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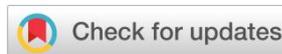
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Research Article

## Effect of *Rhizobium*-entomophilous insect interaction on groundnut production in Doba, southern Chad

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### Abstract

This study investigated the combined effect of *Rhizobium* inoculation and flower-visiting insects on the productivity of groundnut (*Arachis hypogaea*) in Doba, southern Chad. Groundnut is a nutritionally and economically important crop, particularly in sub-Saharan Africa where protein-rich food is often scarce. Improving yields through eco-friendly practices like biofertilization and biological pollination can significantly enhance food security. A field experiment was conducted using three treatments : plots with *Rhizobium*, plots with chemical fertilizer, and control plots. Additionally, flowers were either left open to insect visits or protected to determine the effects of pollinators. Insects such as *Amegilla calens*, *Apis mellifera*, and *Braunsapis* sp. were identified as key pollinators, with *Amegilla* sp. being the most frequent visitor. Key parameters analyzed included number and weight of pods and seeds. Results showed that both *Rhizobium* inoculation and pollinator activity significantly increased yields. In plots exposed to pollinators, *Rhizobium* inoculation led to a marked improvement in pod and seed production compared to controls. The combined presence of *Rhizobium* and floral insects significantly enhanced groundnut yield components such as pod number, seed number, and seed weight. The findings underscore the potential of integrated agroecological practices specifically the synergistic use of biofertilizers and insect pollinators to sustainably boost crop productivity in regions like southern Chad.

**Keywords :** *Rhizobium*, entomophilous, insect, *Arachis hypogaea*, Doba.

## INTRODUCTION

Worldwide, 144 children under the age of 5 are stunted <sup>1</sup> and around 795 million people are undernourished, one in four in sub-Saharan Africa, suffering from inadequate energy intake combined with protein, vitamin and mineral deficiencies <sup>2</sup>. Unsatisfactory supplies and the high shortage of protein-rich food are stimulating research into new sources of protein to supplement or replace existing proteins. Crop production in Africa is characterised by low productivity due to low input use and land degradation <sup>3</sup>. Land degradation undermines yield improvements for millions of farmers in developing countries who struggle to feed their families. The use of biofertilisers would be a more accessible and less costly alternative for small-scale producers and would make it possible to

increase production while protecting the environment by reducing the need for prolonged use of chemical fertilisers <sup>4,5,6,7</sup>. *Rhizobia* are bacteria capable of fixing atmospheric nitrogen in association with host legumes such as groundnut, soybean and cowpea <sup>4</sup>. A mass supply of symbiotic microorganisms in the form of inoculum to plants can improve crop growth by helping them to obtain nutrients <sup>6,8,9</sup>. Groundnut is a crop of great nutritional and economic importance in most tropical and subtropical regions. In Chad, cowpea and groundnut production in 2017 was estimated at 142,087 tonnes and 920,067 tonnes respectively <sup>10</sup>. In Chad, as in other countries around the world, biofertilisers are known to increase yields of certain legumes <sup>4,5,7</sup>. Studies on the impact of floricultural insects on the fruit and grain yields of certain plants have been carried out in Chad by several authors <sup>11</sup> and

work has been done on the profitability of *V. unguiculata* and *A. hypogaea* grown under the effect of *Rhizobium* 5,6,12, and also the study of the cumulative effect of insects and biofertilisers on the yield of certain legumes 13,14,15. To our knowledge, little work has been done on the influence of *Rhizobium* and flowering insects on these plants in the province of Logone Occidentale in southern Chad. The present work is a contribution to the improvement of yields through the interaction between *A. hypogaea*, insects and *Rhizobium* in order to fight for food security, which is the key to a healthy population.

## MATERIALS AND METHODS

### Materials

### Study site

The work was carried out in Doba in the province of Logone Oriental in Chad. The geographical coordinates are as follows: latitude: 8°68; longitude: 16°84 and altitude: 389.7m. The climate is Sudano-Guinean, mild and cool, with two seasons: a rainy season (April to October) and a dry season (November to March). Annual rainfall is 434 mm. Temperature extremes range from a low of 29°C to a high of 39°C, then from 22°C to 26°C. Soils are ferruginous, ferralitic or hydromorphic. The vegetation of the Eastern Logone is made up of isolated trees in agricultural plots or tree parks, forest galleries, shrub savannahs and relics of tree savannahs.

