



Visit of *Apis mellifera* Linne (Hymenoptera: Apidae) on the flowers of *Justicia secunda* (Acanthaceae), *Persicaria maculosa* (Polygonaceae), *Salvia leucantha* (Lamiaceae) and *Tithonia diversifolia* (Asteraceae) in urban areas of Ngaoundere (Cameroon)

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1 ABSTRACT

At Ngaoundéré (Adamaoua, Cameroon), from March to February 2016 and 2017, the foraging activity of *Apis mellifera* (Hymenoptera : Apidae), was studied on the flowers of four plant species : *Justicia secunda* (Acanthaceae), *Persicaria maculosa* (Polygonaceae), *Salvia leucantha* (Lamiaceae) et *Tithonia diversifolia* (Asteraceae). The flowers of each plant species were observed two days per week (Thursday and Sunday), between 07 am and 18 pm, with three daily time brackets : 07 - 11 am, 11 - 15 pm and 15 - 18 pm, for recording the pollen and / or nectar foraging behaviour of the honeybees. The main objective of this research was to assess the apicultural value of each plants species, for its possible use in the development of beekeeping in the Adamaoua Region. Results showed that all this plants studied (*Justicia secunda*, *Salvia leucantha*, *Persicaria maculosa* and *Tithonia diversifolia*), were visited slightly for pollen and highly for nectar. The mean duration of a visit per flower for nectar collection varied from 1.14 sec for *P. maculosa* to 13.28 sec for *J. secunda* (2016/2017). The mean duration of a flower visit for harvesting the pollen varies from 1.17 sec for *P. maculosa* and *T. diversifolia* (2017/2018) to 4.29 sec for *S.leucantha* (2016/2017). The average duration of a visit per flower slightly varied from one year to another. The highest number of workers foraging simultaneously per flower was one for each plant species. the abundance by 1000 flowers were 80 to 610 for *J. secunda*, from 20 to 170 for *P. maculosa*, from 20 to 120 for *S. leucantha* and from 20 to 449 for *T. diversifolia*. Abundance per plant varied from 8 to 232 for *J. secunda*, from 2 to 35 for *P. maculosa*, from 2 to 52 for *S. leucantha* and 8 to 214 for *T. diversifolia*. The mean concentration in total sugar of *J. secunda* was 37.90 % in 2016/2017 and 30.90 % in 2017/2018. The mean concentration in total sugars of *P. maculosa* was 25.02 % in 2016/2017 and 25.04 % in 2017/2018. The mean concentration in total sugars of *T. diversifolia* was 40.82 % in 2016/2017 and 41.10 % in 2017/2018. All the plant species studied could be preserved for the nutrition and the maintenance of honeybee colonies. On each plant species, *A. mellifera* workers carried pollen frequently from flower to flower and can thus be considered as pollinators.



2 INTRODUCTION

Around the world, honey is one of the most popular natural products thanks to its medicinal and nutritional advantages (Bakenga & Mapatano, 2000). Melliferous bees, *Apis mellifera* visit the flowers of various plants species and harvest nectar, pollen or the two products (McGregor, 1976 ; Pesson & Louveaux, 1984). The nectar is transformed into honey ; pollen and nectar are stored in the hive for subsequent consumption (Crane, 1999). The production of domestic bees colonies is proportional to the abundance and attractiveness of nectariferous and polliniferous bee plants in the environment of the apiary (Villières, 1987 ; Sgeren *et al.*, 1996 ; Winfree *et al.*, 2007). Thus, the proper operation of beekeeping in a given geographical area requires in-depth knowledge of the apicultural value of the plant species there present (Bakenga *et al.*, 2000 ; Tchuenguem, 2005 ; Pohl, 2008). In Cameroon, beekeeping production is low, while the demand in honey and other hive products is constantly increasing (INADES, 2000). The largest amounts of honey and other products in the hive consumed or marketed come from the Adamaoua Region with a climate particularly favourable to the proliferation of bees

3 MATERIAL AND METHODS

3.1 Study site and biological material:

The present study was carried out from March 2016 to February 2018 in the urban area of Ngaoundéré, Adamawa Region of Cameroon. This Region is situated between the 6th and 8th degrees of North latitude and between the 11th and 15th degrees of East longitude; it belongs to the high-altitude Guinean Savannah agro-ecological zone (Djoufack *et al.*, 2012). The climate is tropical and characterized by two seasons: a rainy season (April to October) and a dry season (November to March) (Tchuenguem, 2005). The annual rainfall is about 1500 mm. The mean annual temperature is 22 °C, while the mean annual relative humidity is 70% (Amougou, *et al.*, 2015). Plants chosen for observations were located in an area of three

(INADES, 2000). Nevertheless, this region remains susceptible to challenges related to beekeeping (Kombo, 1989; INADES, 2000). In order to sustainably improve this apicultural production and participate in the sustainable development of beekeeping in the Adamawa Region in particular and in Cameroon in general, it would be wise to intensify research in beekeeping, then popularize the results of these works with producers. With this mind, the investigations were carried out in Ngaoundere, from March 2016 to February 2018, on the exploitation of the flowers of *Justicia sucunda* (Radié-divin), *Persicaria maculosa* (Renouée persicaire), *Salvia leucantha* (Sauve du mexique) and *Tithonia diversifolia* (Tournesol mexicain) by *Apis mellifera*. This work aims at mastering the relationships between *A. mellifera* and *J. sucunda*, *P. maculosa*, *S. leucantha* and *T. diversifolia* growing in urban areas in Ngaoundere, for their optimal management. For each of these plant species, the work consisted in studying the activity of *A. mellifera* on flowers, estimating its apicultural value and evaluating the possibilities of intervention of this bee in pollination.

kilometres in diameter, centred on the Ngaoundere Urban Community (latitude: 07°19.064' N, longitude: 13°34.622' E, altitude: 1133 m a.m.). Table 1 describes plant species studied. Table 2 gives the relative abundance of opened flowers per month for each of these plants during the two observation periods. Animal material included *A. mellifera* and other insect species naturally present in the environment. The number of honey bee colonies in the area varied from 18 in March 2016 to 25 in February 2017 and from 23 in March 2017 to 44 in February 2018. The vegetation was represented by crops, ornamental plants, hedge plants and native plants of savannah and gallery forests.



