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Integrated impact of mycorrhiza (*Glomus* sp) and pollinating insects on growth and yield of *Vigna subterranea* (L.) Verdcourd (Fabaceae)

Mohamadou Moussa*, Ngakou* Albert, Tchuenguem Fohouo Fernand-Nestor
Department of Biological Science, Faculty of Science, University of Ngaoundere, P.O. Box 454 Ngaoundere, Cameroon
***Corresponding author:** alngakou@yahoo.fr, Tel: (+237) 677909201*
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1 SUMMARY

Bambara groundnut (*Vigna subterranea)* is an important component of some dietary staples in many African countries. Although it is a crop legume, its yields are generally low. It is well known that, by providing pollination, anthophilous insects including bees, generally increase fruit and seed yields of many plant species. Therefore, the effect of insect pollinators and mycorrhiza on growth and yield parameters of *Vigna subterranea* was assessed from April to September 2015 and 2016 at Dang (Ngaoundere, Cameroon). The experiment was set up in a complete randomized block design with three treatments: plots applied with mycorrhiza; plots applied fertilizer-NPK; plots applied neither with mycorrhiza, nor with fertilizer-NPK. Two other treatments were formed by flowers protected against insects and free pollinated flowers. Parameters such as reproduction mode, frequency of floral entomofauna, activity of insects on flowers, cumulative effect of treatments on yield were assessed. Data were analysed using student test, chi-square and Pearson correlation. Results indicate that root nodules formed by the host plant in plots that received mycorrhiza were significantly higher than those from positive and negative controls. *Vigna subterranean* was revealed as an allogamous-autogamous plant with the predominance of autogamy. In 2015 and 2016, 3205 and 1565 visits from five insect species were recorded on *V. subterranea* flowers respectively. *Eurema eximia* and *Halictus* sp. were the most frequently insect species observed in the field, with 25.62 and 23.58% visits respectively. The comparison of yields between plants with flowers left in free pollination to those with flowers protected from insects indicated 32.80% increase in fructification index and 21.55% increase in the number of seeds per pod due to insects. The synergistic effect of insects and mycorrhiza increased the number of seeds per pod by 28.8% and the percentage of normal seeds by 30.03%. This study results suggest that inoculation of Bambara groundnut seeds at sowing with mycorrhiza and installation of hives close to field could be recommended for a sustainable pods and seed yield improvement of this crop.

2 INTRODUCTION

Bambara groundnut (***Vigna subterranea*** L. Verdc.) is one of the main income sources of income for women in producing areas (Ndiang *et al.*, 2014). In Africa, after groundnut and cowpea, it is ranked as the third most important food legume (Bamshaiye *et al.*, 2011). Bambara groundnut is intercropped with cereals and root crops in many traditional farming systems (Ntundu, 1997). However, the drought tolerance and ability to produce yield in soils that are too poor are the agronomic values of this crop (Anchirinah *et al.*, 2001, Azam-Ali *et*

al., 2001). It enriches the soil with nitrogen for other crops by contributing to atmospheric nitrogen fixation, through the established symbiosis with *Rhizobium*, and is therefore beneficial in crop rotation and inter-cropping (Mukurumbira, 1985, Karikari, 1971). In many African countries, Bambara groundnut is also an important source of protein in the diet (Linnemann and Azam-Ali, 1993). Nutritionally, it contains sufficient quantities of protein (19%), carbohydrate (63%), fat (6.5%) and essential amino acids (Amarteifio *et al.*, 2006). Despite these attributes, pest attacks, diseases and nutrient deficiencies, particularly phosphorus and nitrogen are the main constraints to crop yield (Kumar, 1991). Although chemical fertilizers are often used, they are toxic to human and his environment (Margni *et al.*, 2002). One way to prevent this is the use microbial inoculants such as rhizobia and Arbuscular mycorrhizal fungi (AMF) that have a direct beneficial effect on the host plants (Arshad and Frankenberger, 1993, Denison and Kiers, 2004, Ngakou *et al.*, 2012). In Cameroon, Bambara groundnut is an important component of some dietary staples. This plant is grown for human consumption and has many other uses in the food (Berchie *et al.*, 2010), and industrial sectors. The nuts can for example be used in cosmetic and pharmaceutical factories due to their high content in proteins, fatty acids, and carbohydrates (Ibrahin and Ogunwusi, 2016). Currently, the production of *V. subterranea* in the country is low, whereas the demand for seeds is high (MINADER, 2012). Therefore, it is important to investigate on the possibilities of increasing the production of this valuable plant. In Cameroon, few researches have been reported on the morphological characterization of some local varieties (Ndiang *et al.*, 2012), on the varietal resistance of *V. subterranea* to insect pest in the far North Region (Kouninki *et al.*, 2014), and the plant potentials in response to dual inoculation *Rhizobium*-mycorrhiza has been carried out (Ngakou *et al.*, 2012). However, research on anthophilous insects has been increased because of their vital importance in the pollination of food crops (Kengni *et al.*, 2015, Nepide and Tchuenguem, 2016). It is well known that, by providing pollination, anthophilous insects, including bees, generally increase fruit and seed yields of many plant species (Sabbahi *et al.*, 2005, Klein *et al.*, 2007, Tchuenguem *et al.*, 2009). Prior to this study, in Adamawa Region, no previous research on the relationships between mycorrhiza, anthophilous insects and yield production of *V. subterranea* has been reported. The main objective of this research was to gather more data on the relationships between *V. subterranea*, mycorrhiza and flower visiting insects for the optimal management of pollination services. The registration of the activity of insects on *V. subterranea* flowers, the evaluation of the impact of flowers visiting insects on pollination, pods and seeds yields of this Fabaceae, the estimation of the impact of mycorrhiza on *V. subterranea* and the evaluation of the impact of the cumulative action of mycorrhiza and flowers visiting insects are discussed.

3 MATERIALS AND METHODS

3.1 Study site, experimental plot and biological material: The experiment was carried out in the field from April to September 2015 and 2016 at Dang (latitude 07°42.22'N, longitude 13°53.76'E and altitude 1054 m above sea level) in Ngaoundéré, Adamaoua region of Cameroon. This region belongs to the high-altitude Guinean savannah agro-ecological zone. The climate is characterized by a rainy season (April to October) and a dry season (November to March), with an annual rainfall of about 1500 mm. The mean annual temperature is 22°C, while the mean annual relative humidity is 70% (Amougou *et al.*, 2015). The animal material was mainly represented by insects naturally present in the environment and 48 colonies of *Apis mellifera* Linnaeus (Hymenoptera: Apidae) located close to the experimental field. The flora surrounding *V. subterranea* field had various unmanaged and cultivated species. Mycorrhizal inoculant used was of the genus *Glomus*, provided by the

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Biotechnology Centre of the University of Yaoundé I. Approximately 10g of mycorrhiza were applied at as a layer into sowing hole before sowing (Ngakou, 2007). ***Vigna subterranea*** seeds of Creams ivory variety with fine brown spots (fig. 1) were provided by the Institute of Research for Agricultural Development (IRAD) at Wakwa-Ngaoundere. The fertilizer-NPK used was of the formula 10:10:20, purchased from a local phytosanitary store. It was applied at 14 days after sowing, at a rate of 10g within the rhizosphere of each plantlet.

Fig.1: ***Vigna subterranea*** Seeds of Creams ivory variety with fine brown spots

3.2 Sowing and weeding: Experimental plot was cleaned on May 10, 2015 and divided into nine subplots, each measuring 10 m^2. The field was replicated on May 17, 2016. Three subplots were inoculated with mycorrhiza, three with chemical fertilizer-NPK and three others left unapplied neither with mycorrhiza nor with fertilizer-NPK. Two seeds were sown per hole on five lines per plot, for a total of 13 holes per line. Bambara groundnut seeds were inoculated as described by Ngakou *et al.* (2012). Holes were separated 40 cm from each other, while lines were 20 cm apart. Weeding was performed manually as necessary to maintain subplots weed-free.