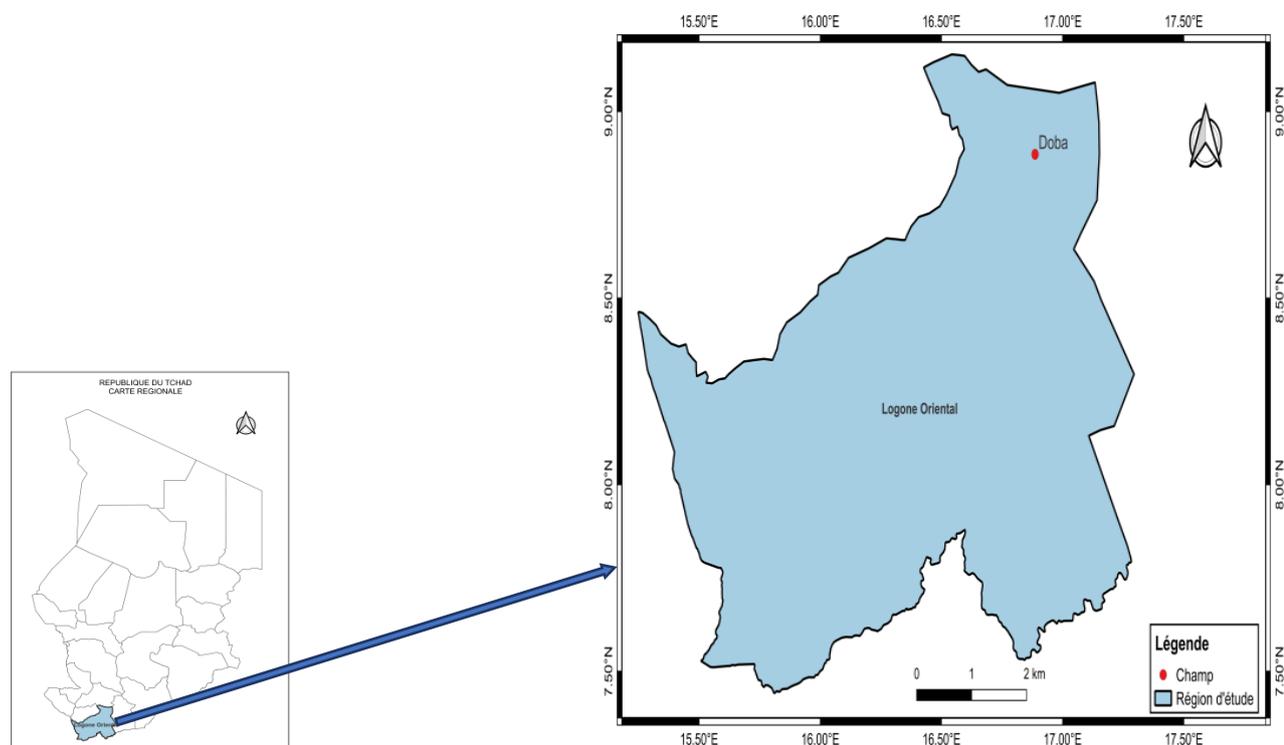


Figure 1: Map of the study area

### Biological materials

#### Plant material

The groundnut (*Arachis hypogaea* L.) seed comes from the ITRAD seed production centre in Bébédja, Chad. The seed used is the JL variety, which has a life cycle of about 75 days. It is an erect plant with yellow flowers and pinnate leaves, producing two (very rarely three) seeds per pod. Pods with one seed can also be found.



Figure 2: Groundnut seed (*Arachis hypogaea*), variety JL 24

#### Animal material

This is a summary of the insects in the natural environment and the bees in the various hives located in Doba.

#### Bacterial material

This consists of *Rhizobium* strains.

