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## Pollination efficiency of *Apis mellifera* (Hymenoptera: Apidae) on *Solanum nigrum* (Solanaceae) at Meskine (Maroua, Cameroon)

Jean MAMOUDOU<sup>1</sup>, Sidonie Fameni TOPÉ<sup>1\*</sup>, Emmanuel BASGA<sup>2</sup> and Fernand-Nestor Tchuengem FOHOUO<sup>3</sup>

<sup>1</sup>Department of Biological Sciences, Faculty of Science, University of Maroua,  
P.O. Box 814, Maroua, Cameroon.

<sup>2</sup>Department of Earth and Life Sciences, Higher Teachers' Training College, University of Maroua,  
P. O. Box 46, Maroua, Cameroon.

<sup>3</sup>Department of Biological Sciences, Faculty of Science, University of Ngaoundéré,  
P. O. Box 454, Ngaoundéré, Cameroon.

\*Corresponding author; E-mail: [sidofameni@gmail.com](mailto:sidofameni@gmail.com); Tel. (+237)697438668 / (+237)678396121

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### ABSTRACT

Most plant species require pollen transfer to produce fruits and set seeds. Although some insects visit flowers for nectar or pollen, not all flowering insects bring about pollination. To evaluate the impact of *Apis mellifera* Linnaeus on fruit and seed yields of *Solanum nigrum* Linnaeus, foraging and pollinating activities of worker bees were studied at Meskine in January 2019 and February 2020. The experiments were carried out on 540 flowers divided in four treatments: two treatments differentiated according to the presence or absence of protection of flowers regarding *A. mellifera* and other insect visits; the third with flowers protected and uncovered when they were opened, to allow *A. mellifera* visits and the fourth with flowers protected then uncovered and reprotected without the visit of insects or any other organism. The foraging behaviour of *A. mellifera* on flowers, its efficiency pollination, the fruiting rate, the number of seeds per fruit and the percentage of normal seeds were evaluated. Results indicate that among 27 insect species recorded on flowers, *A. mellifera* ranked first accounting for 34.16% of 2652 visits. This worker bee intensely harvested nectar and pollen. The mean foraging speed was 10.39 flowers / min. The mean abundance per 1000 flowers was 519.46. For the two years, throughout its pollination efficiency, *A. mellifera* increased the fruiting rate by 16.29%, as well as the percentage of normal seeds by 15.57%. Hence, the installation of honeybee colonies close to *S. nigrum* fields is recommended to improve fruit yields, seed quality, pollen as a hive product and honey production.

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**Keywords:** Honeybee, Far North, apicultural value, flowers, yields.

### INTRODUCTION

Pollinators play an important role in the sustainability of ecosystems and agriculture (Klein et al., 2007). Nearly 80% of the commercial crops are pollinated by insects

(Free, 1993). Honeybees are well adapted to pollination because their sense of smell, eyes, mouthparts, and numerous branched body hairs are ideally suited for searching food sources, sipping nectar, collecting and distributing

pollen (Abrol, 2012). Pollination is an ecosystem service in that wild pollinators, in particular wild bees, contribute significantly to the pollination of a large array of crops (Winfree et al., 2008; Mohamadou et al., 2018). In the natural environment and in agro ecosystems, flowering insects in general and Apoidea in particular have great ecological and economical importance because they have a positive influence on food production (Dounia and Tchuenguem, 2013; Basga et al., 2018). Besides, the activity and diversity of flowering insects of a plant vary with place and period (Abrol, 2012; Tchuenguem et al., 2014).

*Solanum nigrum* occupies an important place in the world economy and contributes effectively to food security (Asseng et al., 2017). It is grown widely in temperate and tropical region (Olmstead et al., 2008). In Africa, mainly in Ivory Coast and Cameroon, it is grown in small farms and vegetable gardens for market supply. *Solanum nigrum* is an excellent source of iron, calcium, proteins, vitamins A and C, iodine and zinc (Schippers, 2000). It generally provides few calories to the human body (Schippers, 2000) and contains soluble fibers (Agbo et al., 2009) which facilitates digestion. The plant extracts are used as an analgesic, antispasmodic, anti-inflammatory and vasodilator (Duke and Ayensu, 1985).

*Solanum nigrum* is an annual plant (Edmonds and Chweya, 1997). Its flowers have whitish petals that surround bright yellow anthers (Edmonds and Chweya, 1997). The flower is hermaphrodite and produce both nectar and pollen, which attract insects (Schippers, 1998).

The flowers remain on the plants for ten days acting as a visual 'flag' to the pollinators (Edmonds and Chweya, 1997). In Cameroon, *S. nigrum* is cultivated as vegetable (Schippers, 1998) and there are no reliable statistics on production and trade (Aboubakar et al., 2020). Nevertheless, there are exports of *S. nigrum* leaves from Cameroon to Nigeria and Gabon.

In Cameroon, Aboubakar et al. (2020) have reported lack of seeds due to soil degradation. In Nigeria, Okafor (1997) also revealed lack of seeds and seedling mortality

which is a direct effect of poor seed quality and vigour. In this country, *S. nigrum* flowers were reported to produce fewer seeds per fruit in the absence of efficient pollinators (Oyelana and Ogunwenmo, 2012). Therefore, to widespread cultivation of *S. nigrum* as leafy vegetables, it is important to investigate on the possibilities of increasing seed production of this plant. Informations obtained on the interaction between *S. nigrum* and its anthophilous insects will allow farmers to develop management strategies that can contribute to increase the quantity and quality of *S. nigrum* yields. During preliminary observations on flower-insect relationships in Maroua before 2017 (unpublished data), *A. mellifera* has been seen intensively visiting flowers of *S. nigrum*. Investigations on foraging and pollinating activities of *A. mellifera* on *S. nigrum* flowers in Cameroon could provide essential data for their optimal management. Indeed, *Solanum nigrum* is one of the many plants species for which informations on pollination by insects in Cameroon remain unknown.

## MATERIALS AND METHODS

### Study site, experimental plot and biological material

The experiments were carried out from 5<sup>th</sup> January to 8<sup>th</sup> February 2019 and from 4<sup>th</sup> February to 8<sup>th</sup> March 2020 at Meskine (latitude: 10° 32' 26" N; longitude: 14° 14' 53" E; altitude: 410 m above sea level), a Western suburb of Maroua in the Far North Region of Cameroon. This Region belongs to the ecological zone with three phytogeographical areas (Sudano-Sahelian, Sahelian and Sudanian altitude) periodically flooded, with unimodal rainfall (Letouzey, 1985). The climate is characterized by two seasons: a dry season (November to May) and a rainy season (June to October); August is the wettest month of the year (Letouzey, 1985). Annual rainfall varies from 400 to 1100 mm; the annual average temperature varies between 29 °C and 38 °C and a daily temperature range between 6 °C and 7 °C (Letouzey, 1985). The experiment plot was a field of 437 m<sup>2</sup>. The animal material included *A. mellifera* and other insect species naturally occurring in the environment of the

study site. The vegetation was represented by wild and cultivated species. The plant material was represented by *S. nigrum* whose seeds were provided by the Institute of Agricultural Research for Development of Garoua.