Table 1: Scientific name, botanic family, biotope, some characteristics and strength of different plants studied at Ngaoundere in 2016 and 2017.

| Scientifique name | Family | Biotope | FP | DCOF | Strength* | |
|---|--------------|----------------|---------------------------------------|--------|-----------|---------|
| | | | | | 2016 | 2017 |
| <i>Justicia secunda</i> Vahl (++ ; he) | Acanthaceae | Gallery forest | January - April; August - December | Red | 208000 | 232000 |
| <i>Persicaria maculosa</i> Gray (++) ; he) | Polygonaceae | Lawn | January - December | White | 2110000 | 2360000 |
| <i>Salvia leucantha</i> Cavanilles (+ ; he) | Lamiaceae | Caward garden | January - December | Purple | 58900 | 61600 |
| <i>Tithonia diversifolia</i> (Hamsley) Gray (++ ; he) | Asteraceae | Gallery forest | January - December | Yellow | 2760000 | 2915000 |

+: cultivated plant; ++: spontaneous plant; sh: shrub; he: herbaceous; FP: Flowering Period; DCOF: Dominant Colour of Open Flower; *: Number of blooming individuals

Table 2: Relative abundance of opened flowers on each plant species per month during the investigation periods.

| Plant species | Study periods (March to February) | Months | | | | | | | | | | | |
|------------------------------|--------------------------------------|--------|----|----|----|----|----|----|----|----|----|----|----|
| | | Ma | Ap | My | Jn | Jl | Au | Se | Oc | No | De | Ja | Fe |
| <i>Justicia secunda</i> | 2016 - 2017 | | | | 1 | 2 | 3 | 4 | 4 | 4 | 3 | 2 | 1 |
| | 2017 - 2018 | 1 | | | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 2 | 2 |
| <i>Persicaria maculosa</i> | 2016 - 2017 | | 2 | 3 | 4 | 4 | 2 | 1 | 1 | | | | |
| | 2017 - 2018 | | 2 | 3 | 4 | 4 | 4 | 4 | 4 | 2 | 1 | 1 | |
| <i>Salvia leucantha</i> | 2016 - 2017 | | | 2 | 3 | 4 | 4 | 4 | 3 | 2 | 1 | 1 | |
| | 2017 - 2018 | | 1 | 2 | 2 | 3 | 4 | 4 | 4 | 3 | 1 | | |
| <i>Tithonia diversifolia</i> | 2016 - 2017 | 1 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 4 | 2 | 1 | 1 |
| | 2017 - 2018 | 2 | 2 | 2 | 3 | 3 | 4 | 4 | 4 | 3 | 2 | 1 | 1 |

Ja: January; Fe: February; Ma: March; Ap: April; My: May; Jn: June; Jl: July; Au: August; Se: September; Oc: October; No: November; De: December; *: ≤ 100 flowers = rare; **: > 100 and ≤ 500 flowers = little abundant; ***: > 500 and ≤ 1000 flowers = abundant; ****: > 1000 flowers = very abundant (Tchuenguem *et al.*, 2007).

3.2 Recording of the foraging activity of *Apis mellifera* on flowers : From March 2016 to February 2017 and from March 2017 to February 2018, the foraging behaviour of *A. mellifera* workers was recorded on flowers of different plant species. Data were taken during two days per week, between 7 am and 6 pm, according to three time slots: 07 am-11am, 11 am-3 pm and 3 pm-6 pm (Tchuenguem *et al.*, 2007). Table 3 provides information on the

number of observation days for each plant species. For a given plant species visited by a honey bee and for each investigation date, the following parameters were registered for each daily time slot and this, whenever possible: floral products (nectar or pollen) harvested, abundance of foragers (highest number of individual bees foraging simultaneously on a flower and on 1000 flowers) (Tchuenguem, 2005), duration of individual flower visit,



influence of the fauna (disruptions of foragers by competitors and/or predators) and impact of the surrounding flowers (attractiveness of other plant species to *A. mellifera* workers) (Tchuenguem *et al.*, 2010). The influence of the competitive flowers was determined by two methods: (a) direct observation of the nectar and/or pollen collection behaviour of workers on a given plant and other flowering plant species under observation and (b) analysis of the pollen loads carried by honey bee workers captured on flowers (Tchuenguem *et al.*, 2010). During each of the three days of full flowering, two pollen foragers were caught on the flowers of each plant species; pollen loads of each worker was then removed from pollen baskets and submitted to the microscopic analysis for the evaluation of the pollen profile (Tchuenguem *et al.*, 2010).

3.3 Evaluation of the concentration in sugar content of the nectar of different plant species: The concentration in total sugars of the nectar is an important parameter for the attractiveness of the honey bee to many flowers (Philippe, 1991). This parameter was evaluated from March 2016 to February 2017 and from March 2017 to February 2018, with a handheld refractometer (0-90% Brix) and a thermometer that gives the ambient temperature. *Apis mellifera* workers in full activity of nectar harvest were captured on flowers and anesthetized by their introduction into a small bottle containing cotton moistened with chloroform (Djonwangwé *et al.*, 2011). Nectar was thereafter removed from honey bee crop by exerting pressure on the abdomen placed between the thumb and the forefinger of the experimenter; the nectar in the mouth was then expelled and its concentration in total sugars measured (in g/100 dry matter) (Nye and Pedersen, 1962). The registered values obtained were corrected

according to the ambient temperature, using a table provided by the device leaflet.

3.4 Evaluation of the apicultural value of different plant species : The apicultural value of each plant species was assessed using data on the flowering intensity, the degree of attractiveness of *A. mellifera* workers for nectar and/or pollen (Villières, 1987; Thuenguem *et al.*, 2007; Thuenguem *et al.*, 2008a, 2008b; Djonwangwé *et al.*, 2011).

3.5 Evaluation of the impact of *Apis mellifera* on pollination : This parameter was recorded while recording the duration of visits. For each studied plant species, we were noting, during the nectar or pollen collection, the number of times where the body of the bee got into contact with the stigma of the visited flower (Jacob-Remacle, 1989). This approach makes it possible to highlight the possibilities of intervention of this bee in self-pollination and cross-pollination (Zumba *et al.*, 2013).

3.7 Evaluation of the influence of *Apis mellifera* on pollination : To measure the ability of *A. mellifera* to act as a pollinator of each plant species, during nectar or pollen harvest, the number of times a forager comes into contact with the stigma of the visited flower was noted (Jacob-Remacle, 1989). This approach allows highlighting the involvement of *A. mellifera* in self-pollination and cross-pollination (Zumba *et al.*, 2013; Potts *et al.*, 2015).

3.8 Data analysis: Data were subjected to descriptive statistics, ANOVA (*F*) for the comparison of means of more than two samples, student's *t*-test for the comparison of the mean of two samples, the Pearson correlation coefficient (*r*) for the study of the association between two variables, chi-square (χ^2) for the comparison of percentages, using Microsoft Excel 2010 software and R commander, version R.13.2.0.