#### Method

##### Preparation of the experimental field

The field is a rectangle 17.5 m long and 11 m wide, ploughed and divided into 9 plots 4 m long and 2.5 m wide, divided into three blocks. The plots are separated from each other by a 1 m wide path. The experimental set-up is a completely randomised block, consisting of three treatments, each repeated four times : the PN treatment (negative control) and the PR treat. The seed

was sown in rows on the plots, with 5 rows per plot. The spacing was 30 cm between two successive seedpots on the rows and 50 cm between the rows, i.e. 60 seed pots for each plot. The seeds were planted at a rate of one seed per plot and at a depth of around 3 cm. Sowing began with the control plots, followed by those treated with chemical fertiliser and then those treated with *Rhizobium* to avoid contamination. The soil was regularly weeded three times. The 17g *Rhizobium*

inoculum was diluted in 400mL of water and mixed with a 26g sachet of milk (Nido). The milk acted as an adhesive between the inoculum and the seeds. The contents of the container were mixed by hand to homogenise the solution. The seeds intended for inoculation were placed in the solution and mixed to coat their integument with the inoculum, then directly sown



Figure 3: Plot of *A. hypogaea*

#### Determination of the mode of reproduction of *A. hypogaea*

On 10 August 2023, 60 flower buds of *A. hypogaea* were labelled on each of the two plots of treatments PR, PN and two treatments were thus constituted :

Treatment 1 (T1): 60 free labelled flower buds;

Treatment 2 (T2): 60 flower buds labelled and carefully protected from insects using gauze bags <sup>16</sup> .



Figure 4 : Free flower (*A. hypogaea*)



Figure 5 : Protected flower (*A. hypogaea*)

At the end of flowering, the number of pods formed was counted in each treatment. For each treatment, the fruiting index (Ifr) was calculated using the following formula:  $Ifr = (F2/F1)$ , where F2 is the number of pods formed and F1 is the number of viable flowers initially borne <sup>17</sup> . The difference between the fruiting indices of the two treatments made it possible to assess the rates

of allogamy (TC) and autogamy (TA) according to the following formulae <sup>18</sup> :  $TC = \{[(IfrX - IfrY) / IfrX] * 100\}$ , where IfrX and IfrY are the average fruiting indices in the treatment with flowers left to pollinate freely (X) and in the treatment with flowers protected from insects (Y) respectively;  $TA = [100 - TC]$ .

## Study of insect activity on the flowers of *A. hypogaea*

### • Frequency of visits

From 9 August 2023 to 19 August 2023, insects were counted on the flowers of treatment 1 according to four time slots: 8 - 9 am, 10 - 11 am, 12 - 1 pm, 2 - 3 pm. We passed over each flower once during each of the time slots. At each pass, the various insects encountered were counted. The data on the frequency of visits by the various floricultural insects that were counted made it possible to determine the place of *A. mellifera* in the entomofauna of *A. hypogaea* <sup>16</sup>. The frequency (Fi) of each insect species was determined using the following formula:  $F_i = V_i/VI \times 100$ , where  $V_i$  = number of visits by the insect and  $VI$  = number of visits by all insects to the same plants <sup>16</sup>.

### • Floral products collected

The aim was to note whether insects collect pollen, nectar or both from a given flower. An individual that plunges its proboscis into a flower is a nectar harvester; if it scratches the anthers with its mandibles and metathoracic legs, it is a pollen harvester.

The floral products collected by the insects were systematically noted when recording the duration of visits per flower.

### • Abundance of visitors

Abundance per flower was recorded following direct counts. The aim was to count the greatest number of individuals simultaneously active on one flower or on 1000 flowers in full bloom <sup>16</sup>. These parameters were recorded during the same dates and time slots as the frequency of visits. Abundance per flower was recorded following direct counts. For abundance per 1000 flowers (A1000), insects were counted on a known number of flowers. A1000 was calculated using the formula:  $A1000 = [(A_x / F_x) \times 1000]$ , where  $F_x$  and  $A_x$  are respectively the number of open flowers and the number of visitors actually counted on the open flowers of treatment 1 at time  $t$  <sup>16</sup>.

### • Visit duration

The duration of visits per flower is the time taken by the bee to collect a product (pollen and/or nectar) from a flower <sup>16</sup>. The data were recorded at the following times: 10 - 11 h, 12 - 13 h and 14 - 15 h.

### • Foraging speed

The foraging speed according to Jacob-Remacle (1989) corresponds to the number of flowers visited per minute. It was calculated according to the following formula:

$V_b = (F_i/d_i) \times 60$ , where  $d_i$  is the duration given by the stopwatch (in seconds) and  $F_i$  is the number of flowers

corresponding to  $d_i$  <sup>16</sup>. It was recorded at the same dates and daily periods as for the duration of visits. The influence of the surrounding flora and fauna was systematically recorded during the timing of the duration of visits per flower. The temperature and hygrometry of the study station were recorded every 30 minutes, using a portable HT - 9227 thermohygrometer installed in the shade throughout the observation period.