### Sowing and weeding

From October 12<sup>th</sup> to 19<sup>th</sup> 2019 and from October 22<sup>nd</sup> to 30<sup>th</sup> 2020, the experimental plot was divided into 8 subplots of 8\*4.5 m<sup>2</sup> each. Three seeds were sown per hole on six lines per subplot. There were 16 holes per line. Holes were separated 50 cm from each other, while lines were 75 cm apart (Djakbé et al., 2017).

### Determination of the mode of reproduction of *Solanum nigrum*

On January 4<sup>th</sup> 2019, 240 flowers of *S. nigrum* at bud stage were labeled and divided in two treatments: 120 unlimited flowers (treatment 1) access by all visitors and 120 flowers bagged using gauze bags net to avoid insect visits (treatment 2) (Tchuenguem et al., 2001). Similarly, on February 3<sup>rd</sup> 2020, 240 flowers at the budding stage were labeled of which 120 flowers were left unprotected (treatment 5), while 120 were bagged (treatment 6). For each cropping year, after harvest, the number of fruits formed in each treatment was assessed.

For each treatment, the fruiting index ( $P_i$ ) was then calculated as described by Tchuenguem et al. (2001):  $P_i = F_b / F_a$ , where  $F_a$  the number of viable flowers initially set and  $F_b$  the number of formed fruits. For each year, 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);  $Atr = \{[(P_iX - P_iY) / P_iX] \times 100\}$ , where  $P_iX$  and  $P_iY$  are the fruiting indexes in treatments X and Y respectively;  $Alr = 100 - Atr$ .

### Estimation of the frequency of *Apis mellifera* visits on *Solanum nigrum* flowers

Observations were conducted on 120 individual opened pollinated flowers of treatments 1 and 5, each day, from 18<sup>th</sup> January

to 26<sup>th</sup> January 2019 and from 12<sup>th</sup> to 30<sup>th</sup> February 2020, according to six daily time frames: 6 - 7 a.m., 8 - 9 a.m., 10 - 11 a.m., 12 - 13 p.m., 14 - 15 p.m. and 16 - 17 p.m. In a slow walk along all labelled flowers of treatments 1 and 5, the identity of all insects that visited *S. nigrum* flowers was recorded. Specimens of all insect taxa were caught using insect net on unlabelled flowers and conserved in 70 % ethanol, excluding butterflies that were preserved dry (Borror and White, 1991), for further taxonomic identification. All insects encountered on flowers were registered and the cumulated results expressed as the number of visits to determine the relative frequency of *Apis mellifera* in the anthophilous entomofauna of *S. nigrum* (Tchuenguem et al., 2009a). Data obtained were used to determine the frequency of visits ( $F_i$ ) of each insect species on *S. nigrum* flowers. For each study period,  $F_i = [(V_i / V_t) \times 100]$  (Tchuenguem et al., 2001), where  $V_i$  is the number of visits of insect  $i$  on treatment with unprotected flowers and  $V_t$ , the total number of insect visits of all the recorded insect species on these flowers.

### Study of the foraging activity of *Apis mellifera* on *Solanum nigrum* flowers

#### Floral products harvested

In addition to the determination of the flower visiting insect frequencies, direct observation of the foraging activity of *Apis mellifera* on flowers was made in the experimental field. Workers were categorized based on their specific foraging behaviour. Nectar foragers were expected to be seen extending their proboscis between the base of the corolla and stamens, while pollen gatherers were supposed to scratch the anthers using their mandibles and legs (Jean-Prost, 1987). During the same time that the duration of *A. mellifera* visits on flowers was registered, the type of floral product harvested by the honeybee was noted (Tchuenguem, 2005). In the morning of each sampling day, the number of opened flowers was counted. Data obtained were used to determine the relationship between the number of visits of *A. mellifera* and the number of opened flowers (Tchuenguem, 2005).

### **Duration of visits and foraging speed**

During the same days as for the registration of the frequency of visits, the duration of individual flower visit was recorded (using stopwatch) according to six-time frames: 7 - 8 a.m., 9 - 10 a.m., 11 - 12 p.m., 1 - 2 p.m., 3 - 4 p.m. and 5 - 6 p.m. Moreover, the number of visits during which the bee came into contact with the stigma (Jacob-Renacle, 1989) was registered.

Regarding the foraging speed ( $F_s$ ) which is the number of flowers visited by an individual bee per minute (Jacob-Renacle, 1989), data were registered during the same dates and according to the same time frames 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 (Tchuenguem, 2005). The stopwatch was stopped as soon as the visitor was lost to sight or when it left *S. nigrum* flower for another plant species. The foraging speed ( $F_s$ ) was calculated using the following formula:  $F_s = (Nf / d_v) * 60$ , where  $d_v$  is the duration (in sec) given by stopwatch and  $Nf$  the number of flowers visited during  $d_v$  (Tchuenguem, 2005). During the observation, when a forager returned to previously visited flower, counting was performed as on two different flowers (Tchuenguem, 2005).

### **Abundances per flower and per 1000 flowers**

The abundances of foragers (highest numbers of individuals foraging simultaneously) (Tchuenguem et al., 2004) per flower and per 1000 flowers ( $A_{1000}$ ) were recorded on the same dates and daily time frames as that for the registration of the duration of visits. Abundance per flower was recorded as a result of direct counting (Tchuenguem et al., 2004). For determining the abundance per 1000 flowers, foragers were counted on a known number of opening flowers and  $A_{1000}$  was calculated using the following formula:  $A_{1000} = [(Ax / Fx) \times 1000]$ , where  $Fx$  and  $Ax$  are respectively the number of flowers and the number of foragers effectively counted on these flowers at time  $x$  (Tchuenguem et al., 2004).

### **Foraging ecology**

The disruption of the activity of foragers by competitors or predators and the attractiveness exerted by other plant species on *A. mellifera* was assessed by direct observations (Tchuenguem, 2005). For the second parameter, the number of times that the bee left this Solanaceae flowers to another plant species and vice versa was noted through the investigation period (Tchuenguem, 2005).