4 RESULTS

4.1 *Apis mellifera* foraging activity on flowers

4.1.1 Floral products harvested, intensity and frequency of collection of different products:

The identity of collected foodstuff by *A. mellifera* workers on the flowers of each of the plant species under investigation as well as the intensity and the frequency of harvest of the different foodstuffs are summarised in tables 3 and 4. Here are the main results:

- i) *A. mellifera* foragers harvested nectar and pollen on each plant species (Fig. 1);
- ii) Foragers were intensely and regularly harvesting the nectar of each of these plant species
- iii) *Persicaria maculosa* was intensely visited for pollen;
- iv) *Justicia secunda* and *Salvia leucantha* were slightly visited for pollen;
- v) *Tithonia diversifolia* was very slightly visited for pollen;

vi) overall, the intensity (very low, low, high and very high) of nectar or pollen collection varied with plant species and time;

vii) on plant species where honey bees harvested nectar, the related frequency (percentage of the number of days where collection of nectar was observed, compared with the number of investigation days) was 100% for each plant species;

viii) on plant species where honey bee harvested pollen, the related frequency varied from 20.31 % for *T. diversifolia* to 100 % for *P. maculosa* (Tableau 3).

The type of product harvested from flowers (nectar or pollen) by *A. mellifera* on a given plant species varied with time slots. This bee harvested nectar or pollen in the morning and midday (*S. leucantha*) or throughout the day (*J. secunda*, *P. maculosa* et *Ti. diversifolia*).

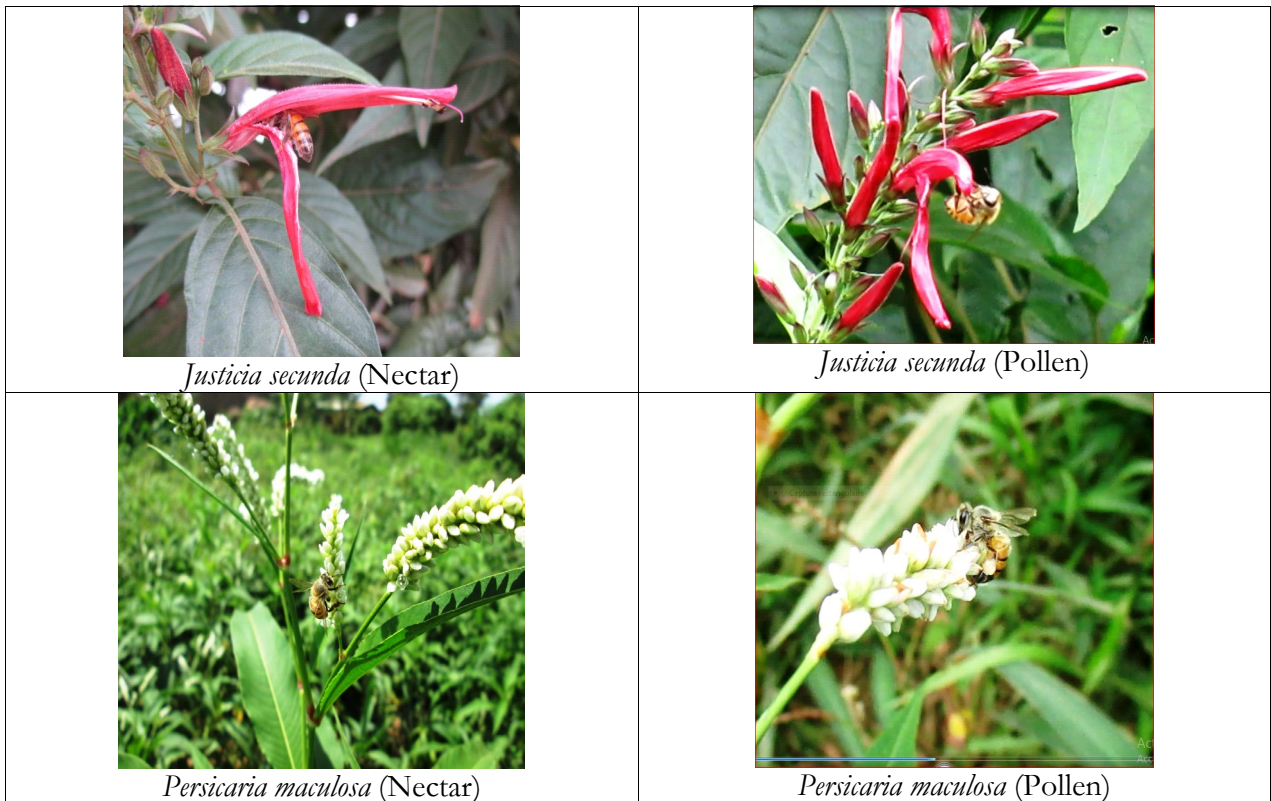




Figure 1: *Apis mellifera* collecting nectar or pollen on a studied plant species flower.



Table 3: Floral products harvested by *Apis mellifera* from the flowers of four plant species according to time, harvesting intensity and collection frequency of each food.

| Plant species | Study periods (March to February) | Months | | | | | | | | | | | | TD | nDNe | pDNe | nDPo | pDPo |
|------------------------------|-----------------------------------|----------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|----------------|-----|------|-------|------|-------|
| | | Ma | Ap | My | Jn | Jl | Au | Se | Oc | No | De | Ja | Fe | | | | | |
| <i>Justicia secunda</i> | 2016 - 2017 | | | | N ² | N ² | N ³ P ¹ | N ⁴ P ¹ | N ⁴ P ² | N ⁴ P ² | N ⁴ P ² | N ² P ¹ | N ² | 126 | 126 | 100,0 | 58 | 46,03 |
| | 2017 - 2018 | N ¹ | | | N ³ P ¹ | N ⁴ P ² | N ⁴ P ² | N ⁴ P ² | N ⁴ P ¹ | N ⁴ P ² | N ³ P ¹ | N ³ P ¹ | N ² | | | | | |
| <i>Persicaria maculosa</i> | 2016 - 2017 | | N ² P ² | N ³ P ² | N ⁴ P ³ | N ⁴ P ³ | N ⁴ P ³ | N ³ P ¹ | N ² P ¹ | | | | | 134 | 134 | 100,0 | 134 | 100,0 |
| | 2017 - 2018 | | N ² P ¹ | N ³ P ³ | N ⁴ P ² | N ⁴ P ⁴ | N ⁴ P ⁴ | N ⁴ P ⁴ | N ⁴ P ³ | N ³ P ² | N ² P ¹ | N ¹ P ¹ | | | | | | |
| <i>Salvia leucantha</i> | 2016 - 2017 | | | N ² P ¹ | N ³ P ² | N ⁴ P ⁴ | N ⁴ P ⁴ | N ⁴ P ⁴ | N ³ P ³ | N ² P ² | N ² P ¹ | N ² P ² | | 108 | 108 | 100,0 | 99 | 91,67 |
| | 2017 - 2018 | | N ² P ² | N ³ P ² | N ³ P ² | N ⁴ P ⁴ | N ⁴ P ⁴ | N ⁴ P ³ | N ⁴ P ⁴ | N ³ P ³ | N ² P ¹ | | | | | | | |
| <i>Titbonia diversifolia</i> | 2016 - 2017 | N ² | N ³ | N ³ | N ⁴ P ¹ | N ⁴ P ¹ | N ⁴ | N ⁴ | N ⁴ P ¹ | N ⁴ | N ³ | N ² | N ² | 192 | 192 | 100,0 | 39 | 20,31 |
| | 2017 - 2018 | N ³ | N ³ | N ³ | N ⁴ P ¹ | N ⁴ | N ⁴ P ¹ | N ³ P ¹ | N ⁴ | N ⁴ P ¹ | N ⁴ | N ² | N ² | | | | | |