### Evaluation of the impact of anthophilous insects on yields

This is based on the impact of anthophilous insects on pollination, the impact of pollination on fruiting of *A. hypogaea*, and a comparison of fruit and seed yields (fruiting rate and percentage of normal seeds) of treatments T1 and T2 in the control subplots. The fruiting rate (Fi) due to the influence of flowering insects was calculated using the formula:  $F_i = \{(F_1 - F_2) / F_1\} \times 100$  where  $F_1$  and  $F_2$  are the fruiting rates in treatments T2 (free flowers) and T1 (protected flowers) respectively. For a treatment  $x$ , the fruiting rate ( $F_x$ ) is:  $F_x = [(number\ of\ fruits / number\ of\ flowers) \times 100]$  <sup>16</sup>.

### Estimation of the cumulative action of *Rhizobium* and insects on *A. hypogaea* yields

It was based on the comparison of fruit and grain yields (fruiting rate, average number of seeds per pod and percentage of normal seeds) of treatment T2 of the PR subplots and treatment T1 of the PN subplots.

### Statistical analysis

The data was analysed using :

- Descriptive statistics (calculation of means, standard deviations and percentages) ;
- Three tests: Student's t-test to compare two means, Chi-square ( $\chi^2$ ) to compare percentages, correlation coefficient ( $r$ ) to study linear relationships between two variables <sup>19</sup> ; Excel 2010; ANOVA to compare more than two means.

## RESULTS

### Reproductive system of *A. hypogaea*

The fruiting index was 0.47 and 0.36 in treatments T1 and T2 respectively in the neutral plots for *A. hypogaea* and 0.93 and 0.85 in treatments T1 and T2.

### Rank of some insects in the floricultural entomofauna of *Arachis hypogaea*

During observation, 153 visits by 08 insect species were recorded on 1155 flowers of *Arachis hypogaea* (Table 1).

Table 1: Number and percentage of visits by different insects recorded on *Arachis hypogaea* flowers.

Order	Family	Insects		Total			
		Genus	species subspecies	PR	PN	N	P(%)
Hymenoptera	Apidae	<i>Braunsapis</i>	<i>sp(Ne)</i>	07	09	16	10,45
		<i>Apis</i>	<i>mellifera(Ne)</i>	23	13	36	23,52
		<i>Amegilla</i>	<i>sp(Ne)</i>	23	08	31	20,26
Lepidoptera	Pieridae	<i>Eurema</i>	<i>eximia(Ne)</i>	11	04	15	9,80
	Nymphalidae	<i>Danaus</i>	<i>plexippus(Ne)</i>	06	10	16	10,45
	Papilionidae	<i>Papilio</i>	<i>demodocus(Ne)</i>	06	02	8	5,22
	Pieridae	<i>Catopsilia</i>	<i>sp(Ne)</i>	08	09	17	11,11
	Pieridae	<i>Catopsilia</i>	<i>sp.1(Ne)</i>	09	05	14	09,15
<b>Total</b>			08	93	60	153	99,96

PR: plots with *Rhizobium*; PN: plots without *Rhizobium* or chemical fertiliser; n: number of visits; P: percentage of visits equal to : (n /218) x 100.

Table 1 shows that *Apis mellifera* is the main flower-feeding insect on *Arachis hypogaea*, accounting for 23.52% of visits.

or chemical fertiliser attracted 60 visits (27.52%). The difference between these two percentages is highly significant ( $\chi^2 = 11.59$ ;  $P > 0.05$ ).

Table 1 also shows that plots with *Rhizobium* attracted 95 insect visits (43.57%) and plots without *Rhizobium*

**Floral products collected**



Figure 6: *Eurema* sp taking nectar in the *A. hypogaea* flower



Figure 7: *Braunsapis* sp taking nectar in the *A. hypogaea* flower

**Daily frequency of visits to *A. hypogaea* flowers**

From 48 to 54 days after sowing (DAS), 23 and 13 visits of *A. mellifera* were counted on the flowers of treatment 1 of plots with *Rhizobium* (PR), and plots without *Rhizobium* or chemical fertiliser (PN) respectively. The figure shows the daily distribution of visits by *A.*

*mellifera* to the flowers. Figure 8 and 9 shows that this insect visits the flowers from 8 a.m. to 5 p.m., with a peak of activity between 12 noon and 1 p.m. in the PR and PN plots and between 10 a.m. and 11 a.m. These times correspond to the time of day when this plant's nectar is most available in the flowers.