During each observation date, temperature and relative humidity in the station were registered every 30 minutes using a mobile thermo-hygrometer installed in the shade (Tchuenguem, 2005), from 6 a.m. to 6 p.m.

### **Evaluation of the apicultural value of *Solanum nigrum***

The beekeeping value of *Solanum nigrum* was assessed using data on its flowering intensity and the attractiveness of workers of *A. mellifera* to its pollen and nectar (Tchuenguem et al., 2008; Wekééré et al., 2020).

### **Evaluation of the impact of flowering insects including *Apis mellifera* on *Solanum nigrum* yields**

Parallel to the implementation of treatments 1, 2, 5 and 6, 600 flowers at bug stage were labelled in 2019 and in 2020, to form two treatments:

- Treatment 3 in 2019 or 7 in 2020: 200 flowers protected using gauze bag nets to prevent insect or any other organism visits and destined to be visited exclusively by *A. mellifera*; as soon as each flower of these treatments was opened, the gauze bag was delicately removed and this flower was observed for up to 10 minutes; the flower visited once by *A. mellifera* was then reprotected (Tchuenguem and Népidé, 2018);

- Treatment 4 in 2019 or 8 in 2020: 100 flowers protected using gauze bag nets and destined to be uncovered then rebagged without the visit of insects or any other organism (Diguir et al., 2020); as soon as each flower of these treatments was opened, the gauze bag was removed and this flower was observed for up to 10 minutes, while avoid

insect or any other organism visits (Diguir et al., 2020).

At the maturity, fruits were harvested and counted from each treatment. The fruiting rate, the number of seeds per fruit and the percentage of normal (well developed) seeds were then determined for each treatment (Tchuenguem et al., 2009b). The evaluation of the effect of insects including *A. mellifera* on *S. nigrum* production was based on the impact of flowering insects on pollination, the impact of pollination on *S. nigrum* fruiting, and the comparison of the fruiting rate, the number of seeds per fruit and the percentage of normal seeds of treatments 1, 2, 4, 5, 6 and 8. For each year, the fruiting rate due to the foraging insects including *A. mellifera* (*Fri*) was calculated using the following formula (Diguir et al., 2020):  $Fri = \{[(FX - FZ) / (FX + FY - FZ)] \times 100\}$ , where *FX*, *FY* and *FZ* 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 (*Fr*) is  $Fr = [(b / a) \times 100]$ , where *b* is the number of fruits formed and *a* the number of viable flowers initially set (Tchuenguem et al., 2001). The impact of flower visiting insects including *A. mellifera* on the number of seeds per fruit and the percentage of normal seeds was evaluated using the same method as mentioned above for the fruiting rate.

#### **Assesment of the pollination efficiency of *Apis mellifera* on *Solanum nigrum***

The contribution of *A. 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 for 2019 and those of treatments 7 and 8 for 2020. For each observation year, the contribution of *A. mellifera* on the fruiting rate (*FrA*) was calculated using the following formula:  $FrA = \{[(FA - FZ) / FA] \times 100\}$ , where *FA* is the fruiting rate in treatment *A* (flowers bagged then uncovered, visited exclusively by *A. mellifera* and rebagged) (Djakbé et al., 2017). The impact of *A. mellifera* on the fruiting rate,

the number of seed per fruit and the percentage of normal seeds were evaluated using the same method as mentioned above for the fruiting rate.

#### **Data analysis**

Statistical analyses were performed using: R commander; version i386 3.2.0.; descriptive statistics; Microsoft Excel 2010; 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; chi-square ( $\chi^2$ ) for the comparison of percentages.

## **RESULTS**

#### **Reproduction mode of *Solanum nigrum***

The fruiting indexes of *S. nigrum* were 0.92, 0.81, 0.9 and 0.8 for treatments 1, 2, 5 and 6 respectively. Thus, in 2019, the allogamy rate was 11.96% whereas the autogamy rate was 88.04%. In 2020, the corresponding figures were 11.11% and 88.89%. For the two cumulated years, the allogamy rate was 11.54% and the autogamy rate was 88.46%. It appears that *S. nigrum* has a mixed reproduction mode that is allogamous – autogamous breeding regime, with the predominance of autogamy over allogamy.

#### **Place of *Apis mellifera* in *Solanum nigrum* floral entomofauna**

Among the 694 and 1958 visits of 17 and 25 insect species recorded on *S. nigrum* flowers in 2019 and 2020 respectively, *A. mellifera* ranked first with 288 visits (41.50%) and 618 visits (31.56%), in 2019 and 2020, respectively (Table 1). The difference between the percentages of *A. mellifera* visits for two years is highly significant ( $\chi^2 = 25.08$ ; *df* = 1; *P* < 0.001).

#### **Activity of *Apis mellifera* on *Solanum nigrum* flowers**

##### **Floral product harvested**

From field observations, *A. mellifera* workers were found to collect pollen (Figure 1) and nectar regularly and intensively on *S.*

*nigrum* flowers. For 792 and 730 visits counted on these flowers in 2019 and 2020 respectively, 721 (91.04%) and 620 (84.93%) were for pollen collection whereas 71 (8.96%) and 110 (15.07%) were for nectar collection, respectively in 2019 and 2020. For the total of 1522 visits recorded during the two seasons, the number of visits allocated to pollen harvest was 1341 (88.11%) and that for nectar collection was 181 (11.89%) (Table 2).

#### **Rythm of visits according to the flowering stages**

*Apis mellifera* visits were most numerous in the *S. nigrum* field when the number of opened flowers was highest. Furthermore, a positive and significant correlation was found between the number of *A. mellifera* visits and the number of *S. nigrum* opened flowers in 2019 ( $r = 0.69$ ;  $df = 4$ ;  $P < 0.01$ ) (Figure 2A) as well as in 2020 ( $r = 0.99$ ;  $df = 4$ ;  $P < 0.01$ ) (Figure 2B). This result highlights the good attractiveness of the pollen and nectar of *S. nigrum* towards *A. mellifera*.

#### **Daily rythm of visits**

*Apis mellifera* foraged on *S. nigrum* flowers throughout the blooming period, with a peak of activity between 8 a.m. and 9 a.m. in 2019 as well as in 2020. Ambient temperature and relative humidity did not influenced the activities of *A. mellifera* on *S. nigrum* (Figure 3). In 2019, the correlation was not significant ( $r = -0.22$ ;  $df = 2$ ;  $P > 0.05$ ) between the number of *A. mellifera* visits and the temperature, and between the same number of visits and the relative humidity ( $r = 0.33$ ;  $df = 2$ ;  $P > 0.05$ ) (Figure 3A). In 2020, the correlation was equally not significant ( $r = -0.4$ ;  $df = 2$ ;  $P > 0.05$ ) between the number of *A. mellifera* visits and the temperature, and between the same number of visits and the relative humidity ( $r = 0.76$ ;  $df = 2$ ;  $P > 0.05$ ) (Figure 3B).