3.3 Determination of the reproduction mode of *Vigna subterranea* : The experiment was carried out between June-July, 2015. Hence, six subplots carrying 360 plants inoculated and uninoculated with 5613 flowers at budding stage were labelled. Three subplots carrying 180 plants with 2720 flowers were left opened to insects visits (treatment 1) (Fig. 2) and three others carrying 2893 flowers were protected using white gauze cages (1 mm^2 mesh) to prevent insect or other pollinator visits (treatment 2) (Fig. 3). Between June-July, 2016, the experiment was repeated. In treatment 3, there were three subplots carrying 180 plants with 2565 flowers, while in treatment 4, the three subplots with 180 plants had 2290 flowers. The number of pods was calculated for each treatment, thirty days after shading of the last flower. The podding index (Pi) was evaluated as described by Tchuenguem *et al.* (2001): $P_i = F_2/F_1$, where F_2 is the number of pods formed and F_1 the number of viable flowers initially set. The allogamy rate (Alr) from which derives the autogamy rate (Atr) was expressed as the difference in podding indexes between treatment X (unprotected flowers) and treatment Y (bagged flowers) as described by Demarly (1977): $Alr = [(P_rX-P_iY)/PiX]*100$, where P_rX and P_iY are respectively the mean podding indexes of treatments X and Y. $Atr = 100-Alr$.

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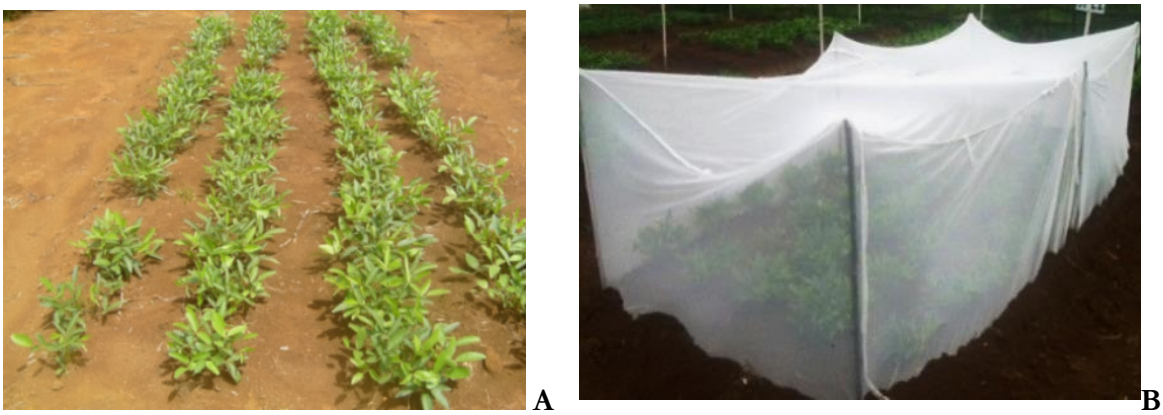

A **B**

Fig. 2: *Vigna subterranea* subplot showing plants unprotected (A), or protected with gauze (B).

3.4 Assessment of the influence of mycorrhiza on nodulation and biomass of *Vigna subterranea:* For each uninoculated subplot (treatment a), subplot inoculated with mycorrhiza (treatment b), and (subplot applied with fertilizer-NPK (treatment c), 15 plants were labelled. At 35 days after planting (DAP), root nodules were harvested per plant, counted, sun dried, and weighed. Plants were dried in an oven at 72°C for 12 hours and weighed (Ngakou *et al.*, 2007b). Plant biomass and nodulation were evaluated on the same 45 individual plants of treatments a, b and c.

3.5 Frequency of floral entomofauna of *Vigna subterranea* : The frequency of insect visits on *V. subterranea* flowers was determined based on observations scheduled on four daily time frames (09.00 - 10.00 a.m., 11.00 - 12.00 a.m., 13.00 - 14.00 p.m. and 15.00 - 16.00 p.m.) in all treatments. From July 10th to August 16th 2015 and from July 12th to August 20th 2016, flowers were completely opened at 09.00 a.m. and closed before 16.00 p.m., corresponding to the period of insects activity. All insect visits were recorded on flowers of treatment 1. Specimens of all insect taxa (3 to 5 per species) caught with an insect net on flowers were conserved in 70% ethanol, except Lepidoptera that was kept in curls for subsequent taxonomy determination. All insects encountered on flowers were registered, and the cumulated results expressed in number of visits to determine the relative frequency of each insect species in the anthophilous entomofauna of *V. subterranea* (Tchuenguem, 2005). In addition to the determination of the floral insects' frequency, direct observations of the foraging activity on flowers were made on each insect species in the experimental field. Nectar or pollen harvested by insects during each floral visit was registered based on their foraging behavior (Kengni *et al.*, 2015). The number of opened flowers was counted, whereas the duration visits of each insect was recorded (using a stopwatch) for at least three times during each of the following daily time frames: 10.00-11.00 a.m., 12.00 a.m.-13.00 p.m. and 14.00 a.m.-15 p.m. at every sampling dates. Moreover, the number of pollinating visits (Tchuenguem, 2005), the abundance of foragers (Tchuenguem *et al.*, 2004) and the foraging speed referring to the number of flowers visited by an insect per minute (Jacob-Remacle, 1989) were evaluated. The abundance of insects per flower and per 1000 flowers (A_{1000}) were recorded following the direct counting on the same dates and daily periods as for the registration of the duration of visits. (A_{1000}) was calculated by the formula: $A_{1000} = ((A_x/F_x)*1000)$, where F_x and A_x are the number of opened flowers and the number of insects effectively counted on these flowers at time x (Tchuenguem *et al.*, 2004). The foraging speed was calculated by this formula: $Vb = (Fi/di)*60$, where *di* is the time (sec) given by a stopwatch, and *Fi*, the number of flowers visited during *di*. Around experimental plot, the disruption of the activity of each insect forager

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by competitors and the attractiveness exerted by other plant species on *V. subterranea* insect foragers were assessed. At each observation date, every 30 minutes, using a portable thermo-hygrometer (HT-9227), ambient temperature and relative humidity were recorded.

3.6 Assessment of the cumulative effects of anthophilous insects and mycorrhiza on *Vigna subterranea* yields: The estimation of this parameter was based on the effect of both mycorrhiza and insects on *V. subterranea* yield. The comparison of productivity (fruiting rate, mean number of seeds per pod and percentage of normal seeds) of treatment 5 and 6 to those of treatments 1 and 3 were assessed.

4 Data analysis : To analyze the data we used Microsoft Excel and four test: Student's (t) for comparison of means of two samples, correlation coefficient (r) to determine the linear relationship between two variables, Chi-square ($\chi 2$) to compare two percentages and STATGRAGHICS CENTURION for the comparison of means of more than two samples.

5 RESULTS

5.1 Reproduction mode of *Vigna subterranean:* The allogamy rate was 17.38% and 47.45%, respectively in 2015 and 2016, whereas the autogamous rate was 82.62% and 52.55% respectively in 2015 and 2016 (table 1). Thus, the *V. subterranea* variety used in this experiment has a mixed autogamous-allogamous reproduction mode with the predominance of autogamy.

Table 1: Allogamy and autogamy rates of ***Vigna subterranea*** in years 2015 and 2016.

Years	Number of studies flowers	Number of pots formed	Autogamous rate (%)	Allogamous rate (%)
2015	5613	3337	82.62	17.38
2016	4855	2512	52.55	47.45
Mean (2015/2016)	5234	2925	67.58	32.41

PM: subplot with mycorrhiza; PE: subplot with fertilizer-NPK; PN: uninoculated subplot.

5.2 Impact of mycorrhiza on the number of flowers, nodulation and biomass of *Vigna subterranean:* In 2015 and 2016, plants inoculated with mycorrhiza at sowing produced a significantly greater number of nodules, nodule dry weight and plant biomass compared to uninoculated plants (Table 2).

Table 2: Number of nodules, plant biomass and number of flowers of ***Vigna subterranea*** as affected by mycorrhiza in 2015 and 2016

Years	Treatments	Number of nodules per/plant	Weight of dry nodules (g/plant)	Plant biomass (g/plant)
2015	PM	$(66.2 \pm 0{,}72)_a$	$(1.52 \pm 0.01)_a$	$(15.25 \pm 1.38)_a$
	PE	$(43.21 \pm 0{,}72)_b$	$(0.76 \pm 0.01)_b$	$(9.12 \pm 1.38)_b$
	PN	$(20.22 \pm 0{,}72)_c$	$(0.2 \pm 0.01)_c$	$(4.48 \pm 1.38)_c$
	P. value	< 0.001	< 0.001	< 0.001
2016	PM	$(70.84 \pm 1.08)_a$	$(1.75 \pm 0.02)_a$	$(29.87 \pm 1.29)_a$
	PE	$(54.44 \pm 1.08)_b$	$(0.88 \pm 0{,}02)_b$	$(23.74 \pm 1.29)_b$
	PN	$(16.4 \pm 1.08)_c$	$(0.33 \pm 0.02)_c$	$(19{,}1 \pm 1.29)_c$
	P. value	< 0.001	< 0.001	< 0.001

5.3 Frequency of floral entomofauna of *Vigna subterranea* : Among the 3205 and 1565 visits of seven species recorded on 2720 and 2565 flowers in 2015 and 2016 respectively, *Eurema eximia* and *Halictus* sp. were the most represented insects with 25.62% and 23.58% visits in 2015 and 2016 for all the treatment respectively (Table 3). Besides, *E. eximia* was the most representative insect the first year, whereas *Halictus* sp. took over the second year.

