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Insect floral visitors of pigeon pea (*Cajanus cajan* L. Millsp.) and impact of the foraging activities on the pollination, pod and seed yields at Maroua - Cameroon

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Abstract

To evaluate the abundance, diversity and the impact of the insect on the pod and seed yields of pigeon pea, in this study, its foraging and pollinating activities were examined in Maroua. Observations were made on 100 inflorescences per treatment. The treatments included unlimited floral access to all visitors and bagging of flowers to prevent access to all visitors. In addition, information on all floral visitors was recorded. Out of the 2206 individual insects (16 species) collected 64.91% were bees whereas 35.09% were non-bee species including butterflies (34.00%) and flies (01.09%). Their activity was highest in the morning hours (11:00 a.m.–12:00 a.m.). The foraging resources of flower visitors collected as well as their activities on the pigeon pea flowers suggest pollen movement which could lead to cross pollination. Insects were effective pollinator, and of course their visits increased fruiting rate, seeds/pod, normal seed and weight seed. Insect foraging resulted in a significant increment of the fruiting rate by 71.64 %, as well as the number of seeds/pod by 09.11 %, the percentage of normal seeds by 24.00 % and the percentage of weight seed by 23.40 % in Maroua. Then, the foraging activity of insects mainly bees on *C. cajan* appears as the limiting factor in the production of this crop. Therefore, the installation and the conservation of the *M. bituberculata* nests close to *C. cajan* field are recommended to maintain and improve yields in the region.

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Introduction

Pollinators play an important role in sustainability and continuity of the ecosystem and agriculture (Klein *et al.*, 2007). Among the pollinators, about 80 % of the commercial crops are pollinated by the insects (Free, 1993). The main group of these insect are the bees, wasps, butterflies, moths, flies and beetles (Greenleaf and Kremen, 2006). It is an ecosystem service in that wild pollinators, in particular wild bees, contribute significantly to the pollination of a large array of crops (Winfree *et al.,* 2008).

Pigeon pea is one of the major grain legume crops grown in tropics and subtropics (Saxana *et al.,* 2002). It contains more minerals, ten times more fat, five times more vitamin A and three times more vitamin C than ordinary peas (Madeley, 1995). As human food, pigeon pea seeds can be used in almost any imaginative form. The green pods and seeds are the most utilized form in Africa though dry seeds are increasingly gaining popularity (Damaris, 2007). There are currently major efforts to promote the introduction of dehulling methods used in India in order to increase diversity of pigeon pea use in Africa (Amaefule *et al.*, 2006). In many parts of Eastern Africa, dhal is becoming a popular meal. Some potential uses of pigeon pea for human consumption in Africa include the production of noodle (Singh *et al.,* 1989), tempe (Mugula and Lyimo, 2000) an other fermented products (Onofiok *et al.,* 1996). Elsewhere, pigeon pea is used as a flour additive to other foods in soups and with rice (Centre for New Crops and Plants Products, 2002). Pigeon pea flour is an excellent component in the snack industry and has been recommended as an ingredient to increase the nutritional value of pasta without affecting its sensory properties (Torres *et al.,* 2007). Millet /pigeon pea biscuits are reportedly highly nutritious and provide a cheaper alternative to wheat imports in Nigeria (Eneche, 1999). Pigeon pea leaves have been used to treat malaria (Aiyeloja and Bello, 2006) in Nigeria, while in Southern Africa, pigeon pea is currently one of the indigenous crops being promoted for potential medicinal use (Mander *et al.,* 1996).

In Cameroon, the cultivation of pigeon pea is mainly faced with the challenge of pests and diseases and for that matter many pigeon pea farmers spray their farms in order to obtain good yield (Kumar, 1991; Niyonkuru, 2002). Ignorantly, such pest management practices result in poisoning and killing of beneficial insects including pollinators (Pando *et al.,* 2011). However, understanding the timeline of activities of flower visitors of pigeon pea and the role they may play in productivity of the crop or other economically important crops could inform farmers and researchers on proper management practices to conserve most of the beneficial insects. This current work was aimed at assessing the activity period, diversity and abundance of the main insect visitors of pigeon pea flowers. The information gained on the interaction of pigeon pea flowers and insect floral visitors will enable farmers to develop management plans that will increase the overall quality and quantity of pigeon pea yield.

Material and methods

Site and biological materials

The study was carried out in Teving (Latitude 10° 593'17''N, Longitude 14°204'39'' E and altitude 439 m), a Western suburb of Maroua in the Far North Region of Cameroon, from June 2015 to February 2016. This Region belongs to the ecological zone with three phytogeographical areas (Sahel-Sudanian, Sahelian and Sudanian altitude) periodically flooded, with unimodal rainfall (Letouzey, 1985). It has a Sahel-Sudanian climate type, characterized by two annual seasons: a long dry season (November to May) and a short rainy season (June to October); August is the wettest month of the year (Kuete *et al.*, 1993). Annual rainfall varies from 400 to 1100 mm (Kuete *et al.,* 1993). The annual average temperature varies between 29 and 38° C and a daily temperature range between 6 and 7 °C (Kuete *et al.*, 1993). The experimental plot is an area of 588 m². The animal material was represented by insects naturally present in the environment. Vegetation was represented by wild species and cultivated plants. The plant material was represented by the seeds of *Cajanus cajan* provided IRAD.