Ja: January; Fe: February; Ma: March; Ap: April; My: May; Jn: June; Jl: July; Au: August; Se: September; Oc: October; No: November; De: December; TD: Total number of observation days; nDNe: number of days where collection of nectar was observed; pDNe: percentage of days where collection of nectar was observed; nDPo: number of days where collection of pollen was observed; pDP: percentage of days where collection of pollen was observed; N: Nectar; P: Pollen; 1, 2, 3 and 4 in superscripts indicate very low, low, high and very high collections respectively (Tchuenguem *et al.*, 2008b)

Table 4: Floral products harvested by *Apis mellifera* from the flowers of four plant species according to daily time slots.

| Plant species | Daily time slots | | |
|------------------------------|-------------------|-------------------|-------------------|
| | 07.00 - 11.00 | 11.00 - 3.00 | 3.00 - 6.00 |
| <i>Justicia secunda</i> | Nectar and pollen | Nectar | Nectar |
| <i>Persicaria maculosa</i> | Nectar and pollen | Nectar and pollen | Nectar and pollen |
| <i>Salvia leucantha</i> | Nectar and pollen | Nectar and pollen | - |
| <i>Tithonia diversifolia</i> | Nectar and pollen | Nectar | Nectar |

4.2 Density of foragers: The highest number of workers foraging simultaneously per flower was one for each plant species. Table 5 indicates that the abundance by 1000 flowers were 80 to 610 for *J. secunda*, from 20 to 170 for *P. maculosa*, from 20 to 120 for *S. leucantha* and from 20 to 449 for *T. diversifolia*. Abundance per plant varied from 8 to 232 for *J. secunda*, from 2 to 35 for *P. maculosa*, from 2 to 52 for *S. leucantha* and 8 to 214 for *T. diversifolia* (Table 6). The abundance per plant is globally proportional to the number of blossoming flowers and more precisely to the offer of nectar and/or pollen.

4.3 Duration of visits per flower: The analysis of Table 7 suggests that overall, the average duration of a flower visit varied with plant species and for a given plant species, with

the type of floral product taken. For example, the mean duration of a flower visit for harvesting nectar varies from 1.14 sec for *P. maculosa* to 13.28 sec for *J. secunda* (2016/2017). The mean duration of a flower visit for harvesting the pollen varies from 1.17 sec for *P. maculosa* and *T. diversifolia* (2017/2018) to 4.29 sec for *S. leucantha* (2016/2017). The average duration of a visit per flower slightly varied from one year to another. The difference between the average duration of nectar and that of pollen collection is very highly significant for *J. secunda* (2016/2017 : $t = 22.95$, $df = 411$, $P < 0.001$; 2017/2018 : $t = 21.65$, $df = 428$, $P < 0.001$), *S. leucantha* (2016/2017 : $t = 3.72$, $df = 229$, $P < 0.001$) and *T. diversifolia* (2016/2017 : $t = 12.96$, $df = 343$, $P < 0.001$; 2017/2018 : $t = 14.75$, $df = 324$, $P < 0.001$).

Table 5 : Abundance of *Apis mellifera* foragers per 1000 flowers depending on the plant species and the months.

| Plant species | Study periods (March to February) | Months | | | | | | | | | | | |
|------------------------------|-----------------------------------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| | | Ma | Ap | My | Jn | Jl | Au | Se | Oc | No | De | Ja | Fe |
| <i>Justicia secunda</i> | 2016 - 2017 | | | | 90 | 310 | 490 | 580 | 600 | 568 | 485 | 328 | 94 |
| | 2017 - 2018 | | | | 593 | 610 | 602 | 600 | 584 | 500 | 549 | 96 | 80 |
| <i>Persicaria maculosa</i> | 2016 - 2017 | | 20 | 162 | 170 | 170 | 166 | 35 | 20 | | | | |
| | 2017 - 2018 | | 95 | 118 | 154 | 160 | 157 | 160 | 146 | 76 | 28 | 20 | |
| <i>Salvia leucantha</i> | 2016 - 2017 | | | 66 | 107 | 119 | 120 | 120 | 110 | 88 | 36 | 20 | |
| | 2017 - 2018 | | 20 | 47 | 82 | 95 | 100 | 99 | 98 | 100 | 20 | | |
| <i>Tithonia diversifolia</i> | 2016 - 2017 | | 20 | 168 | 195 | 409 | 402 | 400 | 410 | 380 | 263 | 97 | 84 |
| | 2017 - 2018 | 232 | 245 | 219 | 449 | 298 | 448 | 449 | 428 | 396 | 276 | 20 | 36 |

Ja: January; Fe: February; Ma: March; Ap: April; My: May; Jn: June; Jl: July; Au: August; Se: September; Oc: October; No: November; De: December **Comparison of the mean abundances per 1000 flowers:** A1000_{2016/2017} : $F = 350.71$ ($df1 = 3$; $df2 = 830$; $P < 0.001$; VHS); A1000_{2017/2018} : $F = 292.33$ ($df1 = 3$; $df2 = 1027$; $P < 0.001$; VHS)

Table 6: Abundance of *Apis mellifera* workers per plant (maximum individuals simultaneously active on open flowers for two observation periods depending) on the plant species and the months.