#### **Abundance of *Apis mellifera***

In 2019, the highest mean number of *A. mellifera* workers simultaneous in activity was 1 per flower ( $n = 536$ ;  $s = 1.44$ ) and 515.50 per 1000 flowers ( $n = 536$ ;  $s = 282.99$ ). In 2020, the corresponding figures were 1 per flower ( $n = 399$ ;  $s = 1.46$ ) and 523.43 per 1000 flowers ( $n = 399$ ;  $s = 287.34$ ). There is no difference

between these two means ( $t = 0.42$ ;  $df = 943$ ;  $P > 0.05$ ). For the two cumulated years the mean number of foragers per 1000 flowers was 519.46 ( $n = 935$ ;  $s = 285.16$ ).

#### **Duration of visits per flower**

In 2019, the mean duration of a visit was 7.97 sec ( $n = 71$ ;  $s = 3.72$ ;  $maxi = 21$ ) for nectar collection, against 8.35 sec ( $n = 721$ ;  $s = 3.73$ ;  $maxi = 49$ ) for pollen harvest. The difference between the duration of a visit for nectar and pollen harvest in 2019 is not significant ( $t = 0.82$ ;  $df = 790$ ;  $P > 0.05$ ). In 2020, the corresponding figures were 7.82 sec ( $n = 110$ ;  $s = 2.84$ ;  $maxi = 21$ ) for nectar, against 8.2 sec ( $n = 620$ ;  $s = 3.76$ ;  $maxi = 68$ ) for pollen. The difference between the duration of a visit for nectar and pollen harvest in 2020 is equally not significant ( $t = 1.22$ ;  $df = 728$ ;  $P > 0.05$ ). The difference between the duration of a visit for nectar harvest in 2019 and 2020 is not significant ( $t = 0.04$ ;  $df = 179$ ;  $P > 0.05$ ), the difference between the duration of a visit for pollen collection in 2019 and 2020 is equally not significant ( $t = 0.04$ ;  $df = 1339$ ;  $P > 0.05$ ). For the two cumulated years, the mean duration of a flower visit was 7.89 sec ( $n = 181$ ;  $s = 3.28$ ) for nectar collection and 8.27 sec ( $n = 1341$ ;  $s = 3.74$ ) for pollen harvest. The difference between these two later means is not significant ( $t = 0.10$ ;  $df = 1520$ ;  $P > 0.05$ ).

#### **Foraging speed**

In *S. nigrum* field, the mean foraging speed of *A. mellifera* was 9.25 flowers per minute ( $n = 85$ ;  $s = 3.61$ ) in 2019 and 11.54 flowers per minute ( $n = 60$ ;  $s = 3.89$ ) in 2020. The difference between these two means is highly significant ( $t = 21.45$ ,  $df = 143$ ;  $P < 0.001$ ). For the two cumulated years, the mean foraging speed was 10.39 flowers per minute ( $n = 145$ ;  $s = 3.75$ ) (Table 2).

#### **Influence of fauna**

Workers of *A. mellifera* were disturbed in their foraging activity by other foragers of the same species or those from other species that were the competitor for *S. nigrum* nectar and / or pollen. In 2019, for 288 visits of *A. mellifera*, 2 (0.69%) were interrupted by *A. mellifera* and 5 (1.73%) by *Xylocopa* sp. 1. In 2020, for 618 visits, 4 (0.64%) were interrupted

by *A. mellifera* and 8 (1.29%) by *Xylocopa* sp. 1.

### **Influence of neighbouring flora**

During the flowering period of *S. nigrum*, flowers of many other plant species surrounding the field of this Solanaceae were visited by *A. mellifera*, for nectar (ne) and / or pollen (po) (Table 3). During the two years of study, we observed no passage of *A. mellifera* from *S. nigrum* flowers to flowers of another plant species and vice versa. Hence during foraging trips on *S. nigrum*, individuals of *A. mellifera* were faithful to this Solanaceae.

### **Apicultural value of *Solanum nigrum***

During the observation periods of flowering of *S. nigrum*, a well elaborated activity of *A. mellifera* workers was registered on its flowers. In particular, there were a good daily and seasonal frequency of visits, high density of workers per 1000 flowers, very good nectar and pollen harvest, fidelity of the workers to the flowers. These data highlight the good attractiveness of *S. nigrum* nectar and pollen to *A. mellifera*. Therefore, *S. nigrum* is a highly polliniferous and nectariferous bee plant.

### **Impact of anthophilous insects including *Apis mellifera* on *Solanum nigrum* yields**

The fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds in the different treatments of *S. nigrum* are shown in Table 4.

This table shows that :

a) The fruiting rate was 91.67%, 80.83%, 83.55%, 68, 99%, 79.29%, 73.47%, 80.59% and 67.09% in treatments 1 to 8 respectively. The differences between these eight percentages are globally highly significant ( $\chi^2 = 253.52$ ;  $df = 7$ ;  $P < 0.001$ ). The two-to-two comparisons showed that the difference observed is highly significant between treatments 1 and 2 ( $\chi^2 = 5.96$ ;  $df = 1$ ;  $P < 0.001$ ), as well as between treatments 5 and 6 ( $\chi^2 = 4.72$ ;  $df = 1$ ;  $P < 0.001$ ). Consequently, in 2019 and 2020, the fruiting rate of unprotected flowers (treatments 1 and 5) was

higher than that of protected flowers (treatments 2 and 6).

b) The mean numbers of seeds per fruit were 79.14, 73.40, 80.59, 66.99, 79.30, 73.48, 80.59 and 67.09 in treatments 1 to 8 respectively. The differences between these eight means are globally highly significant ( $F = 846.44$ ;  $df_1 = 7$ ;  $df_2 = 798$ ;  $P < 0.001$ ). The two-to-two comparisons showed that the difference observed is highly significant between treatments 1 and 2 ( $t = 49.72$ ;  $df = 205$ ;  $P < 0.001$ ), as well as between treatments 5 and 6 ( $t = 47.42$ ;  $df = 202$ ;  $P < 0.001$ ). Consequently in 2019, as well as in 2020, the mean number of seeds per fruit of unbagged flowers (treatments 1 and 5) was higher than that of bagged flowers (treatments 2 and 6).

c) The percentages of normal seeds were 97.74%, 94.82%, 91.84%, 86.64%, 97.76%, 94.83%, 96.62% and 94.79% in treatments 1 to 8 respectively. The differences between these eight percentages are globally highly significant ( $\chi^2 = 49.57$ ;  $df = 7$ ;  $P < 0.001$ ). The two-to-two comparisons showed that the difference observed is highly significant between treatments 1 and 2 ( $\chi^2 = 96.79$ ;  $df = 1$ ;  $P < 0.001$ ), as well as between treatments 5 and 6 ( $\chi^2 = 96.71$ ;  $df = 1$ ;  $P < 0.001$ ). Hence, in 2019 as well as in 2020, the percentage of normal seeds of exposed flowers (treatments 1 and 5) was higher than that of bagged flowers (Treatments 2 and 4).

