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Pollination efficiency of Apis mellifera (Hymenoptera: Apidae) on Annona senegalensis (Annonaceae) flowers at Dang (Ngaoundere, Cameroon)

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ABSTRACT

To evaluate the apicultural value of *Annona senegalensis* and the impact of *Apis mellifera* on fruit and seed yields of this Annonaceae at Dang, its foraging and pollinating activities were studied, from March to June in 2017 and 2020. Observations were carried out on 540 flowers divided in four treatments: 120 flowers accessible to all insects; 120 flowers bagged to avoid all visits; 200 flowers protected then uncovered during anthesis, to allow only *Ap. mellifera* visits; 100 flowers bagged then uncovered and rebagged without the visit of insects or any other organism. Workers daily rhythm of activity, their foraging behavior on flowers and their pollination efficiency were evaluated. Results showed that, honeybee foraged on *An. senegalensis* flowers throughout its whole blooming period. This bee intensely and exclusively harvested pollen. The greatest number of workers foraging simultaneously was 3 per flower and 340 per 1000 flowers. The mean foraging speed for each studied year was 3 flowers/min. Through its pollination efficiency on *An. senegalensis*, *Ap. mellifera* increased the fruiting rate by 26.19%, the mean number of seeds per fruit by 16.33% and the percentage of normal seeds by 22.41%. Hence, the installation of *Ap. mellifera* hive close to *An. senegalensis* plantations is recommended for increase beebread production as a hive product, and to improve fruit and seed yields of this plant.

Keywords: *Apis mellifera*, *Annona senegalensis*, pollination efficiency, yields, Dang.

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INTRODUCTION

Beekeeping is an income - generating activity and contributes to improve agricultural yields through pollination by bee (Vacher, 2007)[1]. Indeed, bees collect pollen and nectar from flowers for food (Axelrod, 1960; Ollerton *et al*., 2011)[2, 3]. However, *Ap. mellifera* transports this substance to the hives in specialized structures of its legs (corbicula), in which pollen, moistened with nectar and saliva, is packaged forming pasty ‘pellets’, called ‘pollen loads’ (Thorp, 2000)[4]. Nectar is transformed into honey (Tchuenguem *et al*., 2004)[5]. These substances have been exploited by humans for thousands of years (Crane, 1999)[6]. Furthermore, pollen is the main source of protein for the honeybee colonies (Zerbo *et al*., 2001)[7] and also contain vitamins, carbohydrates and fats (Omran *et al*., 2017)[8]. It is necessary for the development of larvae and the longevity of the colony (Schmidt *et al*., 1987; Roulston & Cane, 2000)[9, 10]. Further, pollen and honey production depends mainly on the abundance of some plant species and their attractiveness to honey bee (Williams & Carreck, 1994; Segeren *et al*., 1996)[11, 12]. Thus, sustainable beekeeping in a given Region requires a detailed knowledge of the apicultural value of the plant species that grow in the environment of the apiaries (Leven *et al*., 2005)[13].

Annona senegalensis Pers. (Annonaceae) is a tropical, aromatic shrub with edible fruit under domestication (Pinto *et al*., 2005) [14]. The plant is 2 - 6 m, or occasionally up to 11 m, in height (Orwa *et al*., 2009) [15]. The species has alternate, simple, and oblong to ovate leaves. Its flowers are solitary or in groups of 2 - 4, arising above the leaf axils (Okhale *et al*., 2016)[16]. The fruits are formed from many fused carpels, turning yellow to orange on ripening (Okhale *et al*., 2016)[16]. The seeds are numerous, cylindrical, oblong, and orange-brown (Orwa *et al*., 2009)[15]. *Annona senegalensis* has a high nutritional, medicinal, and economic importance for African rural communities across its geographical distribution range (Orwa *et al*. 2009; Mustapha, 2013; Okhale *et al*. 2016)[15, 17, 16]. Fruits, leaves, and flowers of *An. senegalensis* are used for human and livestock consumption and medical treatments (Achille *et al*., 2020)[18]. The food value varies considerably, but most forms have an abundance of carbohydrates, proteins, calcium, phosphorus, iron, thiamine, niacin and riboflavin; while some are rich in magnesium, ascorbic acid and carotenes (Yoa & Wickramaratne, 1995)[19]. Phytochemical screening revealed the presence of various secondary metabolites including tannins (Jada *et al*., 2014)[20], flavonoid (Jada *et al*., 2015)[21], saponins (Afolabi *et al*., 2013)[22], alkaloids (Jou *et al*., 1995)[23], glycosides, steroids (Ijaiva *et al*., 2014)[24], volatile oil (Ngamo *et al*., 2007)[25] and anthocyanins (Mpiana *et al*., 2012)[26]. In traditional medicine applications, the leaves have been used in treating of yellow fever, tuberculosis, and small pox (Mustapha *et al*., 2013) [16]. The stem bark is used in snakebite and hernia treatment (Dambatta *et al*., 2011)[27].

Before our research, some studies have been documented on *An. senegalensis* floral entomofauna.