| Plant species | Study periods (March to February) | Months | | | | | | | | | | | |
|------------------------------|-----------------------------------|--------|----|----|-----|-----|-----|-----|-----|-----|-----|----|----|
| | | Ma | Ap | My | Jn | Jl | Au | Se | Oc | No | De | Ja | Fe |
| <i>Justicia secunda</i> | 2016 - 2017 | | | | 8 | 25 | 88 | 184 | 218 | 200 | 194 | 39 | 12 |
| | 2017 - 2018 | | | | 223 | 198 | 204 | 215 | 232 | 186 | 159 | 88 | 94 |
| <i>Persicaria maculosa</i> | 2016 - 2017 | | 8 | 13 | 35 | 32 | 19 | 6 | 2 | | | | |
| | 2017 - 2018 | | 12 | 18 | 21 | 25 | 27 | 27 | 20 | 25 | 4 | 2 | |
| <i>Salvia leucantha</i> | 2016 - 2017 | | | 3 | 6 | 18 | 15 | 16 | 7 | 4 | 3 | 2 | 52 |
| | 2017 - 2018 | | 2 | 5 | 5 | 8 | 20 | 18 | 18 | 14 | 2 | | |
| <i>Titbonia diversifolia</i> | 2016 - 2017 | 8 | 22 | 19 | 34 | 31 | 115 | 196 | 188 | 190 | 46 | 18 | 20 |
| | 2017 - 2018 | 40 | 38 | 40 | 88 | 100 | 149 | 214 | 195 | 135 | 21 | 13 | 10 |

Ja: January; Fe: February; Ma: March; Ap: April; My: May; Jn: June; Jl: July; Au: August; Se: September; Oc: October; No: November; De: December

Table 7 : Duration of *Apis mellifera* visits on flowers of four plant species according to the study periods and harvested products.

| Plant species | Visiting time per flower (sec) | | | | | | | | Comparison of means of the two study periods | | |
|----------------------------------|--------------------------------|----------------------|-------------|-------------|-----------------------------|----------------------|-------------|-------------|--|-----------|------------------------|
| | March 2016 to February 2018 | | | | March 2016 to February 2018 | | | | | | |
| | <i>n</i> | <i>m</i> ± <i>sd</i> | <i>mini</i> | <i>maxi</i> | <i>n</i> | <i>m</i> ± <i>sd</i> | <i>mini</i> | <i>maxi</i> | <i>t</i> | <i>df</i> | <i>p-value</i> |
| <i>Justicia secunda</i> (N) | 298 | 13.28 ± 8.98 | 2 | 37 | 314 | 12.52 ± 9.10 | 1 | 56 | 1,04 | 610 | > 0.05 ^{NS} |
| <i>Justicia secunda</i> (P) | 115 | 1.28 ± 0,40 | 1 | 3 | 116 | 1.33 ± 0.54 | 1 | 3 | 0,75 | 229 | > 0.05 ^{NS} |
| <i>Persicaria maculosa</i> (N) | 205 | 1.14 ± 0.35 | 1 | 2 | 277 | 1.22 ± 0.43 | 1 | 3 | 2,24 | 480 | < 0.05 ^S |
| <i>Persicaria maculosa</i> (P) | 145 | 1.24 ± 0.45 | 1 | 3 | 166 | 1.17 ± 0.40 | 1 | 3 | 1,44 | 309 | > 0.05 ^{NS} |
| <i>Salvia leucantha</i> (N) | 119 | 3.40 ± 1.11 | 2 | 8 | 101 | 3.03 ± 1.14 | 1 | 7 | 2,42 | 218 | < 0.05 ^S |
| <i>Salvia leucantha</i> (P) | 112 | 4.29 ± 2.28 | 2 | 19 | 96 | 2.73 ± 1.22 | 1 | 8 | 6,24 | 206 | < 0.001 ^{THS} |
| <i>Titbonia diversifolia</i> (N) | 285 | 2.89 ± 1.39 | 1 | 8 | 274 | 2.52 ± 1.23 | 1 | 7 | 3,33 | 557 | < 0.001 ^{THS} |
| <i>Titbonia diversifolia</i> (P) | 60 | 1.37 ± 0.64 | 1 | 4 | 52 | 1.17 ± 0.38 | 1 | 2 | 2,02 | 110 | < 0.05 ^S |

n: number of visits studied; *m*: mean; *sd*: standard deviation; *mini*: minimum; *maxi*: maximum; *df*: degree of freedom; *ne*: nectar collection visits; *po*: pollen collection visits; NS: non-significant difference

Comparison of means duration of visits: Nectar_{2016/2017} : $F = 295.52$ ($df1 = 3$; $df2 = 903$; $P < 0.001$; VHS); Nectar_{2017/2018} : $F = 285.77$ ($df1 = 3$; $df2 = 962$; $P < 0.001$; VHS); Pollen_{2016/2017} : $F = 88.93$ ($df1 = 3$; $df2 = 428$; $P < 0.001$; VHS); Pollen_{2016/2017} : $F = 116.07$ ($df1 = 3$; $df2 = 426$; $P < 0.001$; VHS)



This difference is significant for *P. maculosa* in 2016/2017 ($t = 2.23$, $df = 348$, $P < 0.05$), but not significant for *S. leucantha* in 2017/2018 ($t = 1.77$, $df = 195$, $P > 0.05$) and *P. maculosa* in 2017/2018 ($t = 1.23$, $df = 441$, $P > 0.05$).

4.4 Influence of other flowering insects:

Apis mellifera foragers were disturbed during their foraging activity on each studied plant species by other individuals from the same species or from other insect species that were competing for nectar or pollen, or predators. Thus, for 843 timed visits on the flowers of *J. secunda* in 2016/2017 and 2017/2018, 115 were interrupted by *A. mellifera* workers (38 visits), *Belonogaster juncea* (34 visits) and *Ceratina* sp. (43 visits). For the 793 timed visits on *P. maculosa* flowers in 2016/2017 and 2017/2018, 67 were interrupted by *A. mellifera* workers (22 visits), *M. ferruginea* (19 visits) and *B. juncea* Fabricius (26 visits). For the 428 timed visits on *S. leucantha* flowers in 2016/2017 and 2017/2018, 49 67 were interrupted by *A. mellifera* workers (24 visits), *B. juncea* Fabricius (19 visits) and

Polyrachis sp. (6 visits). For the 671 timed visits *T. diversifolia* flowers in 2016/2017 and 2017/2018, 98 were interrupted by *A. mellifera* workers (27 visits), *X. olivacea* (19 visits), *X. inconstans* (38 visits) et *S. jucunda* (14 visits).