In 2019, the numeric contribution of anthophilous insects in the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds of *S. nigrum* were 12.84%, 6.56% and 8.29% respectively. In 2020, the corresponding figures were 12%, 6.53% and 0.07%. For the two cumulated years, the numeric contribution of anthophilous insects including *A. mellifera* were 12.42%, 6.54% and 4.18% for the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds of *S. nigrum* respectively.

### **Pollination efficiency of *Apis mellifera* on *Solanum nigrum***

During a single flower visit of *A. mellifera* for nectar or pollen harvest on *S.*

*nigrum* flowers, this bee always came into contact with anthers and stigma, increasing the possibility of this Solanaceae pollination.

The comparison of fruiting rates (Table 5) shows that the difference observed was highly significant between treatments 3 and 4 ( $\chi^2 = 11.63$ ;  $df = 1$ ;  $P < 0.001$ ), as well as between treatments 7 and 8 ( $\chi^2 = 9.96$ ;  $df = 1$ ;  $P < 0.001$ ).

The comparison of the mean numbers of seeds per fruit (Table 5) shows that the difference observed was significant between treatments 3 and 4 ( $t = 2.02$ ;  $df = 195$ ;  $P < 0.05$ ), as well as between treatments 7 and 8 ( $t = 2.05$ ;  $df = 193$ ;  $P < 0.05$ ).

The comparison of the percentage of normal seeds (Table 5) shows that the difference observed was highly significant

between treatments 3 and 4 ( $\chi^2 = 25.10$ ;  $df = 1$ ;  $P < 0.001$ ), as well as between treatments 7 and 8 ( $\chi^2 = 29.36$ ;  $df = 1$ ;  $P < 0.001$ ).

Hence, in 2019 and 2020, the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds of flowers visited once by *A. mellifera* was higher than that of flowers bagged then uncovered and rebagged without insect or any other organism visit.

In 2019, the numeric contribution of *A. mellifera* on the fruiting rate, the percentage of the number of seeds per fruit and the percentage of normal seeds were 16.29%, 5.19% and 15.63% respectively. In 2020, the corresponding figures were 16.66%, 1.06% and 15.52% respectively. For the two cumulated years, the corresponding figures were 16.29%, 3.45% and 15.57% respectively.

**Table 1:** List of insects collected on *Solanum nigrum* flowers in 2019 and 2020 at Meskine, number and percentage of visits of different insects.

Insects			2019		2020		Total	
Order	Family	Genus and species	$n_1$	$p_1$ (%)	$n_2$	$p_2$ (%)	$n_t$	$pt$ (%)
Coleoptera		(sp. 1) (ne, po)	-	-	4	0.20	4	0.15
	Scarabeidae	(1 sp.) (ne, po)	-	-	1	0.05	1	0.04
Diptera	Muscidae	<i>Chrysomia chloropyga</i> (ne)	2	0.29	3	0.15	5	0.19
	Syrphidae	(1 sp.) (ne, po)	-	-	6	0.31	6	0.23
Hymenoptera	Apidae	<i>Apis mellifera</i> (ne, po)	288	41.50	618	31.56	906	34.16
		<i>Amegilla calens</i> (ne)	14	2.02	205	10.47	219	8.26
		<i>Amegilla</i> sp. 1 (ne, po)	2	0.29	74	3.78	76	2.87
		<i>Amegilla</i> sp. 2 (ne, po)	5	0.72	2	0.10	7	0.26
		<i>Amegilla</i> sp. 3 (ne, po)	2	0.29	3	0.15	5	0.19
		<i>Braunsapis</i> sp. (ne, po)	77	11.10	156	7.97	233	8.79
		<i>Ceratina</i> sp. 1 (ne, po)	6	0.86	-	-	6	0.23
		<i>Ceratina</i> sp. 2 (po)	4	0.58	-	-	4	0.15
		<i>Xylocopa olivacea</i> (ne, po)	20	2.88	101	5.16	121	4.56
		<i>Xylocopa</i> sp. 1 (ne, po)	197	28.39	467	23.85	664	25.04
		<i>Xylocopa</i> sp. 2 (ne, po)	5	0.72	17	0.87	22	0.83
		Formicidae	<i>Camponotus brutus</i> (ne)	-	-	105	5.36	105
	<i>Polyrachis</i> sp. (ne, po)	-	-	4	0.20	4	0.15	



	Halictidae	(1 sp.)	-	-	14	0.72	14	0.53
		<i>Lasioglossum</i> sp. 1 (ne, po)	5	0.72	17	0.87	22	0.83
		<i>Lasioglossum</i> sp. 2 (ne, po)	4	0.58	3	0.15	7	0.26
		<i>Lipotriches azarensis</i> (ne, po)	55	7.93	128	6.54	183	6.90
	Megachilidae	(1 sp.)	5	0.72	3	0.15	8	0.30
	Vespidae	<i>Amophilia sabulosa</i> (ne, po )	-	-	2	0.10	2	0.08
		<i>Belonogaster juncea</i> (ne)	-	-	7	0.36	7	0.26
		<i>Sceliphron</i> sp. (ne, po )	-	-	3	0.15	3	0.11
Lepidoptera	Papilionidae	<i>Acraea acerata</i> (ne)	-	-	4	0.20	4	0.15
	Pieridae	<i>Eurema</i> sp. (ne)	3	0.43	11	0.56	14	0.53
<b>TOTAL</b>			<b>694</b>		<b>1958</b>		<b>2652</b>	
			<b>17 species</b>		<b>25 species</b>		<b>27 species</b>	

$n_1$  and  $n_2$ : number of visits on 120 flowers in 2019 and 2020;  $p_1$  and  $p_2$ : percentages of visits in 2019 and 2020; **sp.**: undetermined species; **ne**: collection of nectar; **po**: collection of pollen;  $p_1 = (n_1 / 694) \times 100$ ;  $p_2 = (n_2 / 1958) \times 100$ .