In Brazil, Gottsberger (1989)[28] and Deroin (1989)[29] report that the Annonaceae flowers produce no nectar, but rewards for pollinators include pollen, and stigmal exudates. The floral visitors of *An. senegalensis* were small diurnal Chrysomelidae and Curculionidae beetles (Gottsberger, 1989)[28].

In Mexico, the main insects families in this Annonaceae flowers are Nitidulidae and Chrysomelidae, while species of Apidae and Formicidae (Hymenoptera) are abundant on the external surfaces of the flowers where contact can not occur with the flowers' sexual structures (Vidal, 1997)[30].

In Cameroon, Tchuenguem *et al*. (2008)[31] have noticed that *Ap. mellifera* harvested pollen and nectar in *An. senegalensis* flowers.

Despite these important researches, the pollination efficiency of *Ap. mellifera* on this Annonaceae has not been reported in previous research to our knowledge. Hence, the main objective of this research was to study of the relationships between *An. senegalensis* and *Ap. mellifera*, for their optimal management in Cameroon. It has five main specific objectives: (1) determine the place of *Ap. mellifera* on *An. senegalensis* floral entomofauna; (2) study the activity of *Ap. mellifera* on *An. senegalensis* flowers; (3) estimate the apicultural value of this plant; (4) assess the impact of the flowering insects on fruit and seed yields of this Annonaceae; (5) evaluate the pollination efficiency of *Ap. mellifera* on this essence.

MATERIALS AND METHOD

Study site

Experiments were carried out from March 15 to June 24, 2017 and from March 13 to June 20, 2020 at Dang (Latitude 7°25.365 N, Longitude 13°32.572 E and Altitude 1083 m) above the see level, a village located in the Adamawa Region of Cameroon. This Region belongs to the high altitude Guinean Savannah agro-ecological zone; the climate is characterized by two seasons: a rainy season (April to October) and a dry season (November to March) (Yonkeu, 1993)[32]. According to Amougou *et al*. (2015)[33], the annual rainfall is about 1500 mm, the mean annual temperature is 22 °C, the mean annual relative humidity is 70%. The vegetation is represented by crops, ornamental plants, hedge plants and native plant species of the savannah and gallery forests (Adamou *et al*., 2014)[34].

Biological materials

The plant material was represented by *An. senegalensis* naturally presents in the study site and the animal material included many insects species naturally present in the environment. The number of *Ap. mellifera* colonies located in the study site was 72 in 2017 and 36 in 2020.

Determination of the reproduction mode of *Annona senegalensis*

The methodology used was modified from that of Tchuenguem *et al*. (2001)[35]. On March 15, 2017, 240 flowers were tagged at bud stage and divided into two treatments: 120 unprotected flowers (treatment 1) and 120 bagged flowers using gauze bags net to avoid all visits (treatment 2). Similarly, on March 13, 2020, 240 flowers at the budding stage were tagged among which 120 were left unprotected (treatment 1'), while 120 were bagged (treatment 2'). For each year of study, the number of fruits was assessed in each treatment a week after shedding of the last labeled flower. The fruiting index (Pi) was then calculated: Pi = Fb / Fa, where Fa is the number of viable flowers initially set and Fb the number of formed fruits. The allogamy rate (Alr) from which derives the autogamy rate (Atr) was expressed as the difference in fruiting indexes between treatment X (unprotected flowers) and treatment Y (bagged flowers) (Demarly, 1977)[36]: Atr = {[(PiX - PiY) / PiX] * 100}, where PiX and PiY are the fruiting indexes in treatments X and Y respectively; Alr = 100 - Atr.

Determination of the place of *Apis mellifera* **on** *Annona senegalensis* **entomofauna**

The frequency of *Ap. mellifera.* in the flowers of *An. senegalensis* was determined based on observations done every day on flowers of treatment 1 from 16th to 24th March 2017, and treatment 1' from 14th to 21th March 2020, during each of the following daily time frame: 6 - 7 am, 8 - 9 am, 10 - 11 am, 12 am - 1 pm, 2 - 3 pm and 4 - 5 pm. In a slow walk along all flowers of treatment 1 and treatment 1', the identity of all insects that visited *An. senegalensis* was recorded. Specimens of all insect taxa were caught with an insect net on unlabeled flowers. For each species, three to five insect specimens were captured. These insects were conserved in 70% ethanol for subsequent taxonomy determination except for Lepidoptera which were conserved in wrapper (Borror & White, 1991)[37]. All insects encountered on flowers were registered and the cumulated results expressed in number of visits to determine the relative frequency of *Ap. mellifera.* in the anthophilous entomofauna of *An. senegalensis*.

Study of the foraging activity of *Apis mellifera* **on** *Annona senegalensis* **flowers**

Floral product harvested

In addition to the determination of the flower visiting insect frequency, direct observation of the foraging activity of *Ap. mellifera* on flowers was made in the experimental set-up. The floral products (nectar or pollen) harvested by *Ap. mellifera* during each floral visit were recorded based on its foraging behavior. Nectar foragers were expected to extend their proboscis in the flowers bottom, while pollen gatherers were supposed to scratch anthers using mandibles and legs (Jean-Prost, 1987)[38]. During the same time that *Ap. mellifera* visits on flowers were registered, the type of floral product collected by foragers was noted (Tchuenguem *et al*., 2005)[39].