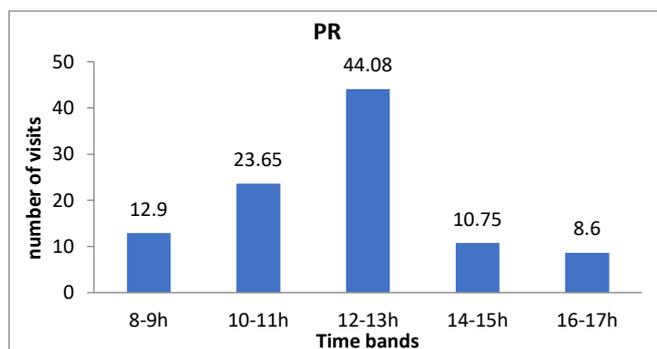


Figure 8 : Daily distribution of insect vists on *A. hypogaea* flowers in plots with *Rhizobium* (PR).

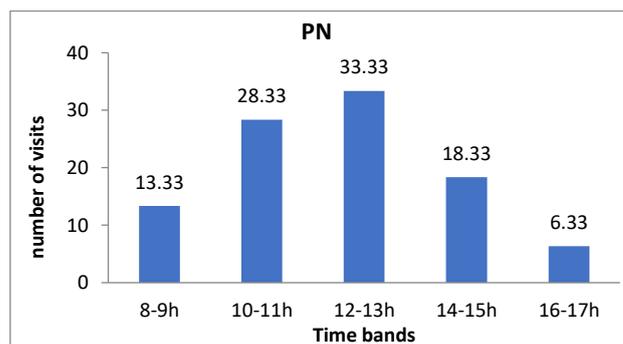


Figure 9 : Daily distribution of insect vists on *A. hypogaea* flowers in neutral plots (PN).

### Insect abundance on *A. hypogaea*

The greatest number of individuals simultaneously active on a flower was 1 in each of the study sites. The mean abundance per 1000 flowers of *A. hypogaea* varied from 23.18 individuals (n = 49; s = 18.81) in *A. mellifera*; 81.86 individuals (n = 120; s = 45.85) in *Amegilla sp*; 55.28 individuals (n = 133; s = 23.84) in *Braunsapis sp* in the PR. The mean abundance per 1000 flowers of *A. hypogaea* varied from 16.03 individuals (n = 34; s = 8.06) in *A. mellifera*; 64.34 individuals (n = 100; s = 33.67) in *Amegilla sp*; 76.23 individuals (n = 100; s = 45.12) in *Braunsapis sp* in the PN. The mean abundance per 1000 flowers of *A. hypogaea* varied from 14.03 individuals (n = 14; s = 8.22) in *Apis mellifera*; 58 individuals (n = 90; s = 32.01) in *Amegilla sp*; 82.43 individuals (n = 124; s = 45.86) in *Braunsapis sp* in EPs. Comparison of mean abundances per 1000 flowers in PR and PN revealed a very highly significant difference in *A. mellifera* (t = 4.02; ddl = 81; P < 0.001), then a highly significant difference in *Amegilla sp* (t = 3.26; ddl = 218; P < 0.01) and *Braunsapis sp* (t = 4.22; ddl = 231; P < 0.01).

### Yields of *A. hypogaea*

**Table 2: Yields of *A. hypogaea***

Treatment	Plots	Number of pods	Pod dry weight	Number of seeds	Seed weight	dry
		m±s	m±s	m±s	m±s	
<b>T1 (Free flowers)</b>	PN	12,97±5,72 <sup>ab</sup>	9,29±3,79 <sup>b</sup>	11,48±5,70 <sup>a</sup>	3,38±1,76 <sup>a</sup>	
	PR	14,17±5,17 <sup>b</sup>	11,39±3,85 <sup>c</sup>	16,34±8,03 <sup>b</sup>	5,64±2,63 <sup>b</sup>	
	P-value	0,09	0,0001	0,0002	0,0001	
<b>T2 (Protected flowers)</b>	PN	16,54±6,18 <sup>a</sup>	12,05±5,23 <sup>a</sup>	15,42±8,79 <sup>a</sup>	5,90±3,21 <sup>a</sup>	
	PR	19,31±7,88 <sup>a</sup>	12,85±5,33 <sup>a</sup>	17,68±10,04 <sup>a</sup>	6,97±4,62 <sup>a</sup>	
	P-value	0,18	0,52	0,4541	0,17	