5.4 Activity of insects on *Vigna subterranea* flowers: The abundance, the foraging speed and the duration of insect visits were focused on the two major flowers insects visiting of *E. eximia* and *Halictus* sp.

5.5 Relationships between insect visits and flowering stages of the plant: The number of insect visits was greater when the number of opened flowers was higher on both uninoculated, mycorrhiza inoculated and fertilizer-NPK applied plants (Fig. 5). A positive and significant correlation was found between the numbers of opened flowers and the number of insect visits on flower of uninoculated plants (r = 0.33; df = 35; P<0.05) in 2015 and 2016 (r = 0.39; df = 35; P<0.05), mycorrhiza inoculated plants in 2015 (r = 0.49; df = 35; P<0.05) and 2016 (r = 0.42; df = 35; P<0.05), and fertilizer-NPK applied plants in 2015 (r = 0.58; df = 35; P<0.05), and 2016 (r = 0.37; df = 35; P<0.05).

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Table 3: Diversity of insects visiting ***Vigna subterranea*** flowers in 2015 and 2016 at Dang (number and percentage of insect visits)

		Insects	2015					2016				
			subplot			Total		subplot			Total	
Order	**Family**	**Genus, species**	**PM**	**PE**	**PN**	**n_1**	**P_1 (%)**	**PM**	**PE**	**PN**	**n_2**	**P_2 (%)**
Hymenoptera	Apidae	*Apis mellifera* (nectar)	208	162	138	508	15.85	-	-	-	-	-
	Halictidae	*Halictus* sp. (pollen)	-	-	-	-	-	148	116	105	369	23.58
		Total Hymenoptera	**208**	**162**	**138**	**508**	**15.85**	**148**	**116**	**105**	**369**	23.58
Diptera	Syrphidae	*Episyrphus* sp. (pollen)	212	175	165	552	17.22	-	-	-	-	-
		(1 sp.) (nectar)	-	-	-	-	-	124	105	85	314	20.07
		Paragus borbonicus (pollen)	208	169	179	556	17.34	139	106	90	335	21.41
		Total Diptera	**420**	**344**	**344**	**1108**	**34,57**	**263**	**211**	**175**	**649**	**41.47**
Lepidoptera	Pieridae	*Eurema eximia* (nectar)	282	276	263	821	25.62	130	100	69	299	19.10
	Lycanidae	(1 sp.) (nectar)	269	252	247	768	23.97	97	89	62	248	15.85
		Total Lepidoptera	**551**	**528**	**510**	**1558**	**48.62**	**227**	**189**	**131**	**547**	**34.96**
Total		**7 species**	**1179**	**1034**	**992**	**3205**	**100**	**638**	**516**	**411**	**1565**	**100**

PM: subplot with mycorrhiza; PE: subplot with fertilizer-NPK; PN: uninoculated subplot; n_1 and n_2: number of visits on 2720 and 2565 flowers in 47 and 46 days respectively; sp.: undetermined species; $P_1$and P_2: percentages of visits P_1= (n_1/3205)*100 and P_2= (n_2/1565)*100; Comparison of percentages of insects visits for two years: x^2_{gobal} = 101.16; *df* = 5; $P < 0.001$.

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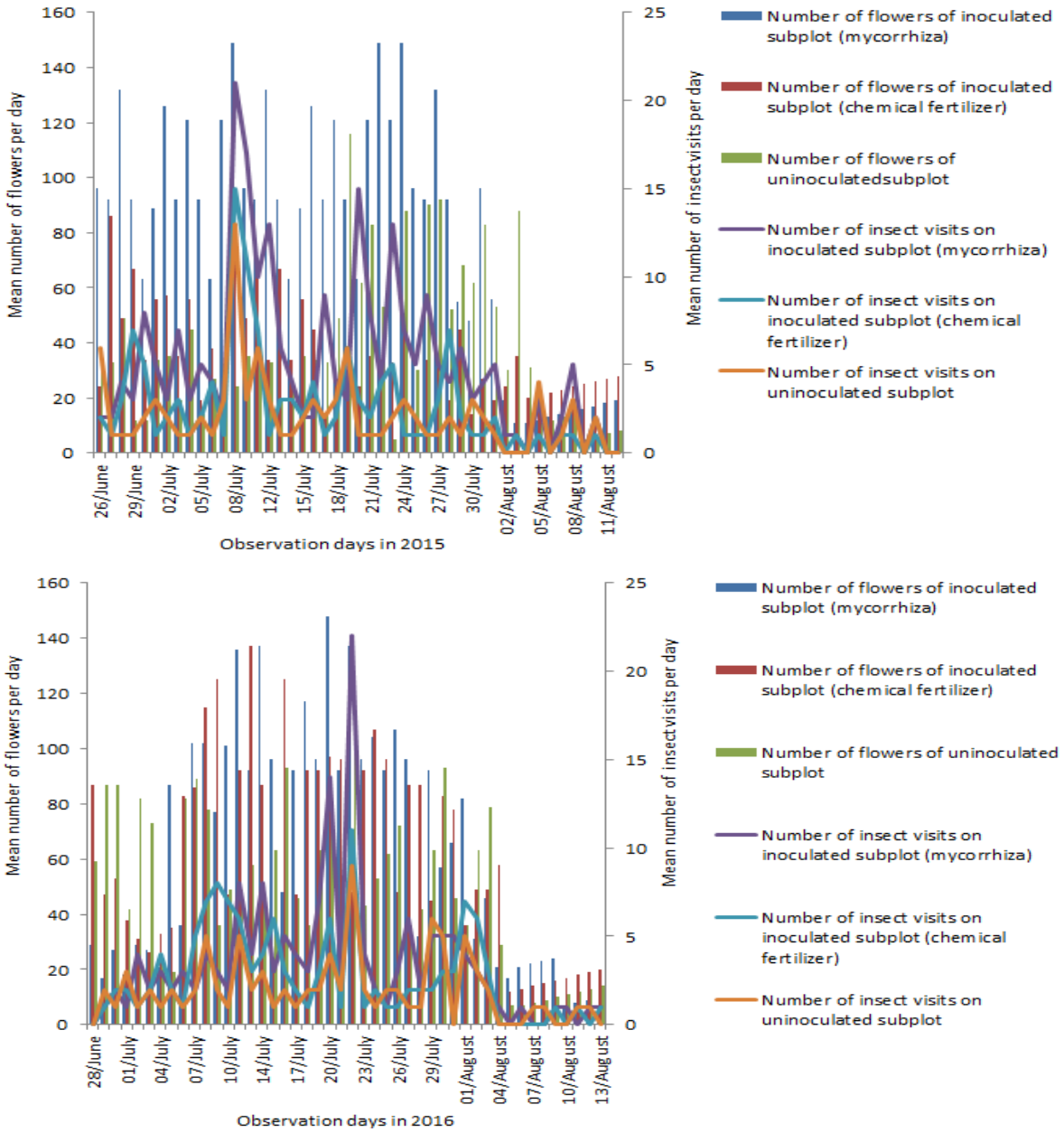

Fig. 3. Variations of the number *of* ***Vigna subterranea*** opened flowers and the number of visits of insects according to the observation days in 2015 and 2016 at Dang

5.6 Diurnal flower visits: Insects foraged on *V. subterranea* flowers daily and throughout the flowering period, with a peak activity between 01.00 p.m. and 02.00 p.m. (table 4). This activity was not influenced neither by temperature, nor hygrometry. In 2015 the correlation between the number of insect visits and the temperature was not significant on uninoculated (r = 0.79; ddl = 3; P > 0.05), mycorrhiza inoculated (r = 0.86; ddl = 3; P>0.05), and fertilizer-NPK applied (r = 0.68; ddl = 3; P>0.05) plants. The relative humidity did not significantly correlate with the number of insect visits on uninoculated (r = -0.40; ddl = 3; P>0.05), mycorrhiza inoculated (r = -0.55; ddl = 3; P>0.05) and fertilizer-NPK applied (r = -0.44; ddl = 3; P>0.05) plants.