Duration of visits and foraging speed

During the same days as for the frequency of visits, the duration of individual flower visits was recorded (using stopwatch) during each of the following daily time frame: 7 - 8 am, 9 - 10 am, 11 am - 12 pm, 13 - 14 pm, 15 - 16 pm and 17 - 18 pm. Moreover, the number of visits during which the foragers bee came into contact with the stigma (Jacob-Remacle,1989)[40] was registered. Regarding the foraging speed (Fs) which is the number of flowers visited by an individual bee per minute (Jacob-Remacle,1989) [40], data were registrated during the same dates and according to the same time frames and daily period as for the duration of visits. The stopwatch, previously set to zero was switched on as soon as an individual landed on a flower and the number of visited flowers was concomitantly counted. The stopwatch was stopped as soon as the visitor was out of sight or when it left *An. senegalensis* flower for another plant species. The foraging speed (Fs) was calculated using the following formula: $Fs = (Nf / dv) * 60$, where dv is the time (sec) given by a stopwatch and Nf the number of flowers visited during dv (Jacob-Remacle, 1989)[40]. During the observation, when a forager returns to previously visited flower, counting is performed as two different flowers (Tchuenguem *et al*., 2005)[39].

Abundance of *Apis mellifera* workers on *Annona senegalensis* flowers

The abundances of foragers (highest numbers of individuals foraging simultaneously) per flower and per 1000 flowers (A_{1000}) were recorded on the same dates and daily time frames as for the registration of duration of visits. Abundance per flower was recorded as a result of direct counting. For determining the abundance per 1000 flowers, foragers were counted on a known number of opened flowers and (A_{1000}) was calculated using the following formula: $A_{1000} = [(Ax / Fx) * 1000]$ (Demelash, 2018)[41], where Fx and Ax are respectively the number of flowers and the number of foragers effectively counted on these flowers at time x.

Foraging ecology

The disruption of the activity of foragers by competitors or predators and the attractiveness exerted by other plant species on *Ap. mellifera* was assessed by direct observations. For the second parameter, the number of times that the bee left *An. senegalensis* flowers to other plant species and vice versa was noted throughout the investigation period. During each daily period of investigation, ambiant temperature and relative humidity in the station were registered every 30 minutes using a mobile thermohygrometer (Technoline WS9119) installed in the shade (Tchuenguem *et al*., 2005)[39].

Evaluation of the apicultural value of *Annona senegalensis*

According to Tchuenguem *el al*., (2005)[39] and Népidé *et al*. (2016) [42], the apicultural value of *An. senegalensis* was assessed using data on the plant flowering intensity and the attractiveness of *Ap. mellifera* foragers with respect to nectar and pollen.

Evaluation of the impact of the flowering insects including *Apis mellifera* **on** *Annona senegalensis* **yields**

Parallel to the constitution of treatments 1, 2, 1’ and 2’, 600 flowers at bud stage were protected in 2017 and 2020, to form two treatments:

- treatments 3 (in 2017) and 3’ (in 2020): 400 flowers protected using gauze bag nets to prevent insect visits and destined to receive one visit of *Ap. mellifera*. Each opened flower of treatments 3 and 3’ were inspected. Hence, gauze bag was delicately removed and this flower was observed for up to 10 minutes; the flowers visited by *Ap. mellifera* were marked and then reprotected. Unvisited flowers by these bees were included in treatment 4 (in 2017) and 4’ (in 2020) (Djakbé *et al*., 2017)[43].

treatments 4 and 4’: 200 flowers protected using gauze bag nets and destined to be uncovered then rebagged without the visit of insects or any other organism. As soon as each flower of treatments 4 and 4’ was opened, the gauze bag was removed and the flower was observed for up to 10 minutes while avoiding the visit by *Ap. mellifera* or any other organism (Djakbé *et al*., 2017)[43].

At maturity, fruits were harvested and counted from each treatment. The mean number of seeds per fruit and the percentage of normal (well developed) seeds (Tchuenguem *et al*., 2009b)[44] were then evaluated. The estimation of the effect of insects on *An. senegalensis* production was based on the impact of flowering insects on pollination, the impact of pollination on *An. senegalensis* fruiting and the comparison of yields (fruiting rate, number of seeds per fruit and percentage of normal seeds) of treatments 1, 2, 4, 1’, 2’ and 4’. For each observations year, the fruiting rate due to the flowering insects including *Ap. mellifera* (Pri) was calculated using the following formula: $Pri = \{[(PX - PZ) / (PX + PY - PZ)] * 100\}$ (Diguir *et al*., 2020)[45], where PX, PY and PZ are the fruiting rates in treatment X (flowers left in free pollination), treatment Y (flowers protected from all insect visits) and treatment Z (flowers bagged then uncovered and rebagged without insect or any other organism visit). The fruiting rate of a treatment (Pr) is giving by the following formula: $Pr = [(b / a) * 100]$, where a is the number of viable flowers initially set and b the number of formed fruits (Tchuenguem *et al*., 2001)[35]. The impact of flower visiting insects including *Ap. mellifera* on the number of seeds per fruit and the percentage of normal seeds were evaluated using the same method as mentioned above for the fruiting rate.