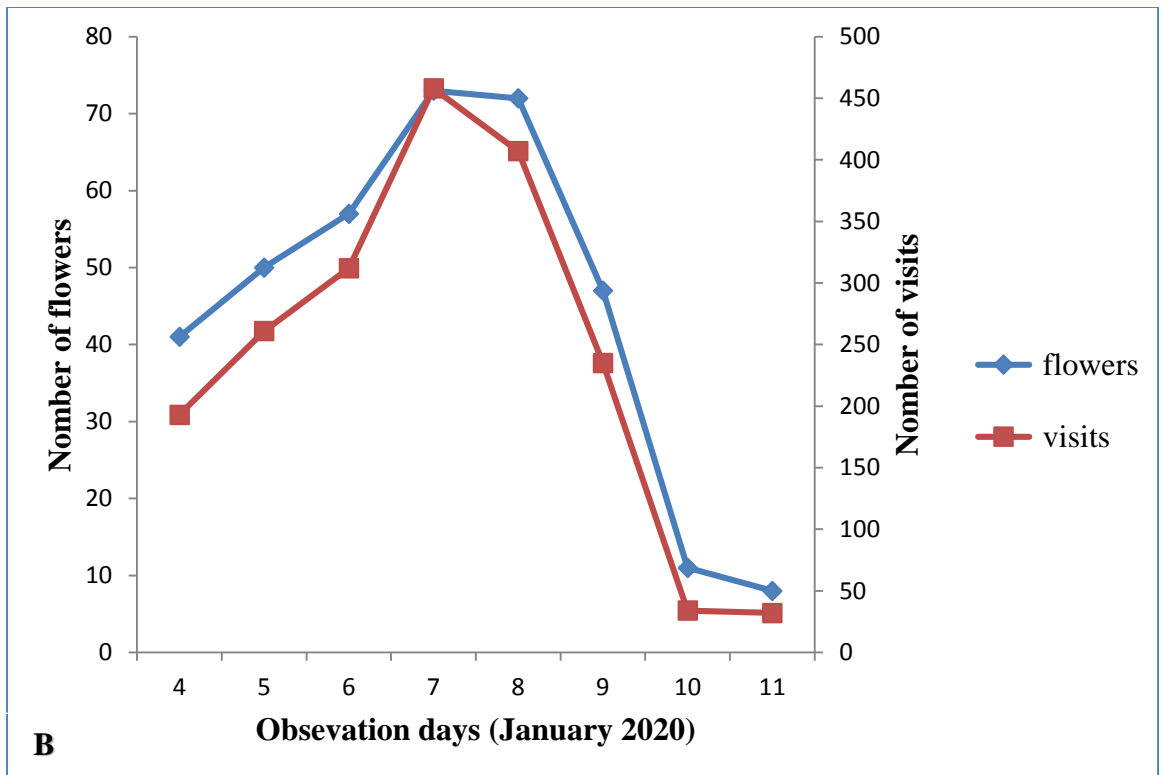
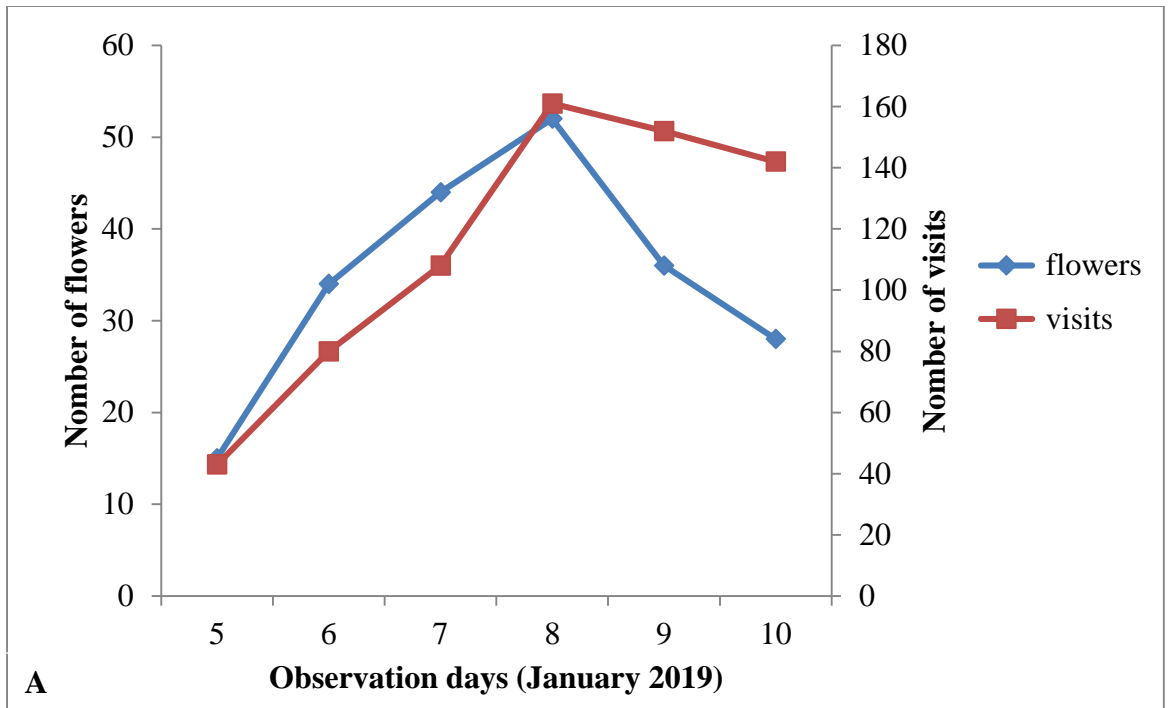