4.5 Influence of neighbouring flora:

Throughout the observation period on each plant species, *A. mellifera* workers visited flowers of many other plant species growing in the study area, for nectar (ne) and/or pollen (po). During the observation period, the passages of foragers from one plant species to another neighbouring were noted (8, 4, 6 and 11 times, for the study period of *J. secunda*, *P. maculosa*, *S. leucantha* and *T. diversifolia* respective Ent). In addition, it emerges from the analysis of pollen charges taken from the baskets of workers that the percentage of pollen grains belonging not only the plant species on which the bees were captured (foreign pollen) varied from 0.39 % (*P. maculosa*) in 2016/2017 to 1.30 % (*T. diversifolia*) in 2017/2018 (Table 8).

Table 8 : Pollen profile of pollen balls harvested in the baskets of a few workers from *Apis mellifera* spinning on the flowers of the four plant species studied according to the period of investigation

| Plant species | Pollen profile of pollen balls | | | | | | | | | |
|------------------------------|--------------------------------|------------|--------------|------------------|--------------------------|----------------------------|------------|--------------|------------------|--------------------------|
| | Mach 2016 to February 2017 | | | | | Mach 2017 to February 2018 | | | | |
| | Number of grains | | | % foreign pollen | Identity of other plants | Number of grains | | | % foreign pollen | Identity of other plants |
| | Total | Host plant | Other plants | | | Total | Host plant | Other plants | | |
| <i>Justicia secunda</i> | 1031 | 1020 | 11 | 1,07 | Cp, Hr | 981 | 972 | 09 | 0,92 | Hr, Va |
| <i>Persicaria maculosa</i> | 7524 | 7495 | 29 | 0.39 | Bu | 8214 | 8176 | 38 | 0,46 | Mp, Pg |
| <i>Salvia leucantha</i> | 6358 | 6315 | 43 | 0.68 | Mp, Ct | 5972 | 5938 | 34 | 0,57 | Dr, Te |
| <i>Tithonia diversifolia</i> | 966 | 956 | 10 | 1.04 | Va | 846 | 835 | 11 | 1,30 | Bp |

Bp: *Bidens pilosa* Linnaeus (Asteraceae); Pg: *Psidium guajava* Linnaeus (Myrtaceae); Mp: *Mimosa pudica* Linnaeus (Fabaceae); Hr: *Hibiscus rosa-sinensis* Linnaeus (Malvaceae); Cp: *Caesalpinia pulcherrima* Linnaeus (Caesalpinaceae); Bu: *Brillantaisia ulgurica* Lindau et Jahrbücher (Acanthaceae); Ct: *Calliandra tweedii* Bentham (Fabaceae); Te: *Tagetes erecta* Linnaeus (Asteraceae); Dr: *Delonix regia* (Bojer ex Hooker) Rafinesque (Fabaceae); Va: *Vernonia adoensis* Schreb. 1791 (Asteraceae)

4.6 Total sugars concentration of nectar for the various plants species:

The Table 9 presents the concentrations in total nectar sugars of studied plant species. The differences between the means concentration in total sugar

of the nectar for these plant species are globally significant ($F = 74.03$; $df1 = 3$; $df2 = 329$; $P < 0.001$) in 2016/2017 as in 2017/2018 ($F = 9.25$; $df1 = 3$; $df2 = 462$; $P < 0.001$). The mean concentration in total sugar of *J. secunda* was



37.90 % in 2016/2017 and 30.90 % in 2017/2018. The difference between these means is very highly significant ($t = 8.68$, $df = 247$, $P < 0.001$). The mean concentration in total sugars of *P. maculosa* was 25.02 % in 2016/2017 and 25.04 % in 2017/2018. The difference between these means is not significant ($t = 0.01$, $df = 124$, $P > 0.05$). The mean concentration in total sugars of *P. maculosa* was 35.72 %

in 2016/2017 and 33.09 % in 2017/2018. The difference between these means is highly significant ($t = 2.81$, $df = 216$, $P < 0.01$). The mean concentration in total sugars of *T. diversifolia* was 40.82 % in 2016/2017 and 41.10 % in 2017/2018. The difference between these means is not significant ($t = 0.35$, $df = 204$, $P > 0.05$).

Table 9: Concentration in total sugars of the nectar of studied plants species.

| Plant species | Concentration of total sugars in nectar | | | | | | | | Comparison of means of the two study periods (t -test) | | |
|------------------------------|---|------------------|--------|--------|----------------------------|------------------|--------|--------|---|-------|------------------------|
| | Mach 2016 to February 2017 | | | | Mach 2017 to February 2018 | | | | | | |
| | n | $m \pm s$ | $mini$ | $maxi$ | n | $m \pm s$ | $mini$ | $maxi$ | t | ddl | p |
| <i>Justicia secunda</i> | 101 | 37.90 \pm 6.94 | 26.24 | 50.64 | 148 | 30.90 \pm 4.98 | 4.79 | 44.79 | 8.68 | 247 | < 0.001 ^{VHS} |
| <i>Persicaria maculosa</i> | 65 | 25.02 \pm 8.09 | 12.58 | 36.72 | 61 | 25.04 \pm 8.88 | 12.58 | 44.72 | 0.01 | 124 | > 0.05 ^{NS} |
| <i>Salvia leucantha</i> | 63 | 35.72 \pm 6.81 | 24.89 | 47.31 | 155 | 33.09 \pm 4.47 | 23.14 | 50.29 | 2.81 | 216 | < 0.01 ^{HS} |
| <i>Titbonia diversifolia</i> | 104 | 40.82 \pm 6.11 | 16.14 | 48.64 | 102 | 41.10 \pm 5.24 | 23.62 | 48.64 | 0.35 | 204 | > 0.05 ^{NS} |

m : mean; sd : standard deviation; NS: non-significant difference; S: significant difference; VHS: very highly significant difference; n : strength; $mini$: minimum; $maxi$: maximum; df : degree of freedom

Comparison of the means concentration in total sugars of the nectar for different plant species: 2016/2017: $F = 74.03$ ($df1 = 3$; $df2 = 329$; $P < 0.001$; VHS); 2017/2018: $F = 9.25$ ($df1 = 3$; $df2 = 462$; $P < 0.001$; VHS)