Assessment of the pollination efficiency of ***Apis mellifera*** **on** ***Annona senegalensis***

The contribution of *Ap. mellifera* on the fruiting rate, the number of seeds per fruit and the percentage of normal seeds was calculated using the data of treatments 3 and 4 and those of treatments 3' and 4'. For each observation year, the contribution of *Ap. mellifera* on the fruiting rate (PrX) was calculated using the following formula: PrX = {[(PC - PZ) / PC] * 100}, where PC is the fruiting rate in treatment C (flowers visited exclusively by *Ap. mellifera*) (Djakbé *et al*., 2017)[43]. The impact of *Ap. mellifera* on the number of seeds per fruit and the percentage of normal seeds were evaluated using the same method as mentioned above for the fruiting rate.

Data analysis

Data were analyzed using descriptive statistics (means, standard deviation and percentages), ANOVA (F) for the general comparison of means of more than two samples, student's t-test for the comparison of means of two samples, Pearson correlation coefficient (r) for the study of the association between two variables and chi-square (χ^2) for the comparison of percentages, using Microsoft Excel 2016 software and Stat Graphics plus 5.0.

RESULTS AND DISCUSSION

Reproduction mode of ***Annona senegalensis***

The fruiting indexes of *An. senegalensis* were 0.80, 0.64, 0.93 and 0.59 for treatments 1, 2, 1' and 2' respectively. Thus in 2017, the allogamy rate was 80% whereas the autogamy rate was 20%. In 2020, the corresponding figures were 63.45% and 36.55%. For the two cumulated years, the allogamy rate was 71.73% and the autogamy rate was 28.27%. It appears that the *An. senegalensis* studied had a mixed reproduction mode, allogamous and autogamous, with the predominance of allogamy over autogamy. This can be attributed, in part, to the temporal separation of female and male function of *Annona* species flowers (Gottsberger, 1989)[28] and the other point by the difference between the diversities of anthophilous insects, which is the factors that can influence the reproduction mode of a plant (McGregor, 1976)[46].

Place of ***Apis mellifera*** **in** ***Annona senegalensis*** **floral entomofauna**

Among 159 and 131 visits of fourth and three insect species recorded on *An. senegalensis* flowers in 2017 and 2020 respectively, *Ap. mellifera* was the most represented insect with 145 visits (91.19%) in 2017 and 124 visits (94.65%) in 2020 (Table 1). The difference between the percentages of *Ap. mellifera* visit for the two years is not significant ($\chi^2 = 0.81$; $df = 1$; $P > 0.05$). Our results are not on line than that obtained in Israel by Nadel & Peña, (1994)[47]. These authors noted that, among the four major pollinators of *Annona* species, *Carpophilus mutilatus* was the most important in terms of abundance on *Annona* flowers, followed by *C. fumatus* and *Haptoncus Luteolus.* Although, one species of Coleoptera is very abundant in the *Annona* grove environment, it rarely visits the flowers. In Florida, about nine species of native and exotic nitidulids visit the *Annona* flowers (Nadel & Peña, 1994)[47]. Therefore, the entomofauna of *Annona* species vary geographically and species may even perform differently in each area.

Activity of *Apis mellifera* on *Annona senegalensis* flowers

Floral product harvested

During each flowering period, individuals of *Ap. mellifera* were found intensively and exclusively harvest pollen on *An. senegalensis* flowers (Fig 1). The present study results are not in line with that of Tchuenguem *et al*. (2008)[31] who found that honeybees harvested nectar and pollen on *An. senegalensis* at Dang. Thus, the type of floral products harvested by *Ap. mellifera* on a given plant species can vary with the year. The observed variations of floral products harvested by *Ap. mellifera* could be mainly explained by the needs of the colonies during the flowering period of *An. senegalensis*.

Figure 1: *Apis mellifera* worker collecting pollen in an *Annona senegalensis* flower at Dang in 2020.

Rhythm of visits according to the flowering stages

Apis mellifera visits were more numerous on treatment 1 and 1’ when the number of opened flowers was highest (Figure 2).