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Table 4: Number and frequency of insect visits on ***Vigna subterranea*** flowers according to daily observation period in 2009 and 2010 at Dang

Insects	Subplot	Daily period days (hours)																	
		2015									2016								
		09 - 10		11 - 12		13 - 14		15 - 16		A	09 - 10		11 - 12		13 - 14		15 - 16		A
		n	*p* (%)	*n*	*p* (%)	*n*	*p* (%)	*n*	*p* (%)		*n*	*p*(%)	*n*	*p* (%)	*n*	*p* (%)	*n*	*P*(%)	
Apis mellifera	PM	22	10.57	40	19.23	97	46.64*	4	1.92	208	-	-	-	-	-	-	-	-	-
	PE	10	6.18	45	27.78	62	38.27*	21	12.97	162	-	-	-	-	-	-	-	-	-
	PN	14	10.14	57	41.3*	49	35.51	18	13.04	138	-	-	-	-	-	-	-	-	-
Halictus sp.	PM	-	-	-	-	-	-	-	-		24	16.21	70	47.29*	48	32.43	6	4.05	148
	PE	-	-	-	-	-	-	-	-		6	5.17	64	55.18*	39	33.63	7	6.04	116
	PN	-	-	-	-	-	-	-	-		2	1.72	13	11.2	87	75*	3	2.59	105
Episyrphus sp.	PM	-	-	66	31.14	142	66.98*	4	1.88	212	-	-	-	-	-	-	-	-	-
	PE	19	10.86	87	49.71*	56	32	13	7.43	175	-	-	-	-	-	-	-	-	-
	PN	-		80	48.48*	75	45.45	10	6.06	165	-	-	-	-	-	-	-	-	-
Syrphidae	PM	-	-	-	-	-	-	-	-	-	2	1.62	30	24.2	62	50*	1	0.8	124
(1sp.)	PE	-	-	-	-	-	-	-	-	-	-	-	73	69.52*	25	23.8	7	6.67	105
	PN	-	-	-	-	-	-	-	-	-	-	-	16	18.82	66	77.64*	3	3.52	85
Eurema	PM	80	28.36	53	18,79	122	43.26*	27	9.57	282	30	23.08	48	36.92	65	50*	2	1.54	130
eximia	PE	82	29.71	43	15.57	133	48.19*	18	6.52	276	13	13	35	35	50	50*	2	2	100
	PN	65	24.72	103	39.16*	88	33.46	7	2.67	263	17	24.64	18	26.08	34	49.27*	-	-	69
Paragus	PM	52	25	69	33.17	78	37.5	9	4.33	208	34	24.46	50	35.97*	46	33.09	4	2.87	139
borbonicus	PE	22	13.02	97	57.39*	47	27.82	3	1.78	169	26	24.53	25	23.6	53	50*	2	1.88	106
	PN	45	25.14	36	20.11	90	50.27*	8	4.46	179	12	13.33	30	33.33	45	50*	3	3.33	90
Lycaenidae (1	PM	67	24.9	110	40.89*	89	33.09	3	1.12	269	9	9.27	64	65.97*	24	24.74	-	-	97
sp.)	PE	59	26.59	60	23.8	128	50.8*	5	1.98	252	4	4.49	46	51.69*	37	39.33	2	2.24	89
	PN	32	12.96	115	46.55*	99	40.09	1	0.41	247	-	-	41	66.13	15	24.2	7	11.3	62
TOTAL		638	19,91*	1061	33,11	1355	**42.27***	151	4.72	**3205**	179	11.44	641	40.95	696	**44.47***	49	3.13	**1565**

n: number of visits in 47 days, *p*: percentage of visits, $p = (n/A) * 100$, A: total number of insect visits, *: daily peak of visit. PM: subplot with mycorrhiza; PE: subplot with fertilizer-NPK; PN: uninoculated subplot

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5.7 Abundance of foraging insect: In 2015, the highest mean number of *E. eximia* simultaneously in activity was 1 per flower for all the treatments, 28.35 per 1000 flowers (n = 188; s = 19.35) on uninoculated plants, 135.41 per 1000 flowers (n = 249; s = 99.54) on mycorrhizal inoculated plants and 32.64 per 1000 flowers (n = 222; s = 28.34) on fertilizer-NPK applied plants. In 2016, the corresponding values were 1 per flower for all the treatments, 37.04 per 1000 flowers (n = 82; s = 33.19), 27.35 per 1000 flowers (n = 76; s = 15.69) and 22.57 per 1000 flowers (n = 57; s = 9.72). In 2016, the abundance of *Halictus* sp. per flower was 1 for all the treatments and 2.59 per 1000 flowers (n = 88; s = 1.53) on negative control plants, 30.13 per 1000 flowers (n = 82; s = 19.87) on mycorrhizal inoculated plants and 24.29 per 1000 flowers (n = 62; s = 14.72) on fertilizer-NPK applied plants.

Table 5: Abundance of *Eurema eximia* and *Halictus* sp. on ***Vigna subterranea*** flowers at Dang in 2015 and 2016

Insects	plot	Year	*n*	Abundance per 1000 flowers				
				m	***sd***	***maxi***	***mini***	**Comparison of means**
Eurema eximia	PM	2015	249	135.41	99.54	666.67	11.49	
		2016	76	27.35	15.69	83.33	11.24	
		$T_{2015/2016}$	325	81.38	57.61	666.67	11.49	
	PE	2015	222	32.64	28.34	176.48	4.66	
		2016	57	22.57	9.72	41.67	10.31	F = 121.44 ; df_1 = 5 ; df_2 = 868 ; P<0.01
		$T_{2015/2016}$	279	27.60	19.03	176.48	4.66	
	PN	2015	188	28.35	19.95	107.15	4.91	
		2016	82	37.04	33.19	200	10.10	
		$T_{2015/2016}$	270	32.69	26.57	107.15	4.91	
	Total		874	141.67	103.21	950.3	21.06	
Halictus sp.	PM	2015	-	-	-	-	-	
		2016	82	30.13	19.87	115.39	10.31	
		$T_{2015/2016}$	82	15.06	9.93	115.39	10.31	
	PE	2015	-	-	-	-	-	
		2016	62	24.29	14.72	90.91	10.31	
		$T_{2015/2016}$	62	12.14	7.36	90.91	10.31	F = 89.32; df_1 = 2 ; df_2 = 229 ; P<0.01
	PN	2015	-	-	-	-	-	
		2016	88	2.59	1.53	8.83	1.03	
		$T_{2015/2016}$	88	1.29	0.76	8.83	1.03	
		Total	232	28.49	18.05	215.13	21.65	

PM: subplot with mycorrhiza; PE: subplot with fertilizer-NPK; PN: uninoculated subplot; *m*: average; *sd*: standard deviation; maxi: maximum; *mini*: minimum; *n*: sample size

5.8 Duration of insect visits per flower: In 2015, the mean duration of *E. eximia* visit on a flower was 3.78 sec (n = 263; s = 1.6) on uninoculated plants, 4.4 sec (n = 282; s = 1.68) on mycorrhizal inoculated plants and 4.35 sec (n = 276; s = 2.32) on fertilizer-NPK applied plants. In 2016, the corresponding values were 3.17 sec (n = 69; s = 2.15), 4.56 sec (n = 130; s = 2.29) and 1.81 sec (n = 100; s = 1.08). In 2016, the mean duration of *Halictus* sp. visit on a flower was 1.65 sec (n = 105; s = 0.86) on uninoculated plants, 1.57 sec (n = 148; s = 0.77) on mycorrhizal inoculated plants and 1.63 sec (n = 163; s = 0.77) on fertilizer-NPK applied plants.