4.7 Apicultural value of the various plant species: During the flowering period, we noted on each of the plants species, a very elaborate activity of *A. mellifera* workers on flower. Particularly, a very strong harvest of nectar on all plants, a very strong collection of pollen on *P. maculosa*, a strong harvest of pollen on *S. leucantha*, a weak collection of pollen on *J. secunda* and a very weak collection of pollen on *T. diversifolia*. In addition, the pollen analysis of honey samples harvested in the experimental environment indicates the presence of the pollen of the plant species studied. The set of data obtained allow to classify these plant species studied in five categories of bee plant : a) those very highly nectariferous (*J. secunda* et *Ti. Diversifolia*) ; b) those strongly nectariferous (*P. maculosa* et *S. leucantha*) ; c) those very highly polliniferous (*P. maculosa*) ; d) those slightly polliniferous (*J. secunda* et *S. leucantha*) and e)

those very slightly polliniferous (*T. Diversifolia*). Table 10 summarizes the most indicated period to harvest honey or pollen in the hives installed in an environment where flowering vegetation is characterized by the predominance of a large population of each of the plants studied with a high value, in the area. A radius of at least 1500 m. Thus, in the urban Ngaoundere, the honey can be harvest in November, December and January, if the host environment is dominated by a strong population of *P. maculosa*, *J. secunda* and/or *S. leucantha* and *T. diversifolia* respectively. In the same environment, pollen can be accessed in the hives during November and December, if the hive's environment is dominated by a strong population of *P. maculosa* and *S. leucantha* respectively.

4.8 Impact of the activity of *Apis mellifera* on pollination : During pollen and/or nectar collection on flowers, *A. mellifera*



foragers were regularly in contact with the anthers and stigma. They could therefore cause a self-pollination, applying the pollen of a flower on his own stigma. For both cumulative study seasons, the frequency of contacts between the bee and the stigma of the flower visited ranged from 56.78 % for *S. leucantha* to 100 % for *P.*

maculosa (Tableau 11). Thus, *A. mellifera* workers carried the pollen from each of the flowers of each plant species studied flower. Therefore, intervene on self-pollination or cross pollination. In short, the foragers of *A. mellifera* greatly increased the pollination possibilities of *J. secunda*, *P. maculosa*, *S. leucantha* and *T. diversifolia*.

Table 10: Apicultural value of various plant species and most favourable period to harvest honey and/or pollen from *Apis mellifera* hives in Ngaoundere.

| Plant species | Apicultural value | | Period of honey and/or pollen collection | |
|------------------------------|-------------------|--------|--|----------|
| | Nectar | Pollen | Honey | Pollen |
| <i>Justicia secunda</i> | 4 | 2 | December | - |
| <i>Persicaria maculosa</i> | 3 | 4 | November | November |
| <i>Salvia leucantha</i> | 3 | 2 | December | - |
| <i>Tithonia diversifolia</i> | 4 | 1 | January | - |

2nd column: 3 = high nectariferous value; 4 = very high nectariferous value; 3rd column: 1 = very low polliniferous value; 2 = low polliniferous value; 4 = very high polliniferous value

Table 11: Number and frequency of contacts between *Apis mellifera* and the stigma during floral visits of four plant species.

| Plant species | Mach 2016 to February 2017 | | | Mach 2017 to February 2018 | | | Total | | |
|------------------------------|----------------------------|----------------------------|--------|----------------------------|----------------------------|--------|--------------------------|----------------------------|--------|
| | Number of studied visits | Visits with stigma contact | | Number of studied visits | Visits with stigma contact | | Number of studied visits | Visits with stigma contact | |
| | | Number | % | | Number | % | | Number | % |
| <i>Justicia secunda</i> | 413 | 259 | 62.71 | 430 | 246 | 57.21 | 843 | 505 | 59.91 |
| <i>Persicaria maculosa</i> | 350 | 350 | 100.00 | 443 | 443 | 100.00 | 793 | 793 | 100.00 |
| <i>Salvia leucantha</i> | 231 | 124 | 53.68 | 197 | 119 | 60.41 | 428 | 243 | 56.78 |
| <i>Tithonia diversifolia</i> | 345 | 312 | 90.43 | 326 | 299 | 91.72 | 671 | 611 | 91.06 |

Global comparison of frequency of visits with stigma contacts of each study periods: a) Mach 2016 to February 2017: $\chi^2_{\text{global}} = 266.05$ ($df = 3$; $P < 0.001^{\text{VHS}}$); b) Mach 2017 to February 2018: $\chi^2_{\text{global}} = 316.91$ ($df = 3$; $P < 0.001^{\text{VHS}}$)

Comparison of frequency of visits with stigmatic contacts of the two study periods: *Justicia secunda*: $\chi^2 = 2.66$ ($df = 1$; $P > 0.05^{\text{NS}}$); *Salvia leucantha*: $\chi^2 = 1.96$ ($df = 1$; $P > 0.05^{\text{NS}}$); *Tithonia diversifolia*: $\chi^2 = 0.34$ ($df = 1$; $P > 0.05^{\text{NS}}$)

NS : non-significant difference; VHS : Very highly significant difference ; df : degree of freedom

5 DISCUSSION

The observed variations for the collection of floral products on the studied plants, would mainly be linked to the availability of nectar and/or pollen about the flowers. The works of Iritie et al. (2014) in Ivory Coast showed that the flowers of *J. secunda* are also visited by *A. mellifera* for pollen harvest. In Cameroon, Dongock et al. (2017a) found that this bee visits the flowers of

Ti. diversifolia for nectar and pollen collection in the Adamawa Region. In contrast, in the eastern area of the Democratic Republic of Congo (Bakenga et al., 2000) and in the western area of Cameroon (Dongock et al., 2004), it was found that *A. mellifera* only forages for pollen on the capitula of this plant. Still in the Democratic Republic of Congo, Bakenga et al. (2000) the



floral products collection by the honey bees on other plant species was also noted: *Brillantaisia (cicatricosa)*: nectar), *Justica (chimperiana)*: nectar and pollen) and *Caesalpinia (decapetala)*: nectar and pollen). In Cameroun, several plant species were identified as highly nectariferous bee plants: *Solanum nigrum* (Mamoudou et al., 2021); *Agave sisalana* (Tchuenguem et al., 2020); *Luffa cylindrica* (Farda & Tchuenguem, 2018); *Gossypium hirsutum* (Mazi et al., 2013). In the same country, other species were identified as highly polliniferous bee plants: *Agave sisalana* (Tchuenguem et al., 2020); *Mimosa pudica* (Dongock et al., 2017a); *Psidium guajava* (Tchuenguem et al., 2007); *Syzygium guineense* var. *macrocarpum* (Tchuenguem, 2005). The high attractivity of the nectar of each of these plants species towards *A. mellifera* workers could be linked to the high availability of food resources on and into the flowers, the quality of floral product and the concentration in total sugars of contained in the nectar. Actually, according to Abrol (2012), *A. mellifera* is constantly on the flowers when nectar's availability and concentration in total sugars are high. The honey bee is therefore a polylectic species which collects nectar and pollen on a large number of plant species belonging to several families, just as many authors like Jacob-Remacle (1990) had already mentioned. *A. mellifera* workers were faithful to the flowers of the studied plant species. This faithfulness called « flower constancy » during the foraging trips is well known with *A. mellifera* (Basualdo et al., 2000). It is explained by the fact that the forager is generally capable of memorizing and recognizing, the colour and the smell of the visited flowers during the previous foraging trips (Wright et al., 2002). Furthermore, the analysis of the pollen balls collected on the *A. mellifera* workers' baskets reveals that the percentage of pollen grains not belonging to the plant species on which the worker has been captured (foreign pollen) varies from 0.45 % to 1.84 % only. For each of the studied plant species, the collected floral product by the honey bees varies with the periods of the day, as indicated in table 4. In Cameroon, the faithfulness of *A. mellifera*