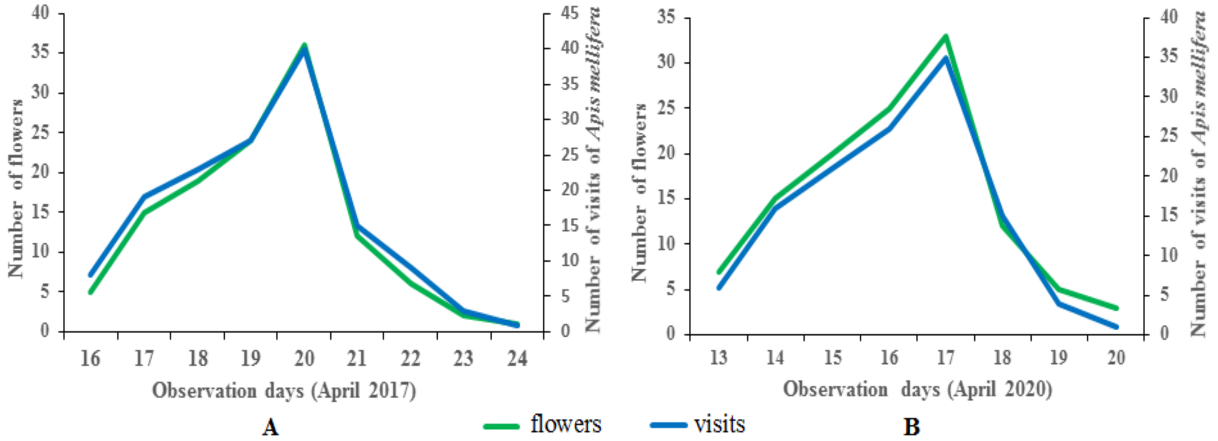

Figure 2: Seasonal variations of the number of *Annona senegalensis* opened flowers and the number of *Apis mellifera* visits on these organs in 2017 (A) and 2020 (B) at Dang.

Moreover, we found a positive and highly significant correlation between the number of *Ap. mellifera* visits and the number of *An. senegalensis* opened flowers in 2017 ($r = 0.99$; $df = 4$; $P < 0.001$) as well as in 2020 ($r = 0.99$; $df = 4$; $P < 0.001$) (Figure 2). This result highlights the good attractiveness of the pollen of *An. senegalensis* towards *Ap. mellifera*.

Daily rythm of visits

Apis mellifera was present on *An. senegalensis* flowers in the morning (6 - 9 am) and in the afternoon (4 - 5 pm) in 2017 as well as in 2020. The peak of activity was situated between 4 and 5 pm in 2017 as well as in 2020 (Figure 3). This peak of activity could be linked to the period of highest availability of pollen on the *An. senegalensis* flowers. Ambient temperature and relative humidity did not influence the activities of *Ap. mellifera* on *An. senegalensis* (Figure 3) but have probably influenced the availability of pollen. During the pistillate phase the *Annona* pollen and stigmas are typically covered by a sticky secretion (Reiss, 1971)[48].

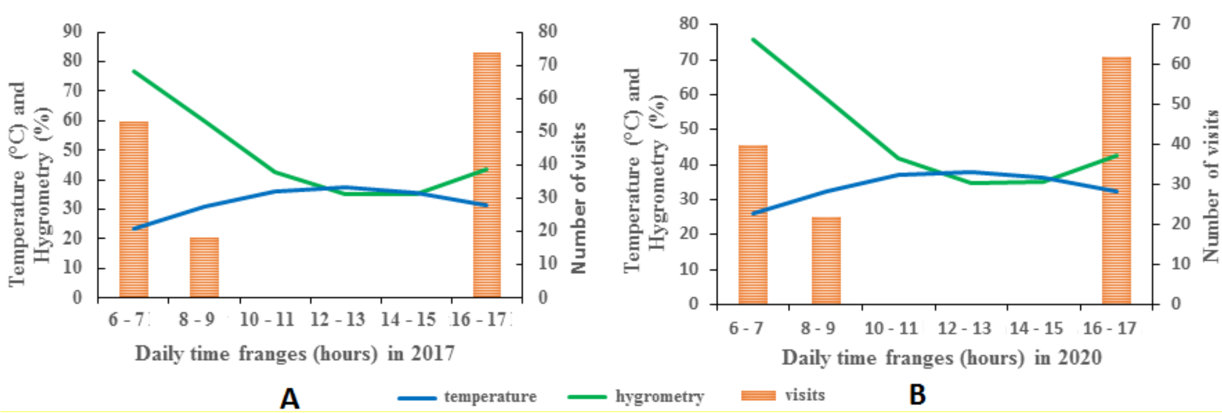

Figure 3: Variations of the ambiant temperature, the relative humidity of the air and the number of *Apis mellifera* visits on *Annona senegalensis* flowers according to the daily time frames in 2017 (A) and 2020 (B) at Dang.

In 2017, the correlation was not significant between the number of *Ap. mellifera* visits and the temperature (*r* = 0.53; *df* = 4; *P* > 0.05), and between the same number of visits and the relative humidity (*r* = - 0.74; *df* = 4; *P* > 0.05). Equally, in 2020, the correlation was not significant between the number of *Ap. mellifera* visits and the temperature (*r* = - 0.47; *df* = 4; *P* > 0.05), and between the same number of visits and the relative humidity (*r* = - 0.72; *df* = 4; *P* > 0.05).