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Table 6: Duration of *Eurema eximia* and *Halictus* sp. on ***Vigna subterranea*** flower at Dang in 2015 and 2016

Insects	plot	Year	*n*	Duration of insects visits per flower				
				m	***sd***	***maxi***	***mini***	**Comparison of means**
Eurema eximia	PM	2015	282	4.4	1.68	9	1	
		2016	130	4.56	2.29	9	1	
		$T_{2015/2016}$	412	4.48	1.98	9	1	
	PE	2015	276	4.35	2.32	9	1	
		2016	100	1.81	1.08	8	1	$F = 11.84$; $df_1 = 5$; $df_2 = 1114$; $P<0.01$
		$T_{2015/2016}$	376	3.08	1.7	9	1	
	PN	2015	263	3.78	1.6	9	1	
		2016	69	3.17	2.15	9	1	
		$T_{2015/2016}$	332	3.47	1.87	9	1	
		Total	1120	11.03	5.55	9	1	
Halictus sp.	PM	2015	-	-	-	-	-	
		2016	148	1.57	0.77	5	1	
		$T_{2015/2016}$	148	0.78	0.38	5	1	
	PE	2015	-	-	-	-	-	
		2016	116	1.63	0.77	4	1	$F = 22.99$; $df_1 = 2$; $df_2 = 366$; $P<0.01$
		$T_{2015/2016}$	116	0.81	0.38	4	1	
	PN	2015	-	-	-	-	-	
		2016	105	1.65	0.86	6	1	
		$T_{2015/2016}$	105	0.82	0.43	6	1	
		Total	369	2.41	1.19	6	1	

PM: subplot with mycorrhiza; PE: subplot with fertilizer-NPK; PN: uninoculated subplot; *m*: average; *sd*: standard deviation; *maxi*: maximum; *mini*: minimum; *n*: sample size

5.9 Foraging speed of insects on *Vigna subterranea* flowers: In 2015, the mean foraging speed of *E. eximia* was 7.8 flowers/min (n = 179, *s* = 5.11) on uninoculated plants, 24.41 flowers/min (n = 200; *s* = 16.96) on mycorrhiza inoculated, and 11.88 flowers/min (n = 195; *s* = 5.05) on fertilizer-NPK applied plants. In 2016, the corresponding values were 11.96 flowers/min (*n* = 96, *s* = 5.48), 27.21 flowers/min (n = 88, *s* = 6.55), and 12.6 flowers/min (n = 65, *s* = 3.9). In 2016, the mean foraging speed of *Halictus* sp. was 12.62 flowers/min (*n* = 104; *s* = 4.89) on uninoculated plants, 35.64 flowers/min (n = 98, *s* = 23.08) on mycorrhiza inoculated and 30.95 flowers/min (n = 80, *s* = 16.45) on fertilizer-NPK applied plants.

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Table 7: Foraging speed of *Eurema eximia* and *Halictus* sp. on ***Vigna subterranea*** flowers at Dang in 2015 and 2016

Insectes	plot	Year	*n*	Mean speed of insects visits on flowers				
				m	*sd*	*maxi*	*mini*	Comparison of means
Eurema eximia	PM	2015	200	24.41	16.96	80	1.39	
		2016	88	27.21	6.55	52.5	12.31	
		$T_{2015/2016}$	288	25.81	11.75	80	1.39	
	PE	2015	195	11.88	5.05	28.5	3.92	
		2016	65	12.6	3.9	25.17	5.21	$F = 94.03$; $df_1 = 5$; $df_2 = 817$; $P<0.01$
		$T_{2015/2016}$	260	12.24	4.47	28.5	3.92	
	PN	2015	179	7.8	5.11	30	1.42	
		2016	96	11.96	5.48	28.89	2.3	
		$T_{2015/2016}$	275	9.88	5.29	29.44	3.92	
		Total	823	47.93	21.51	80	1.42	
Halictus sp.	PM	2015	-	-	-	-	-	
		2016	98	35.64	23.08	165	12.5	
		$T_{2015/2016}$	98	17.82	11.54	165	12.5	
	PE	2015	-	-	-	-	-	
		2016	80	30.95	16.45	97.5	10.28	
		$T_{2015/2016}$	80	15.47	8.22	97.5	10.28	$F = 54.80$; $df_1 = 2$; $df_2 = 279$; $P<0.01$
	PN	2015	-	-	-	-	-	
		2016	104	12.62	4.89	28.89	3.75	
		$T_{2015/2016}$	104	6.31	2.44	28.89	3.75	
		Total	282	39.6	22.2	165	3.75	

PM: subplot with mycorrhiza; PE: subplot with fertilizer-NPK; PN: subplot uninoculated; *m*: average; *sd*: standard deviation; *maxi*: maximum; *mini*: minimum; *n*: sample size

5.10 Influence of neighbouring flora: In the study area, during the observation period, flowers of many other plant species were visited by *Paragus borbonicus* and *Halictus* sp. for either nectar (ne) or pollen (po) collection. Amongst these plant species were *Phaseolus vulgaris* (Fabaceae, ne), *Bidens barteri* (Asteraceae, ne and po), *Cajanus cajan* (Fabaceae, ne), *Tithonia diversifolia* (Asteraceae, ne and po) and *Helianthus annuus* (Asteraceae, ne and po). During the entire observation period, six species of *Paragus borbonicus* and four species of *E. eximia* foraging on *V. subterranea* flowers were observed moving to a neighbouring plant of a different species and vice versa. These insects were regularly interrupted by the wind.

5.11 Impact of insects on the pollination, pod and seed yields of *Vigna subterranea*: In 2015, pod and seed yields from flowers of plants protected and visited by insects (treatment 1) was higher than that of flowers protected from insects (treatment 2) (table 8). In 2016, pod and seed yield from flowers of plants visited by insects (treatment 3) was greater than that of flowers protected from insect visits (treatment 4). The comparison of the percentages of normal seeds shows that the differences were highly significant between treatments 1 and 2 (χ^2 = 239.58; df = 1; $P<0.001$), then treatments 3 and 4 (χ^2 = 389.15; df = 1; $P<0.001$). The fruiting rate due to insect activity was 17.38% in 2015 and 47.45% in 2016. For the two years of study, the fruiting rate attributed to insects was 32.41%. The number of seeds per pod attributed to the activity of insects was 22.84% in 2015 and 20.26% in 2016. For the two experimental periods, the proportion of seeds per pod due to insects was 21.55%. The percentage of normal seeds due to insects was 24.03% in 2015 and 29.39% in 2016. For the two years of study, the percentage of normal seeds per pod attributed to insect activity was 26.71%.

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Table 8: Yield components of different treatments as influenced by protection of ***Vigna subterranea*** flowers from insects in 2015 and 2016 at Dang

Year	Treatments	NF	NFP	FrR (%)	Seeds/pod		TNS	NS	%NS
					m	*sd*			
2015	1(Unprotected flowers)	2720	1878	64.91	1.62	0.49	1798	1617	89.93
	2 (Bagged flowers)	2893	1459	53.63	1.25	0.43	1677	1154	68.82
2016	3(Unprotected flowers)	2565	1710	66.67	1.53	0.51	2236	1974	88.29
	4 (Bagged flowers)	2290	802	35.03	1.22	0.41	1992	1242	62.35

NF: Number of flowers; NFP: Number of formed pod; FrR: Fruiting rate; TNS: Total number of seeds; NS: Normal seeds; %NS: Percentage of normal seeds; *m*: mean; *sd*: standard deviation

Comparison of fruiting rates: $\chi^2_{Global} = 936.43$; $df = 3$; $P<0.001$, Fl_{PN2015}/Fi_{PN2015}: $\chi^2 = 201.45$; $df = 1$; $P<0.001$, Fl_{PN2016} / Fi_{PN2016}: $\chi^2 = 485.21$; $df = 1$; $P<0.001$, Fl_{PN2015} / Fi_{PN2016}: $\chi^2 = 3.42$; $df = 1$; $P>0.05$, **Comparison of mean number of seeds per pod:** $F = 624.96$; $df_1 = 3$; $df_2 = 881$; $P<0.001$, Fl_{PN2015}/Fi_{PN2015}: $t = 694.68$; $df = 3473$; $P<0.001$, Fl_{PN2016} / Fi_{PN2016}: $t = 701.29$; $ddl = 4226$; $P<0.001$, Fl_{PN2015} / Fi_{PN2016}: $t = 178.92$; $df = 4032$; $P<0.001$, **Comparison of percentage of normal seeds:** $\chi^2_{Global} = 890.75$; $df = 3$; $P<0.001$, Fl_{PN2015}/Fi_{PN2015}: $\chi^2 = 239.58$; $df = 1$; $P<0.001$, Fl_{PN2016} / Fi_{PN2016}: $\chi^2 = 389.15$; $df = 1$; $P<0.001$, Fl_{PN2015} / Fi_{PN2016}: $\chi^2 = 2.78$; $df = 1$; $P>0.05$.