**Figure 1:** *Apis mellifera* worker collecting pollen from a *Solanum nigrum* flower at Meskine in 2019.

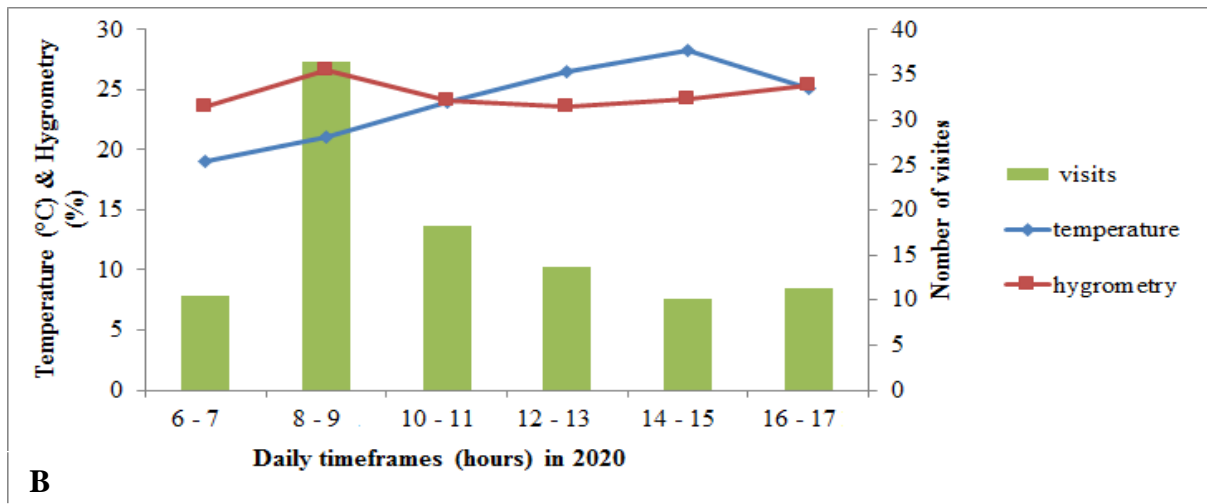
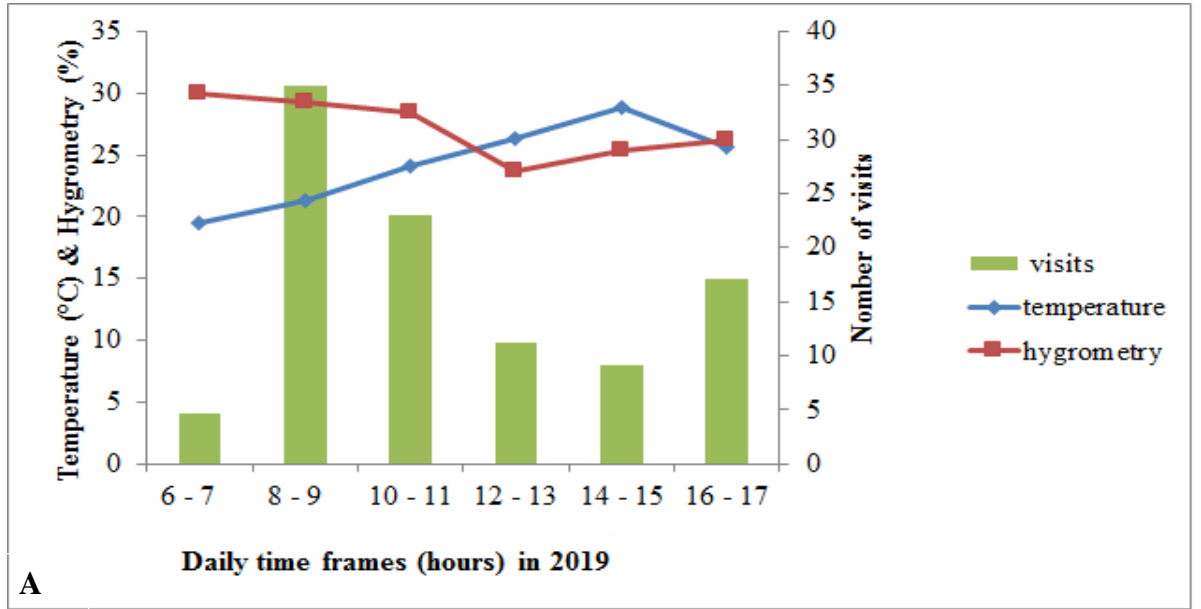
**Table 2:** Foraging speed of *Apis mellifera* on *Solanum nigrum* flowers in 2019 and 2020 at Meskine.

Years	Number of flowers visited per minute					Comparison of means
	<i>n</i>	<i>m</i>	<i>sd</i>	<i>mini</i>	<i>maxi</i>	
<b>2019</b>	85	9.25	3.61	4	25	$t = (21.45; df = 143; P < 0.001)$ VHS
<b>2020</b>	60	11.54	3.89	5	25	
<b>Total</b>	145	10.39	3.75	4	25	

**n**: number of visits studied; **m**: mean; **sd**: standard deviation; **mini**: minimum; **maxi**: maximum; **df**: degree of freedom; **VHS**: very highly significant difference.



**Figure 2:** Seasonal variations of the number of *Solanum nigrum* opened flowers and the number of *Apis mellifera* visits on these organs in 2019 (A) and 2020 (B) at Meskine.



**Figure 3:** Variations of the temperature, the humidity and the number of *Apis mellifera* visits on *Solanum nigrum* flowers according to the daily time frames in 2019 (A) and 2020 (B) at Meskine.

**Table 3:** Plant species surrounding *Solanum nigrum*, floral products and intensity of harvesting by *Apis mellifera* in 2019 and 2020 at Meskine.

Plant species	Family	Floral products	
		Nectar	Pollen
<i>Solanum lycopersicum</i>	Salanaceae	+	+++
<i>Mangifera indica</i>	Anacardiaceae	+++	+
<i>Cosmos sulphureus</i>	Asteraceae	+	+++
<i>Luffa cylindrica</i>	Curcubitaceae	+++	++
<i>Gossypium hirsutum</i>	Malvaceae	+++	+
<i>Hibiscus sabdariffa</i>	Malvaceae	+	+++

+ = low collection; ++ = high collection; +++ = very high collection.

**Table 4:** Fruiting rate, mean number of seeds per fruit and percentage of normal seeds in the different treatments of *Solanum nigrum* in 2018 and 2019 at Mesquine.

Years	Treatments	NFS	NFF	FR (%)	Seeds/fruit			TNS	NNS	%NS
					<i>m</i>	<i>sd</i>	<i>n</i>			
2019	1 (Uf)	120	110	91.67	79.14	4.97	110	8705	8508	97.74
	2 (Pf)	120	97	80.83	73.40	6.76	97	7120	6751	94.82
	3 (Fpva)	105	93	83.55	80.59	4.21	93	7495	7245	91.84
	4 (Fpwv)	148	104	68.24	66.99	8.12	104	6967	6619	86.64
2020	5 (Uf)	120	108	90	79.30	4.90	108	8564	8372	97.76
	6 (Pf)	120	96	80	73.48	6.79	96	7054	6689	94.83
	7 (Fpva)	110	93	83.55	80.59	4.23	93	7495	7242	96.62
	8 (Fpwv)	152	102	68.24	67.09	7.89	102	6844	6488	94.79

Uf: unprotected flowers; Pf: protected flowers; Fpva: flowers protected then uncovered, visited once by *Apis mellifera* and reprotected; Fpwv: flowers bagged then uncovered and rebagged without visit by insect or any other organism; NFS: number of flowers studies; NFF: number of fruits formed; FR: Fruiting rate; TNS: total number of seeds; NNS: number of normal seeds; %NS: percentage of normal seeds.