Abundance of workers

In 2017, the mean number of *Ap. mellifera* workers simultaneously in activity was 1 per flower (*n* = 98; *s* = 0.4; *maxi* = 2) and 108.77 per 1000 flowers (*n* = 98; *s* = 50.71; *maxi* = 340). In 2020, the corresponding figures were 1 per flower (*n* = 89; *s* = 0.35; *maxi* = 3) and 92.33 per 1000 flowers (*n* = 89; *s* = 44.27; *maxi* = 300). For the two cumulated years, the mean number of *Ap. mellifera* individuals simultaneously in activity per 1000 flowers was 100.56. The high abundances per 1000 flowers show the good attractiveness of the pollen of *An. senegalensis* towards *Ap. mellifera*. These high abundances are linked to the ability of honey bees to recruit a large number of foragers to exploit an interesting food resource (Frisch, 1967)[49]. The difference between the mean number of foragers per 1000 flowers in 2017 and 2020 was highly significant (*t* = 15.95; *df* = 185; *P* < 0.001). This difference could be explained also by the variation of the number of *Ap. mellifera* colony over the years. Indeed, during the observation, we registered 72 colonies in 2017 compared to 36 noted in 2020. Some of the results obtained were similar to those reported in others studies at Dang (Tchuenguem *et al*., 2008)[31,] These authors showed that the mean number of *Ap. mellifera* per flower of *An. senegalensis* was 1.

Duration of visits per flower

In 2017 and 2020 the mean duration of *Ap. mellifera* visit per flower was 21.06 sec (*n* = 181; *s* = 12.71; *maxi* = 98) and 20.71 sec (*n* = 191; *s* = 13.04; *maxi* = 138) respectively. The difference between these two means is significant (*t* = 2.51; *df* = 185; *P* < 0.05). This difference could be explained by the availability of pollen in the visited flowers or the variation of diversity of flowering insects from one year to another. For the two cumulated years the mean duration per flower was 20.88 sec. Our results are higher than that reported by Tchuenguem *et al*., (2008)[31]. Those authors obtained a mean duration of 13.5 sec (*n* = 54; *s* = 6.82; *maxi* = 40) for the collection of pollen by *Ap. mellifera* on this Annonaceae.

Foraging speed

The mean foraging speed of *Ap. mellifera* worker was 3 flowers per minute in 2017 (*n* = 128; *s* = 1.14; *maxi* = 9) and in 2020 (*n* = 137; *s* = 1.10; *maxi* =8). The difference between these two means is significant (*t* = 2.35; *df* = 116; *P* < 0.05). For the two cumulated years, the mean foraging speed was 3.11 flowers per minute. The later difference could be explained by the availability of pollen or the distance separating the flowers visited during the various foraging trips.

Influence of the fauna

The duration of visits of *Ap. mellifera* was partially influenced by disruptions due to other anthophilous insects during their foraging activity. Thus for 181 visits recorded in 2017, 14 (12.92 %) were interrupted by others *Ap. mellifera* and four (2.2 %) by *Calliphora* sp.. In 2020, for 191 visits, 27 (14.13 %) were interrupted by *Ap. mellifera*. In order to obtain their optimal load of pollen, the foragers of *Ap. mellifera* victims of such disturbances were certainly obliged to visit a greater number of flowers during the corresponding foraging trips.

Influence of neighboring flora

During the study period, other flowering plants were competing with *An. senegalensis* for visitation by *Ap. mellifera* for either pollen (po) or nectar (ne). These plants included: *Voacanga africana*, *Millettia laurentii*, *Mangifera indica*, *Psidium guajava* and *Tithonia diversifolia*. But, during the flowering period of *An. Senegalensis*, *Ap. millefera* workers were not seen flying from flowers of *An. Senegalensis* to neighbouring plant and vice versa. This result indicates that *Ap. mellifera* shows flowers constancy (Louveaux, 1984; Basualdo *et al*., 2000)[50, 51] on this Annonaceae. This floral constancy is due to the fact that in honey bees, individual foragers are generally capable of memorizing and recognizing the shape, colour and odour of the flowers visited during previous foraging strips (Hill *et al*., 1997; Wright *et al*., 2002)[52,53]. In addition, the analysis of the pollen loads collected from the pollen baskets of some workers bees shows that the percentages of the foreign pollen grain was 0.00 % (table 2).

Table 1: Diversity of flowering insects on *Annona senegalensis* in 2017 and 2020 at Dang.

Insects			**2017**		**2020**		**Total$_{2017 / 2020}$**	
Order	**Family**	**Genus and Species**	n_1	P_1 (%)	n_2	P_2 (%)	n_T	P_T (%)
Hymenoptera	Apidae	*Apis mellifera*	145	91.19	124	94.65	269	92.92
	Formicideae	*Componotus* sp.	3	1.88	2	1,52	5	1.7
Diptera	Calliphoridae	*Calliphora* sp.	2	1.2	5	3,83	6	2.52
Coleoptera		(1 sp.)	9	5.66	-	-	9	2.83
Total	Visits		159	100	131	100	289	100
	Species		4		3		4	

n_1 and n_2: number of visits on 120 flowers in 2017 and 2020; P_1 and P_2: percentages of visits in 2017 and 2020; $P_1 = (n_1 / 159) * 100$; $P_2 = (n_2 / 131) * 100$. sp.: undetermined species

Table 2: Pollen profile of pollen loads collected in the corbiculae of six *Apis mellifera* workers foraging on flowers of *Annona senegalensis* according to the investigation periods at Dang.