workers has also been mentioned on the flowers of several other plants species including *Agave sisalana* (Tchuenguem et al., 2020); *Allium cepa* (Tchindébé & Tchuenguem, 2014). During the day, the nature of floral substances harvested by honey bees on a plant species can vary with the time slot. The observed variations would mainly be linked to the availability of nectar and/pollen into and on the flowers. This result could be justified by the fact that the high air humidity in the morning facilitates the agglutination of the pollen grains of these plants; in the afternoons, their pollen grains are produced in a great quantity and are easily accessible for the bees. Thus, the pollen harvest in these conditions would require less energy expending (Phillipe, 1991). The high abundances per 1000 flowers and by per single plant highlights the satisfactory attractiveness of nectar and/or pollen of the flowers of the corresponding plants towards *A. mellifera* workers. Our results corroborate those of Faegri & Pijl (1979) who indicate that the number of blooming flowers is a key factor in the orientation of insects towards the flowers. The low abundance of insects per 1000 flowers and per plant highlights the attractiveness of nectar and/or pollen of the flowers of the corresponding plants towards *A. mellifera* foragers. On each of the studied plant species, the mean duration of visit for nectar and/ or pollen harvest by *A. mellifera* also varies with the availability of the floral products, as shown in table 7. Honey bees stay longer on flowers that are rich in nectar or in pollen, compared to those poor in these resources. The duration of visit for nectar in a flower with corolla tube filled with that substance is by far superior to that of nectar harvest in a flower which corolla tube is not filled with substance. This result could be explained by the principle of optimal foraging which characterizes the honey bee (Roubik, 1989). The difference between the mean durations of visits for the harvest of floral products seems to be linked also to their accessibility (Tchuenguem, 2005) : all the four plant species pollen under investigation, is produced by the anthers that are located above the filament of the stamen and are therefore



easily accessible to *A. mellifera* workers, while nectar is found in the corolla tube, between the base of the stylet and the stamens which is difficultly accessible in the studied plants species. In order to obtain their optimal nectar and/or pollen charge, the *A. mellifera* workers that faced disturbance were certainly obliged to visit a greater number of flowers or plants during the corresponding foraging trips. These various behaviours among individuals result in the reduction of the duration of visit of some visits per flower, the loss of an amount of pollen carried by *A. mellifera* workers and the increase of some foraging speeds. During a foraging trip, *A. mellifera* workers were overall faithful to the exploited plant species. As for the concentration in total sugars of the studied plants (table 9), it appears that the concentration in total nectar sugars varied with the daily time slots, for each of the two investigation years. Overall, the concentrations in total nectar sugars recorded between 11 am and 3 pm were generally superior to those recorded between 7 am and 11 am or between 3 pm and 6 pm. The set of data showing the presence of pollen grains in the honey suggest that *A. mellifera* workers use the nectar harvested in each of the flowers of these plant species to produce honey. All the studied plant species more or less contribute to the feeding and therefore to the maintenance of bee colonies

6 CONCLUSION

In urban areas in Ngaoundere, *Apis mellifera* workers intensely and regularly harvested nectar in the flowers of *Justicia secunda*, *Persicaria maculosa*, *Salvia leucantha* and *Tithonia diversifolia*. *Persicaria maculosa* flowers were very highly visited for pollen. Those of *Justicia secunda* and *Salvia leucantha* were slightly visited by *A. mellifera* for pollen collection. The Asteraceae, *Ti. diversifolia* was slightly visited for le pollen. The *A. mellifera* foragers were faithful to the flowers of each of the plant species during the foraging trips. Thus, *Justicia secunda* and *Tithonia diversifolia* are highly nectariferous bee plants; *Persicaria maculosa* and *Salvia leucantha*, are highly nectariferous bee plants that can be grown and protected to

during the year. It is therefore important to preserve and/or grow them in the beehive environment. For each of the two observation years, these bees could therefore provoke a self-pollination, dropping a flower pollen in its own stigma. During foraging trips, these bees could also cause cross-pollination, since the foragers regularly went from the flowers of a plant to those of another plant of the same species. Moreover, in Mayel-Ibbé, Dounia (2015) mentioned that the frequency of contacts between *A. mellifera* workers and the stigma were of 100 % and 93.78 % for *Glycine max* and *Gossypium hirsutum* respectively. In Dang, Mazi (2015) mentioned that the frequency of contacts between the honey bee and the stigma vary 77.08 % in 2010 to 69.20 % in 2011 with *Cajanus cajan* and of 76.40 % in 2010 to 72.33 % in 2011 with *Gossypium hirsutum*. In the same study site, as for this last author, Wékéré (2019) found that the frequency of contacts between *A. mellifera* and the stigma was of 100 % with *Acacia sieberiana*, *Ageratum conyzoides*, *Allophylus africanus*, *Senegalia polyacantha*, *Sida rhombifolia*, *Stylosanthes guianensis* and *Terminalia schimperiana*. These observations corroborate those of Philippe (1991); Roubik et al. (2005) and Abrol (2012) who point out that the effective pollinators of a plant species are recruited among the floral insects which are regularly in contact with the stigma and the anther of the visited flowers.

increase honey production. Moreover, *Persicaria maculosa* is a highly polliniferous bee plant that could be cultivated and protected to increase pollen production as a bee hive product in this environment. All the studied plant species more or less contribute to the feeding, and therefore to the maintenance of bee colonies throughout the year. It is therefore necessary to preserve and/or grow them in urban areas in Ngaoundere for the maintenance of the animal and plant biodiversity. *Apis mellifera* workers carry pollen from flower to flower on each of the studied plant species and are therefore considered as pollinators of these plants.



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