5.12 Impact of mycorrhiza on pod and seed yields of *Vigna subterranea:* The comparison of the mean number of seeds per pod (table 9) showed that the differences were highly significant between treatments 5 and 2 ($t = 381.21$; $df = 3587$; $P<0.001$), and between treatments 6 and 4 ($t = 906.56$; $df = 4226$; $P<0.001$). Pod and seed yield from flowers of plants protected and inoculated with mycorrhiza (treatment 5 in 2015; treatment 6 in 2016) was higher than that from flowers of plants protected and uninoculated (treatment 2 in 2015; treatment 4 in 2016). The comparison of the percentages of normal seeds shows that the differences were highly significant between treatments 5 and 2 ($\chi^2 = 14.99$; $df = 1$; $P<0.001$), and treatments 6 and 4 ($\chi^2 = 127.76$; $df = 1$; $P<0.001$). The percentage of normal seeds from flowers of plants inoculated with mycorrhiza (treatment 5) was higher than those protected and uninoculated (treatment 2). The fruiting rate due to mycorrhiza was 17.5% in 2015 and 36.33% in 2016. For both years of study, the fruiting rate attributed to mycorrhiza was 26.91%. The number of seeds per pod attributed to mycorrhiza was 13.79% in 2015 and 22.78% in 2016. For both years of study, the proportion of seeds per pod due by mycorrhiza was 18.28%. The percentage of normal seeds due to mycorrhiza was 7.79% in 2015 and 19.35% in 2016, whereas, the number of normal seeds per pod attributed to mycorrhiza was 13.57%.

Table 9: Yield components of different treatments of ***Vigna subterranea*** as influenced by mycorrhiza in 2015 and 2016 at Dang

Year	Treatments	NF	NFP	FrR (%)	Seeds/pod		TNS	NS	%NS
					m	*sd*			
2015	5 (Bagged flowers inoculates by Mycorrhiza)	2763	1796	65	1.45	0.5	1912	1427	74.63
	2 (Bagged flowers)	2893	1459	53.63	1.25	0.43	1677	1154	68.82
2016	6 (Bagged flowers inoculates by Mycorrhiza)	3325	1829	55.01	1.58	0.5	2843	2198	77.31
	4 (Bagged flowers)	2290	802	35.03	1.22	0.41	1992	1242	62.35

NF: Number of flowers; NFP: Number of formed pod; FrR: Fruiting rate; TNS: Total number of seeds; NS: Normal seeds; %NS: Percentage of normal seeds; *m*: mean; *sd*: standard deviation

Comparison of fruiting rate: $\chi^2_{Global} = 456.11$; $df = 3$; $P<0.001$, Fi_{PM2015}/Fi_{PN2015}: $\chi^2 = 122.80$; $df = 1$; $P<0.001$, Fi_{PM2016}/Fi_{PN2016}: $\chi^2 = 217.52$; $df = 1$; $P<0.001$, **Comparison of mean number of seeds per pod:** $F = 429.25$; $df_1 = 3$; $ddl_2 = 767$; $P<0.001$, Fi_{PM2015}/Fi_{PN2015}: $t = 381.21$; $df = 3587$; $P<0.001$, Fi_{PM2016}/Fi_{PN2016}: $t = 906.56$; $df = 4833$; $P<0.001$, **Comparison of percentage of normal seeds:** $\chi^2_{Global} = 96.54$; $df = 3$; $P<0.001$, Fi_{PM2015}/Fi_{PN2015}: $\chi^2 = 14.99$; $df = 1$; $P<0.001$, Fi_{PM2016}/Fi_{PN2016}: $\chi^2 = 127.76$; $df = 1$; $P<0.001$

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5.13 Impact of fertilizer-NPK on pod and seed yields of *Vigna subterranea:* The comparison of the mean number of seeds per pod (table 10) showed that the differences were highly significant between treatments 7 and 2 (t = 155.68; df = 3521; P<0.001) as well as treatments 8 and 4 (t = 499.09; df = 4435; P<0.001). Pod and seed yields from flowers of plants protected and applied with fertilizer-NPK (treatment 7 in 2015; treatment 8 in 2016) was higher than that from flowers of plants protected and uninoculated (treatment 2 in 2015; treatment 4 in 2016). The comparison of the percentages of normal seeds shows that the differences were highly significant between treatments 7 and 2 (χ^2 = 27.74; df = 1; P<0.001), and treatments 8 and 4 (χ^2 = 69.74; df = 1; P<0.001). The percentage of normal seeds from flowers of plants protected and applied with fertilizer-NPK (treatment 7) was higher than those protected and uninoculated (treatment 2). The fruiting rate due to fertilizer-NPK was 7.67% in 2015 and 24.94% in 2016. For both years of study, the fruiting rate attributed to fertilizer-NPK was 16.30%. The number of seeds per pod attributed to fertilizer-NPK was 6.01% in 2015 and 14.69% in 2016. For both years of study, the proportion of seeds per pod due to fertilizer-NPK was 10.35%. The percentage of normal seeds due to fertilizer-NPK was 10.27% in 2015 and 15.77% in 2016. For the two years of study, the percentage of normal seeds per pod attributed to fertilizer-NPK was 13.02%.

Table 10: Yield components of different treatments of ***Vigna subterranea*** as influenced by fertilizer-NPK in 2015 and 2016 at Dang

Year	Treatments	NF	NFP	FrR (%)	Seeds/pod		TNS	NS	%NS
					m	*sd*			
2015	7 (Bagged flowers inoculates by chemical fertilizer)	2928	1701	58.09	1.33	0.47	1846	1416	76.70
	2 (Bagged flowers)	2893	1459	53.63	1.25	0.43	1677	1154	68.82
2016	8 (Bagged flowers inoculates by chemical fertilizer)	2766	1291	46.67	1.43	0.5	2445	1810	74.03

NF: Number of flowers; NFP: Number of formed pod; FrR: Fruiting rate; TNS: Total number of seeds; NS: Normal seeds; %NS: Percentage of normal seeds; *m*: mean; *sd*: standard deviation
Comparison of fruiting rate: χ^2_{Global} = 279.45; df = 3; P<0.001, Fi_{PE2015}/Fi_{PN2015}: χ^2 = 34.43; df = 1; P<0.001, Fi_{PE2016}/Fi_{PN2016}: χ^2 = 70.11; ddl = 1; P<0.001, **Comparison of mean number of seeds per pod:** F = 216.77; df_1 = 3; ddl_2 = 685; P<0.001, Fi_{PE2015}/Fi_{PN2015} : t = 155.68; df = 3521; P<0.001, Fi_{PE2016}/Fi_{PN2016}: t = 499.09; df = 4435; P<0.001, **Comparison of percentage of normal seeds:** χ^2_{Global} = 101.35; df = 3; P<0.001, Fi_{PE2015}/Fi_{PN2015}: χ^2 = 27.74; df = 1; P<0.001, Fi_{PE2016}/Fi_{PN2016}: χ^2 = 69.74; df = 1; P<0.001

5.14 Cumulative impact of insect pollinators and mycorrhiza on the pollination, pod and seed yields of *Vigna subterranean:* In 2015, the fruiting rate of flowers from plants inoculated and opened to insects (treatment 9) was higher than that of protected flowers (treatment 2) (Table 11). In 2016, the fruiting rate of flowers opened to insects from inoculated plants (treatment 10) was higher than that from flowers of plants protected from insects visits (treatment 4). The number of seeds per pod from flowers of inoculated plants and opened to insects (treatment 9 in 2015; treatment 10 in 2016) was significantly higher (t = 1188.89; df = 3977; P<0.001 in 2015, t = 1320.92; df = 5100; P<0.001 in 2016) than that of flowers protected from insects visits (treatment 2 in 2015; treatment 4 in 2016). The percentage of normal seeds from flowers opened to insects on inoculated plants (treatment 9 in 2015; treatment 10 in 2016) was higher than that of flowers protected from insect visits (treatment 2 in 2015; treatment 4 in 2016). The fruiting rate due to cumulative effects of flowering insects and mycorrhiza activity was 26.26% in 2015, 62.46% in 2016 and 44.36% for the two years of study. The cumulative effect of

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flowering insects and mycorrhiza on the number of seeds per pod was 29.38% in 2015, 28.23% in 2016 and 28.80% for the two years of study. The percentage of normal seeds due to cumulative effects of flowering insects and mycorrhiza activity was 26.32% in 2015, 33.74% in 2016 and 30.03% for the two years of study.