Year	Total number of pollen grains	*Annona senegalensis* pollen	Pollen of other plant species
2017	1418	1418	00
2020	1623	1623	00
Total	3041	3041	00

Apicultural value of *Annona senegalensis*

During the two flowering periods of *An. senegalensis*, we noted a remarkable activity of the workers of *Ap. mellifera* on the flowers of the studied plant. There were in particular very high daily and seasonal frequencies of visits, a high density of foragers per 1000 flowers, a large harvest of pollen and constancy of foragers to flowers during foraging trips. Thus, the pollen of *An. senegalensi* is available in great quantities for *Ap. mellifera* workers. Hence, it is possible to classify *An. senegalensis* in the category of a highly polliniferous bee plant with the flowering period located in the dry season. Our result is not in accordance with the one of Tchuenguem *et al*. (2008)[31]. These authors showed that *An. senegalensis* was highly polliniferous and nectariferous plant. As a results, plantation and protection of this species could be recommended to strengthen *Ap. mellifera* colonies, to improve pollen production as a hive product in the region.

Impact of anthophilous insects on *Annona senegalensis* **production**

During pollen harvest, flowering insects were in regular contact with the anthers and stigma (100 %). Thus, these insects increased the pollination possibilities of this plant species. Table 3 presents the results on the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds in the different treatments.

Table 3: **Fruiting rate, mean number of seeds per fruit and percentage of normal seeds according to different treatments of *Annona senegalensis* in 2017 and 2020 at Dang.**

Years	Treatments	NF	NFr	Frr **(%)**	**Number of seeds / Fruit**		TNS	NS	% NS
					m	*sd*			
2017	1 (Uf)	120	96	80,00	48,49	8,51	4675	4128	88,29
	2 (Pf)	120	77	64,16	48,94	11,30	3769	2514	66,70
	3 (Fpvx)	136	115	84,55	53,69	10,14	6175	5597	90,63
	4 (Fpwv)	164	98	59,75	47,19	12,81	3870	2608	67,39
2020	1’ (Uf)	120	112	93,33	54,22	12,86	5030	4894	97,29
	2’ (Pf)	120	71	59,16	48,45	10,55	2848	2046	71,83
	3’ (Fpvx)	148	124	83,78	56,13	9,63	6671	6156	92,28
	4’ (Fpwv)	152	98	64,47	44,58	10,04	3780	2819	74,57

NF: number of flowers; NFr: number of fruits; Frr: fruiting rate; TNS: total number of seeds; NS: number of normal seeds; % NS: percentage of normal seeds; *m*: mean; *sd*: standard deviation; Uf:

unprotected flowers; Pf: flowers bagged; Fpvx: flowers protected using gauze bag nets then uncovered, visited once by *Ap. mellifera* and rebagged; Fpwv: flowers protected using gauze bag nets, uncovered, then rebagged without the visit of insects or any other organism

From the table 3, we documented the following:

a) In 2017, the contributions of anthophilous insects on the fruiting rate were 80.00 %, 64.16 %, 84.55 % and 59.75 % in treatments 1 to 4 respectively. In 2020, the corresponding figures were 93.33 %, 59.16 %, 83.78 % and 64.47 % respectively. The differences between these eight percentages are highly significant (χ^2 = 63.20; *df* = 7; *P* < 0.001). The two - by - two comparisons showed that the difference observed is significant between treatments 1 and 2 (χ^2 = 7.47; *df* = 1; *P* < 0.01) and highly significant between treatments 1' and 2' (χ^2 = 38.68; *df* = 1; *P* < 0.001). Consequently, in 2017 and 2020, the fruiting rate of unprotected flowers (treatments 1 and 1') was higher than that of protected flowers (treatments 2 and 2').

b) In 2017, the mean numbers of seeds per fruit were 48.49, 48.94, 53.69, and 47.19 in treatments 1 to 4 respectively. In 2020, the corresponding figures were 54.22, 48.45, 56.13 and 44.58 respectively. The differences between these eight means are highly significant (*F* = 19.09; *df1* = 786; *df2* = 4; *P* < 0.001). The two - to - two comparisons showed that the difference observed is not significant between treatments 1 and 2 (*t* = 1.94; *df* = 171; *P* > 0.05) and highly significant (χ^2 = 20.75; *df* = 181; *P* < 0.001) between treatments 1' and 2'. For the two cumulated years, the mean number of seeds per fruit of unprotected flowers was higher than that of protected flowers.

c) In 2017, the percentages of normal seeds were 88.29 %, 66.70 %, 90.63 % and 67.39 % in treatments 1 to 4 respectively. In 2020, the corresponding figures were 97.29 %, 71.83 %, 92.28 % and 74.57 % respectively. The differences between these eight percentages are generally highly significant (χ^2 = 68.79; *df* = 7; *P* < 0.001). Paired wise comparisons showed that the difference observed is highly significant between treatments 1 and 2 (χ^2 = 13.36; *df* = 1; *P* < 0.001) as well as between treatments 1' and 2' (χ^2 = 24.82; *df* = 1; *P* < 0.001). Hence, in 2017 as well as in 2020, the percentage of normal seeds of unprotected flowers was higher than that of protected flowers.