## DISCUSSION

### Place of *Apis mellifera* in the *Solanum nigrum* floral entomofauna

Results obtained from the experiments indicated that among insect species recorded on *S. nigrum* flowers, *A. mellifera* was the most represented insect with 288 visits (41.44%) in 2019 and 618 visits (31.01%) in 2020. Bees and flies in Britain (Edmonds and Chweya, 1997) and *Magachile latimanus* in Nigeria (Oyelana and Ogunwenmo, 2012) have respectively been described as the main pollinators of *Solanum* crop species. *Apis mellifera* was shown to be the most floral visitor of other plant species including *Helianthus annuus* (Egono et al., 2018), *Vitellaria paradoxa* (Basga et al., 2018), *Luffa cylindrica* (Farda and Tchuenguem, 2018), *Sesamum indicum* (Mahfouz et al., 2012; Pando et al., 2013) and *Physalis minima* (Basualdo et al., 2000). The significant difference between the frequencies of *A. mellifera* visits and those of other insects can be explained by the strategies adopted by this bee that consist of recruiting a great number of workers for the exploitation of an interesting nutritional source (von Frisch, 1969;

Louveaux, 1984; Kajobe, 2006). Consequently, there may be a limitation of the number of visits of other insect species due to the occupation of the majority of open flowers by *A. mellifera* workers.

### Activity of *Apis mellifera* on *Solanum nigrum* flowers

The fact that nectar collection occurred early in the morning whereas pollen collection occurred throughout the day is in agreement with the observations of Schippers (1998) who mentioned that anthers of *Solanum* flowers may still contain a large amount of pollen on the second day of anthesis. The activity of the honeybee was high in the morning, with a peak situated between 8 and 9 a.m., which is the daily period of highest availability of nectar and pollen in the flower of *S. nigrum*.

The positive and significant correlation between the number of opened flowers and the number of visits in 2019, as well as in 2020 indicates the good attractiveness of *S. nigrum* nectar and / or pollen with respect to *A. mellifera*. This result corroborates those of Faegri and Pijl (1979) which indicate that the number of opened flowers is an essential factor

which plays an important role in guiding insects towards the flowers.

The high density of workers per 1000 flowers recorded in this study could be due to the natural faculty of honeybees to recruit a high number of workers to exploit an interesting food source (Louveaux, 1984). Honeybees can smell or detect pollen or nectar odors (Free, 1970) using sensory receptors located on the flagellum of their antennae (Free, 1970). In fact, honeybees dance inside the nest after a successful foraging trip in order to communicate to their nest mate's information concerning the food odor, the distance and the direction from the hive to the food source (Frisch, 1967).

The disruption of visits by other insects reduced the duration of certain *A. mellifera* visits. This obliged some workers to visit more flowers during a foraging trip to maximize their pollen or nectar loads. Similar observations have been made in *A. mellifera* workers foraging on the flowers of: *Physalis minima* (Djakbé et al., 2017); *Sesamum indicum* (Otiobo et al., 2016; Tchuenguem and Népidé, 2018) and *Solanum nigrum* (Basualdo et al., 2000).

The present study revealed that during one foraging trip, an individual bee foraging on a given plant species scarcely visited another plant species. This result indicates that *A. mellifera* showed flower constancy (Basualdo et al., 2000) on *S. nigrum*. The fidelity of *A. mellifera* has been demonstrated on flowers of several other plant species including *Physalis minima* (Djakbé et al., 2017), *Sesamum indicum* (Otiobo et al., 2016; Tchuenguem & Népidé, 2018), *Helianthus annuus* (Basualdo et al., 2000; Egono et al., 2018) and *Vigna unguiculata* (Djonwangwé et al., 2017)

#### **Impact of anthophilous insects including *Apis mellifera* on *Solanum nigrum* yields**

The flowers that were exposed to visits provided more fruits and more seeds than protected flowers, in agreement with the

previous results reported on *S. nigrum* in Nigeria (Oyelana and Ogunwenmo, 2012). The significant increase in fruit production in the presence of flowering insects including *A. mellifera* is the consequence of their foraging activity on the pollination of the visited flowers.

The significant contribution of *A. mellifera* and other insects in the fruit (12,42%) and seed (4,18%) yields of *S. nigrum* is in agreement with the findings in Ghana (Amoako and Yeboah-Gyam, 1991) and Nord West Region of Cameroon (Otiobo et al., 2015) where solanaceous crops produce less seeds per fruit in the absence of efficient pollinators.

#### **Pollination efficiency of *Apis mellifera* on *Solanum nigrum***

During the collection of nectar or pollen on each flower, *A. mellifera* workers regularly come into contact with the stigma and anthers. They could thus enhance self-pollination, which has been demonstrated in the past (Edmonds and Chweya, 1997), by applying pollen of one flower on its 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 flowers belonging to a different plant of the same species (geitonogamy) (Edmonds and Chweya, 1997). The positive and significant contribution of *A. mellifera* in the fruiting rate, the percentage of normal seeds of *S. nigrum* is justified by the action of this bee on the pollination of visited flowers.

#### **Conclusion**

At Mesquine (Maroua, Cameroon), *Solanum nigrum* is a plant that benefits from the pollination by insects among which *Apis mellifera* was the most important and harvested nectar and pollen. The comparison of fruit and seed sets of flowers protected then uncovered, visited once by *A. mellifera* and rebagged to those of flowers protected from insects then

uncovered and rebagged without the visit of insects or any other organism underscores the value of this bee in increasing the fruiting rate (16.29%), the percentage of the number of seeds per fruit (3.45%) and the percentage of normal seeds (15.57%). *Solanum nigrum* is a highly polliniferous and nectariferous bee plant that could be cultivated and protected to increase pollen yield as a hive product, improve honey production and strengthen *A. mellifera* colonies. The installation of *A. mellifera* colonies at the vicinity of *S. nigrum* fields is recommended to increase fruit quantity and seed production. Furthermore, insecticide and / or herbicide treatments should be avoided in the field of *S. nigrum* during its flowering period. If these treatments are necessary, the choice of the insecticides that are less toxic for bees or the integrated pest control should be recommended to protect pollinating insects such as *A. mellifera*.

#### COMPETING INTERESTS

The authors declared that they have no competing interests for this article.

#### AUTHORS' CONTRIBUTIONS

JM was the main investigator. He collected data in the field and contributed to bibliographic research, preliminary insect determination and data analysis. SFT designed the project and FNTF supervised it. Both contributed to bibliographic research, insect identification and data analysis. EB contributed to bibliographic research, insect identification and data analysis. Each of these authors participated in the drafting of the manuscript.

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