Table 11: Yield components of different treatments of ***Vigna subterranea*** as influenced by mycorrhiza and insect pollinators in 2015 and 2016 at Dang

Year	Treatments	NF	NFP	FrR (%)	Seeds/pod		NS	NS	%NS
					m	*sd*			
2015	9 (Inoculated flowers open to insects)	3036	2208	72.73	1.77	0.42	2302	2150	93.40
	2 (Bagged flowers)	2893	1459	53.63	1.25	0.43	1677	1154	68.82
2016	10 (Inoculated flowers open to insects)	3526	3291	93.33	1.70	0.46	3110	2927	94.11
	4 (Bagged flowers)	2290	802	35.03	1.22	0.41	1992	1242	62.35

NF: Number of flowers; NFP: Number of formed pod; FrR: Fruiting rate; TNS: Total number of seeds; NS: Normal seeds; %NS: Percentage of normal seeds; *m*: mean; *sd*: standard deviation
Comparison of fruiting rate: $\chi^2_{Global} = 1360.41$; $df = 3$; $P<0.001$, Fl_{PM}/Fl_{PN}: $\chi^2 = 312.07$; $df = 1$ $P<0.001$, Fl_{PM}/Fl_{PN}: $\chi^2 = 2264.39$; $df = 1$ $P<0.001$, **Comparison of mean number of seeds per pod:** $F = 318.67$; $df_1 = 3$; $df_2 = 831$; $P<0.001$, Fl_{PM}/Fl_{PN}: $t = 1188.89$; $df = 3977$; $P<0.001$, Fl_{PM}/Fl_{PN}: $t = 1320.92$; $df = 5100$; $P<0.001$, **Comparison of percentage of normal seeds:** $\chi^2_{Global} = 947.61$; $df = 3$; $P<0.001$, Fl_{PM}/Fl_{PN}: $\chi^2 = 416.26$; $df = 1$; $P<0.001$, Fl_{PM}/Fl_{PN}: $\chi^2 = 820$; $df = 1$; $P<0.001$

6 DISCUSSION

In the Guinea-savannah zone of Cameroon, the number of nodules formed by *V.subterranea* was reported to be low in the absence of inoculum (Ngakou *et al.*, 2012), with nodules starting to degenerate as from 60 days after planting. Successful nodulation of leguminous crops by mycorrhiza largely depends on the presence of a specific and beneficial strain in the soil for a particular legume (Mugabo *et al.*, 2014). There was a significant correlation between nodulation and plant biomass, supporting the improved nitrogen fixation potential of the host crop legume that usually leads to increase soil fertility (Odoh *et al.*, 2017). At Dang, during the first cropping season, the main anthophilous insects visiting *V. subterranea* were of the order of Lepidoptera, the family of Pieridae being the preponderant, whereas with the decreased neighbouring flora during the second year, the order of Hymenoptera was the most represented with the family of Halictidae ranking first. These results are similar to those recently obtained Kingha (2014), who found the order of Hymenoptera as the most abundant on *Arachis hypogaea* at Dang. However, the diversity and abundance of the flower-visiting entomofauna have been reported to vary with plant species (Tchuenguem, 2005, Roubik, 2000). Subplots inoculated with mycorrhiza attracted more insects than uninoculated subplot. This finding could be explained by higher number of flowers on subplot inoculated with mycorrhiza. The peak activity of insects on *V. subterranea* flowers was between 01.00 p.m. and 02.00 p.m., which corresponds probably to the period of higher availability of nectar and/or pollen on *V. subterranea* flowers. The working visit time of insects on flowers was reported to depend on the availability of pollen (Stone *et al.*, 1998), or nectar (Pierre *et al.*, 1996; Suso *et al.*, 2001; Pouvreau, 2004). The weak abundance of insects per 1000 flowers highlights the low attractivity of the nectar and/or the pollen of the corresponding plants to various insects. As far as *A. mellifera* on *V. subterranean* is concerned, it could be related to more interesting and easily accessible food source for this honeybee in the environment of *V. subterranea* at flowering period. Similar results were reported on *Arachis hypogaea* at Nkolbisson

(Tchuenguem, 1993). The significant difference observed between the duration of visits in 2015 and 2016 could be attributed to the availability of nectar or the variation in the diversity of insect visitors from one year to another. During our investigations, the disruption of visits by *Paragus borbonicus* reduced the duration of *A. mellifera* visits. This finding confirms other reported results on *A. hypogaea* (Kingha, 2014) and *Cajanus cajan* (Mazi *et al.*, 2014) at Ngaoundere. The diversity and richness of the neighbouring flora to *V. subterranea* could explain the weak visits frequency of several insect species on flowers. The positive and significant contribution of insects in fruiting rate, number of seed per pod and percentage of normal *V. subterranea* seeds could be justified by the action of pollinators. This lines with other results reported at Ngaoundere on *Cajanus cajan* (Mazi *et al.*, 2014) and *Arachis hypogaea* (Tchuenguem *et al.*, 2014), who revealed that self-pollination of these plants produced little seeds per pod in the absence of efficient pollinators. The high yield in pod and seed from treatments visited by insects compared to treatment bagged indicate that insects were effective in increasing cross-pollination or self-pollination. In our experiment, the uses of both flowering insects and mycorrhiza highly improved the seed and pod yields of *V. subterranea*. Flowering insects contributed to facilitate the liberation of pollen from anthers for optimal occupation of stigma, thus increasing pollination (Kengni *et al.*, 2015). Mycorrhiza was reported to improve nutrient uptake of the host legume that led to increased plant growth and yield (Ngakou *et al.*, 2007a). These results are in agreement with those obtained by Kengni *et al.* (2015), who pointed out the use of both insect pollinators and *Rhizobium* to highly improved seed and pod yields of *Glycine max* at Ngaoundere. In fact, involving pollinating insects and mycorrhiza for the management of plant growth may provide high yield returns to *V.subterranea* investment.

7 CONCLUSION

From our study, *V. subterranea* is a plant species that highly benefits from insect pollinators, among which *Eurema eximia* and *Halictus* sp. are the most important. The comparison of pod and seed sets of unprotected flowers with those of flowers visited by insects underscores the value of these insects in increasing pod, seed yields and quality. Furthermore, the comparison of pod and seeds set of uninoculated and bagged flowers with those of plants inoculated with mycorrhiza and visited by insects indicates the value of cumulative activity of insects and mycorrhiza in increasing pod and seeds yields. It is suggested that sowing Bambara groundnut seeds with mycorrhiza and preserving anthophilous insects close to plants at bloom could be encouraged to significantly improve the production of this underestimated important crop legume.

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9 REFERENCES

Amarteifio JO, Tibe O. & Njogu RM: 2006. The mineral composition of Bambara groundnut (***Vigna subterranea*** (L.) Verdc) grown in Southern Africa. *Afr. J. Biotechn.*, 5(23), 2408-2411.

Amougou JA, Abossolo SA. & Tchindjang M: 2015. Variability of precipitations at Koundja and Ngaoundere based on temperature changes of Atlantic Ocean and El NINO. IvoryCoast *Revi. Sci.Technol.*, 25: 110-124.

Anchirinah VM, Yiridoe EK. & Bennett-lartey JO: 2001. Enhancing sustainable production and genetic resource conservation of Bambara groundnut: A survey of indigenous agricultural knowledge systems. *Outlook Agric.*, 30(4), 281-288.

Arshad M. & Frankenberger JWT: 1993. Microbial production of plant growth regulators. In: Blaine F, Metting J. (eds.). Soil microbial ecology. Marcel and Kekker Inc, New York, USA, pp. 307-347.

Azam-Ali SN, Sesay A, Karikari SK, Massawe FJ, Anguilar-Manjarrez J, Bannayan M. & Hampson KJ: 2001. Assessing the potential of underutilized crop-A case study using Bambara groundnut. *Exp. Agric.*, 37, 433-472.

Bamshaiye OM, Adegbol JA. & Bamishaiye EI: 2011. Bambara groundnut: an under-utilized nut in Africa. *Adv. Agric. Biotech.*, 1, 60-72.

Berchie JN, Sarkodie AJ, Adu-Dapaah H, Agyemang A, Addy S, Asare E, & Donkor J: 2010. Yield evaluation of three early maturing Bambara groundnut (***Vigna subterranea*** L. Verdc) landraces at CSIR-Crops Research Institute-Kumasi, Ghana. *J. Agron.*, 9, 175-179.