For the same parameter, the values in the same column assigned the same letter are not significantly different at the 5% threshold. With regard to the number of pods, there was no significant difference between the plots inoculated with *Rhizobium* and the control when the flowers were protected and left open. However, when the flowers were left to pollinate freely (T1), *Rhizobium* inoculation significantly increased (p=0.09) the total number of pods compared with non-inoculated plots. In terms of pod dry weight, there was no significant difference between plots inoculated with *Rhizobium*, the control and chemical fertiliser when the flowers were protected. However, when the flowers were left to pollinate freely (T1), *Rhizobium* inoculation significantly (p=0.0001) increased the dry weight of the pods compared with non-inoculated plots.

With regard to the number of seeds, there was no significant difference between plots inoculated with *Rhizobium* and the control when the flowers were

### Duration of visits per flower in *A. hypogaea* plots

The average duration of an insect visit per flower of *A. hypogaea* was 2.34 sec (n = 22; s = 1.38) for *A. mellifera*; 8.26 sec (n = 94; s = 4.71) for *Amegilla sp*; 5.85 sec (n = 57; s = 4.41) for *Braunsapis sp* in the PR. The mean duration of an insect visit per *A. hypogaea* flower was 2.98 sec (n = 28; s = 1.87) in *A. mellifera*; 4.26 sec (n = 68; s = 5.32) in *Amegilla sp*; 5.24 sec (n = 70; s = 2.97) in *Braunsapis sp* in the PN.

### Foraging speed on *A. hypogaea* flowers

The average foraging speed varied from 6.18 flowers/min (n = 11; s = 1.70) in *Apis mellifera*; 7.64 flowers/min (n = 103; s = 3.72) in *Amegilla sp*; 22.55 flowers/min (n = 163; s = 9.97) in *Braunsapis sp* in the PR. The average foraging speed varied from 4.10 flowers/min (n = 19; s = 1.20) in *Apis mellifera*; 7.04 flowers/min (n = 94; s = 3.32) in *Amegilla sp*; 16.52 flowers/min (n = 84; s = 10.07) in *Braunsapis sp*; 14.03 flowers/min (n = 100; s = 7.30) in P3 in the NP.

The mean speed was significant for *A. mellifera* in the PR and PN plots (t=3.23; ddl=28; P < 0.001); it was not significant for *Amegilla sp* (t=1.20; ddl=200; P < 0.001).

protected. However, when the flowers were left to pollinate freely (T1), the inoculated plots significantly (p=0.0002) increased the number of seeds compared with the non-inoculated plots.

In terms of dry seed weight, there was no significant difference between plots inoculated with *Rhizobium* and the control when the flowers were protected. However, when the flowers were left to pollinate freely (T1), the inoculated plots significantly (p=0.0001) increased the dry weight of the seeds compared with the non-inoculated plots.

### DISCUSSION

The aim of the study was to determine the combined influence of *Rhizobia* and floricultural insects on the fruit and granary yields of *Arachis hypogaea*. The fruiting index in this work was 0.47 and 0.36 in treatments T1 and T2 respectively in the neutral plots