In 2017, the contribution of anthophilous insects in the fruiting rate and the percentage of normal seeds were 23.99 % and 23.85 % respectively. In 2020, the contribution of flowering insects in the fruiting rate, the number of seeds per fruit and the percentage of normal seeds were 32.78 %, 16.59 % and 24.02 %.

The number of seeds produced in the fruits is directly associated with the number of pollen grains that reach the stigma and fertilize the oosphere and the distance from the forest fragments in relation to the growing area is important when considering the radius of flight of the bees during foraging (Vinícius-Silva *et al*., 2017)[54]. According to Benevides *et al*. (2009)[55] and Garibaldi *et al*. (2011)[56], the short distance between the crops and the natural areas positively influenced the richness of floral visitors and fruit formation. Our results emphasize the importance of pollinators on the quality of fruits produced. In fact, by lying on flowers, the insect could facilitate the release of pollen for the optimal occupation of the stigma. According to McGregor (1976)[46], the fruiting is maintly dependent on pollination intensity. The results obtained were similar to those reported in others studies at Dang (Adamou *et al*., 2014; Népidé *et al*., 2016; Tchuenguem *et al*., 2020)[34, 42, 57]. Data from these authors showed that through its pollinating activity, *Ap. mellifera* significantly increased the fruiting rate by 37.70 % in *Brachiaria brizantha*, 61.85 % in *Croton macrostachyus*, and 96.07 % in *Agave sisalana*. These contributions are high compared to that obtained in *An. senegalensis*. These authors revealed that when collecting pollen, *Ap. mellifera* shakes flowers and this movement could facilitate the liberation of pollen by anthers, for an optimal occupation of the stigma.

Pollination efficiency of *Apis mellifera* on *Annona senegalensis*

For the fruiting rate, the difference was highly significant between treatments 3 and 4 ($\chi^2 = 27.92$; $df = 1$; $P < 0.001$) as well as between treatments 3' and 4' ($\chi^2 = 17.76$; $df = 1$; $P < 0.001$). Therefore, in 2017 and 2020, the fruiting rate of flowers visited by *Ap. mellifera* was higher than that of flowers protected, uncovered and rebagged without the visit of insect or any other organism.

The comparison of the mean number of seeds per fruit (Table 6) showed that the difference was highly significant between treatments 3 and 4 ($t = 29.90$; $df = 211$; $P < 0.001$) as well as between treatments 3' and 4' ($t = 64.13$; $df = 220$; $P < 0.001$).

The comparison of the percentage of normal seeds (Table 6) showed that the difference was highly significant between treatments 3 and 4 ($\chi^2 = 16.28$; $df = 1$; $P < 0.001$) as well as between treatments 3' and 4' ($\chi^2 = 11.34$; $df = 1$; $P < 0.001$). Hence, in 2017 and 2020, the percentage of normal seeds of flowers visited by *Ap. mellifera* was higher than that of flowers protected, uncovered and rebagged without visit of insect or any other organism.

In 2017, the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds due to *Ap. mellifera* were 29.33 %, 12.10 % and 25.64 % respectively. In 2020, the corresponding figures were 23.05 %, 20.57 % and 19.19 %.

For the two cumulated years, the numeric contribution of *Ap. mellifera* via a flower visit on the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds were 26.19 %, 16.33 % and 22.41 % respectively.

In general, our results showed that the impact of *An. mellifera* activities on *An. senegalensis* seeds production is positive, probably because *Ap. mellifera* brought supplementary pollen grains onto the stigma. During the collection of pollen, foragers flew frequently from one flower to another. They could thus enhance self-pollination by applying pollen of one flower on its own stigma. *Apis mellifera* could provide allogamous pollination through carrying of pollen within their hairs, silk, legs, mouthparts, thorax and abdomen, which is then deposited on another flowers belonging to a different plant of the same species (geitogamy) (Philippe, 1991; Roubik, 1995; Abrol, 2012)[58,59, 60].

CONCLUSION

At Dang, *An. senegalensis* is a plant species that benefits highly from pollination by insects, among which *Ap. mellifera* is the most important. The worker bees intensely harvest pollen on flowers of this Annonaceae. The comparison of fruit and seed yields of protected flowers visited exclusively by *Ap. mellifera* with the flowers protected from insects then uncovered and rebagged without the visit of insects or any organism, underscores the value of this bee in increasing fruit and seed production as well as seed quality. The plantation of *An. senegalensis* close to *Ap. mellifera* colonies is recommended to increase beebread production as a hive product, to strengthen *Ap. mellifera* colonies during the dry season and to improve fruit and seed yields of *Annona senegalensis*.

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