Denison RF. & Kiers ET: 2004. Lifestyle alternatives for rhizobia: mutualism, parasitism and forgoing symbiosis. *FEMS Microbiol. Letter*, 237, 187-193.

Ibrahin HD. & Ogunwusi AA: 2016. Industrial Potentials of Bambara Nut. *J. Poverty Invest. Dev.*, 22, 12-18.

Karikari SK: 1971. Economic importance of Bambara groundnut. *World Crops*, 23(4), 195-196.

Kengni BS, Tchuenguem FFN. & Ngakou A: 2015. Pollination and yield attributes of (cowpea) *Vigna unguiculata* L. Walp. (Fabaceae) as influenced by the foraging activity of *Xylocopa olivacea* Fabricius (Hymenoptera: Apidae) and inoculation with *Rhizobium* in Ngaoundere, Cameroon. *Int. J. Agron. Agric. Res.*, 6(2), 62-76.

Kingha TBM: 2014. Foraging and pollination activity of insects on *Arachis hypogaea* and *Phaseolus vulgaris* (Fabaceae) flowers at Dang (Ngaoundere, Cameroon). Ph.D. thesis, Department of Biological Sciences, Faculty of Science, University of Ngaoundere, 139 p.

Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunnigham SA, Kremen C. & Tscharntke T: 2007. Importance of pollinators in changing landscapes for world crops. *Proc. Royal Soc. B.,* 274(2), 303-313.

Linnemann AR, Azam-Ali SN: 1993. Bambara groundnut (*Vigna subterranea*). In: Williams JT. (ed.). Pulses and vegetables. Chapman and Hall, London, pp. 13-57.

Ntundu WH: 1997. Tanzania country report ***Vigna subterranea*** (L.) Verdc. In: Heller J, Begemun F. (eds.). Promoting the conservation and use of underutilized and neglected crop of the workshop on conservation and improvement of Bambara groundnut, Zimbabwe, p. 53-58.

Margni M, Rossier D, Crettag P. & Jolliet O: 2002. Life cycle impact assessment of pesticides on human health and ecosystem. *Agric. Ecosyst. Envi.,* 93(3), 379-392.

Mazi S, Tchuenguem FFN. & Brückner D: 2014. Foraging and pollination behaviour of *Chalicodoma rufipes* L. (Hymenoptera: Megachilidae) on *Cajanus cajan* L. Mill sp. (Fabaceae) flowers at Dang (Ngaoundere, Cameroon). *Int. J. Agron. Agric. Res.*, 4(4), 77-88.

MINADER, 2012. Annual statistics for the Agricultural sector, 2009-2010 cropping campaigns. Direction of Agricultural and Statistical investigations. AGRI-STAT, 17: 123p.

Mugabo JP, Balkrishna SB, Anil Kumar HE, Byiringiro E. Yumnam NS: 2014.

Contribution of Arbuscular Mycorrhizal Fungi (AM Fungi) and *Rhizobium* inoculation on crop growth and chemical properties of rhizospheric soils in high plants. *IOSR J. Agric. Vet. Sci.*, (IOSR-JAVS), 7(9), 45-55.

Mukurumbira LM: 1985. Effects of the rate of fertilizer nitrogen and previous grain legume crop on maize yields. *Zim Agric. J.*, 82(6), 177-179.

Ndiang Z, Bell JM, Missoup AD, Fokam PE. & Amougou A: 2012. Study of the morphological variability of some voandzeia varieties in Cameroon. *J. Appl. Biosci.*, 60: 4410-4420.

Ndiang Z, Bell JM, Fokam PE, Ouattara B, Simo C. & Dibong DS: 2014. Agro-morphological variability in twelve Bambara groundnut (***Vigna subterranea*** (L.) Verdc.) accessions in Cameroon. Sci.Technol. Dev., 16, 38-45.

Nepide NC. & Tchuenguem FFN: 2016. Pollination efficiency of *Apis mellifera adansonii* Latreille (Hymenoptera: Apidae) on *Croton macrostachyus* (Euphorbiaceae) flowers at Dang, Ngaoundéré, Cameroon. *Int. J. Biosci.*, 9 (3), 75-88.

Ngakou A, Nwaga D, Nebane CLN, Ntonifor NN, Tamò M. &. Parh IA: 2007a. Arbuscular-mycorrhizal fungi, rhizobia and *Metarhizium anisopliae* enhance P, N, and Mg, K, and Ca accumulations in fields grown cowpea. *J. Plant Sci.*, 2(5), 518-529.

Ngakou A, Nwaga D, Ntonifor NN, Tamò M, Nebane CLN. & Parh IA: 2007b. Contribution of Arbuscular Mycorrhizal Fungi (AMF), rhizobia and *Metarhizium anisopliae* to cowpea production in Cameroon. *Int. J. Agric. Res.*, 2(9), 754-764.

Ngakou A, Ngo Nkot L, Gomoung D, & Adamou S: 2012. Mycorrhiza-*Rhizobium Vigna subterranean* dual symbiosis: impact of microbial symbionts for growth and sustainable yield improvement. *Int. J. Agric. Biol.*, 14 (6), 915-921.

Odoh NC, Idibia PO. & N'cho OC: 2017. Varietal response of Bambara groundnut (*Vigna subterranea*) to mulching and AMF inoculation in Central Nigeria. *J. Adv. Agric.*, 7(2), 1030-1035.

Pierre J, Le Guen J, Pham Delegue MH, Mesquida J, Marilleau R. & Morin J: 1996. Comparative study of nectar secretion and attractivity to bees of two lines of spring-type faba bean (*Vicia faba* L. var. equina Steudel). *Apidologie*, 27, 65-75.

Pouvreau A: 2004. Les insectes pollinisateurs. Delachaux and Niestle (eds.), Paris, France. 189 p.

Roubik DW: 2000. Pollination system stability in Tropical America. *Conserv. Biol.*, 14(5), 1235-1236.

Sabbahi R, De Oliveira D. & Marceau J: 2005. Influence of honeybee (Hymenoptera: Apidae) density on the production of canola (Crucifera: Brassicaceae). *J. Econ. Entomol.*, 98 (2), 367-372.

Suso MJ, Pierre J, Moreno MT, Esnault R. & Le Guen J: 2001. Variation in outcrossing levels in faba bean cultivars: role of ecological factors. *J. Agric. Sci.*, 136(4), 399-405.

Stone GN, Willmer P. & Rowe JA: 1998. Partitioning of pollinators during flowering in an African *Acacia* community. *Ecology*, 79, 2808-282.

Tchuenguem FFN: 1993. Activity of anthophilous insects and their impact on yields of two cultivated plants in Cameroon: *Zea mays* Linnaeus (Gramineae) and *Arachis hypogaea* Linnaeus (Papilionaceae). 3rd Cycle Doctorate thesis, University of Yaounde I, 133p.

Tchuenguem FFN, Messi J. & Pauly A: 2001. Activité de *Meliponula erythra* sur les fleurs de *Dacryodes edulis* et son impact sur la fructification. *Fruits,* 56, 179-188.

Tchuenguem FFN, Messi J, Brückner D, Bouba G, Mbofung CM. & Hentchoya HJ: 2004. Foraging and pollination behavior of the African honeybee (*Apis mellifera adansonii*) on *Callistemon rigidus* flowers at Ngaoundéré (Cameroon). *J. Cam. Acad.Sci.*, 4, 133-140.

Tchuenguem FFN: 2005. Foraging and pollination activity of *Apis mellifera adansonii* Latreille (Hymenoptera: Apidae, Apinae) on flowers of three plants at Ngaoundere (Cameroon): *Callistemon rigidus* (Myrtaceae), *Sygygium guineense* var. *macrocarpum* (Myrtaceae) and *Voacanga africana* (Apocynaceae). State Doctorate thesis, University of Yaounde I, 103p.

Tchuenguem FFN, Djonwangwe D, Messi J. & Brückner D: 2009. Foraging and pollination behavior of *Apis mellifera adansonii* Latreille (Hymenoptera: Apidae) on *Helianthus annuus* flowers (Asteraceae) at Ngaoundéré (Cameroon). *Cam. J. Exp. Biol.*, 5 (1), 1-9.

Tchuenguem FFN, Kingha TBM. & Brückner D: 2014. Diversity of insects and its impact on fruit and seed yields of *Arachis hypogaea* L. (Fabaceae) at Dang (Ngaoundere-Cameroon). *Int. J. Biol. Chem. Sci.*, 8 (3), 983-997.