for *A. hypogaea* and 0.93 and 0.85 in treatments T1 and T2. These results do not corroborate those in Cameroon on *A. hypogaea*, which found 0.13, 0.12, 0.26 and 0.19<sup>20</sup>, nor those on *conguiculata*, which found 0.95, 0.19, 0.94 and 0.21<sup>7</sup>. This difference is due to the fact that the treatments are free and protected for one (1) year. during the observation, 153 visits from 08 insect species were recorded on 1155 flowers of *Arachis hypogaea*. This result does not corroborate with those in Cameroon who recorded 1217 visits from 07 species on *Vigna subterranea*<sup>15</sup>. This is due to climatic factors, flower morphology and cultivated plant species. During their visits, the insects frequently come into contact with the anthers and stigmas. This explains why they collect both nectar and pollen. This result is similar to that on *Vigna unguiculata* in Garoua, Cameroon, where the insects also collect nectar and pollen<sup>21</sup>. The optimal daily activity period for *A. mellifera* in the *Rhizobium* plot and the neutral plot was from 8 am to 5 pm with a peak from 12 pm to 1 pm. This result differs from that on *Vigna unguiculata* in Garoua, Cameroon. Comparison of mean abundances per 1000 flowers in PR and PN revealed a very highly significant difference in *A. mellifera* ( $t = 4.02$ ;  $ddl = 81$ ;  $P < 0.001$ ), followed by a highly significant difference in *Amegilla sp* ( $t = 3.26$ ;  $ddl = 218$ ;  $P < 0.01$ ) and *Braunsapis sp* ( $t = 4.22$ ;  $ddl = 231$ ;  $P < 0.01$ )<sup>21</sup>. This abundance is almost similar to the results in Cameroon<sup>15</sup>. The high abundance of workers per 1000 flowers of *A. hypogaea* is thought to be linked to the ability of honey bees to recruit a high number of foragers to exploit an interesting food source. The average duration of an insect visit per *A. hypogaea* flower was 2.34 seconds for *A. mellifera*, 8.26 seconds for *Amegilla sp* and 5.85 seconds for *Braunsapis sp* in plots with *Rhizobium*. The average duration of an insect visit per *A. hypogaea* flower was 2.98 seconds in *Apis mellifera*; 4.26 seconds in *Amegilla sp*; 5.24 seconds in *Braunsapis sp* in the neutral plots. These results are almost similar to those of who found an average duration of 10.18 on nectar collection and 4.42 on pollen collection in sesame flower<sup>22</sup>. The average foraging speed varied from 6.18 flowers/minute in *Apis mellifera*; 7.64 flowers/minute in *Amegilla sp*; 22.55 flowers/minute in *Braunsapis sp* in plots with *Rhizobium*. On the other hand, the average foraging speed varied from 4.10 flowers/minute in *Apis mellifera*; 7.04 flowers/minute in *Amegilla sp*; 16.52 flowers/minute in *Braunsapis sp* in the neutral plots. These results do not corroborate those in Cameroon who found an average foraging speed of 21.59 flowers per minute for *Xylocopa olivacea* on the flower of *Solanum lycopersicum*, Rio Grande variety<sup>20</sup>. This difference is due to variations in obsequential foraging speeds, which are linked to the accessibility of pollen or nectar, the availability of these products, the distances separating the flowers exploited during the different foraging trips and, above all, the frequency of interruptions in the visits of each insect. The average yields in terms of number of pods and seeds due solely to insects were 12.99 and 11.48 respectively. Those due to *Rhizobia* were 19.31 and 17.68 respectively. These results are higher than those in Cameroon, who found average yields of pods and seeds due solely to insects to

be 08.91 and 09.18 respectively<sup>23</sup>. Furthermore, those due to *Rhizobia* are different from those who found 8.0 as the average number of pods per plant and 8.15 as the average number of seeds per pod in *Vigna unguiculata*<sup>5</sup>. The cumulative effect of floricultural insects and *Rhizobiums* significantly increases the number of peanut seeds than the number of seeds induced by insects alone, but not significantly to the number of seeds in plots attributed to *Rhizobiums* alone. These results corroborate those obtained with the red variety *Vigna subterranea* in Dang (Ngaoundéré)<sup>15</sup>. Under the right symbiotic conditions, legumes will satisfy most of their requirements for growth, flowering and increased production<sup>4</sup>. In addition, as insects move from flower to flower, they have a positive effect on the pollination of many legumes, increasing pod and/or seed yields<sup>24</sup>.

## CONCLUSION

This study demonstrated the cumulative effect of *Rhizobium* and flower-feeding insects on groundnut yields, and the use of microbial inoculum in the field contributed to an improvement in soil fertility and an increase in groundnut yields in the field. Among the insects that visit the flowers, *Apis mellifera* and *Amegilla sp*. were respectively the most frequent. Inoculation with *Rhizobium* significantly increased the average number of pods and seeds, as well as the dry weight of pods and seeds, compared with insects. Insects and *Rhizobium* significantly improved the yields of this Fabaceae.

**Conflicts of Interest :** For this article, the authors declare that they have no conflict of interest.

**Authors' Contributions :** JD contributed to the literature search, field data collection, data analysis and first draft. MAD and MKK contributed to the first draft and data analysis. DG contributed to the correction and scientific orientation of the draft. NB and MM coordinated all the work.

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