Planting and maintenance of culture

On July 2nd 2015, the experimental plots (that have been previously plowed) was divided into six sub - plots of 10 × 5 m^2 each, with a row of two meters between the left and subplots. This field received seedlings of 6 lines per sub-plot. The seeds were sown in holes at the rate of 2 grains per hole. The spacing was 1 m between rows and 1 m on rows; a hole was 4 cm depth (Niyonkuru, 2002). Four weeks after germination (occurred August 4, 2015), the plants were thinned leaving the stronger. Thinning of the opening of the first flower, which occurred December first 2015, weeding was done with a hoe every three weeks. Weeding was performed manually as necessary to maintain weed-free plots.

Estimation of the frequency of insectsin the flowers of Cajanus cajan

On 29th November 2015, 100 inflorescences of *C. cajan* at the bud stage were labelled, among which 50 inflorescences (1569 flowers) were left unattended (Fig. 1) and 50 inflorescences (1638 flowers) bagged to prevent visitors (Fig. 2). The frequency of insectsin the flowers of *C. cajan* was determined based on observations on flowers of treatment1, three days per week, from 8th December 2015 to 9th January2016, at 7:00–8:00 hours,9:00–10:00 hours, 11:00–12:00 hours, 13:00–14:00 hours, 15:00–16:00 hours and 17:00 – 18:00 hours. Flowers typically were completely opened at 7:00 and closed before 18:00 hours.

In a slow walk along all labelled inflorescences of treatment 1, the identity of all insects that visited *C. cajan* was recorded. For 6–10 min observations, 10 inflorescences were observed before moving to a different treatment. Specimens of all insect taxa were caught with an insect net on unlabelled inflorescences; for each species of insect, 3–5 specimens were captured. These specimens were conserved in 70% ethanol for subsequent taxonomy determination, besides butterflies which were fixed with needle. The insect species were identified in the laboratory of Zoology of the University of Maroua, the use of the identification key of Delvare and Aberlenc (1989), Eardley *et al.* (2010) and Pauly (2014) which are adapted to the insects of the tropical zone. All insects encountered on flowers were registered and the cumulated results expressed in number of visits to determine the relative frequency of each insectin the anthophilous entomofauna of *C. Cajan* (Pando *et al.*, 2011).

Foraging activities and resources of the insects on Cajanus cajan flowers

Daily observations were made between 7:00 am and 6:00 pm on flowers of *C. cajan* for foragers, the resources collected (nectar foragers were seen introducing the head between the stigma or the anther and the corolla, while pollen gatherers directly scratched the anthers with the mandibles or the legs), their abundance, the foraging behaviour and the disruption of the activity of foragers by competitors. All the insects that visited the *C. cajan* flowers were collected using insects weep net. Collected flower visitors were sorted out into their various species and their bodies examined for the presence of pollen.

Evaluation of the effect of insects on Cajanus cajan yields

This evaluation was based on the impact of insects visiting flowers on pollination, the impact of pollination on fructification of *C. cajan* and the comparison of yields (fruiting rate, mean number of seeds per pod and percentage of normal seeds) of treatment X (unprotected inflorescences) and treatment Y (protected inflorescences). The fruiting rate due to the influence of foraging insects (*Fri*) was calculated by the formula: $Fr_i = \{[(Fr_X - Fr_Y) / Fr_X] \times 100\}$, where Fr_X and Fr_Y were the fruiting rate in treatment X and treatment Y. The fruiting rate of a treatment (Fr) is: $Fr = [(F_2 / F_1) \times 100]$, where F_2 is the number of pods formed and F_1 the number of viable flowers initially set. At maturity, pods were harvested from each lot and the number of seeds per pod counted. The mean number of seeds per pod and the percentage of normal seeds (well-developed seeds) were then calculated for each treatment. The impact of flowering insects on seed yields was evaluated using the same method as mentioned above for fruiting rate (Pando *et al.,* 2011).

Data analysis

Data were analysed using descriptive statistics; Student's t test was used for the comparison of means of the two samples paired and Chi-Square (x^2) test was used for the comparison of two percentages using SPSS statistical software (version 19.0; SPSS, Inc., Chicago, Illinois, USA) and Microsoft Excel 2010.

Results

Frequency and diversity of insect flower visitors of Cajanus cajan

A total of 2206 individuals constituting 16 insect species were collected on *C. cajan* flowers over the period (Table 1). Bees belonging to two families (Megachilidae and Apidae) and made up of 1432 individuals (64.91 %) were the major floral visitors collected (Fig. 3). The non-bee insect flower visitors include butterflies (34.00 %) and flies (01.09 %) were also sampled. In terms of numbers collected, butterflies and flies were significantly different ($P \leq 0.01$) (Fig. 4). Bees were the most abundant insects visiting pigeon pea flowers (Fig. 5). The bee fauna comprised of 6 species with *Megachilebi tuberculata*, being the most abundant with 778 individuals (35.26 %), followed *by Chalicodoma cincta cincta* (Fabricius) with 270 individuals (12.9 %) (Fig. 5 and Table 1).

Table 1. The diversity, foraging resource and frequency of insects' visitors on *Cajanus cajan* flowers.

Order	Family	Species	Abundance	n (n-1)	*P* (%)
Hymenoptera	Megachilidae	*Megachilebi tuberculata*N, P	778	604506	35.26
		*Chalicodoma cincta cincta*N, P	270	72630	12.13
	Apidae	*Apis mellifera adansonii*N, P	138	18906	06.25
		*Xylocopa olivacea*N, P	56	3080	02.53
		*Xylocopa torrida*N, P	56	3080	02.53
		Xylocopa sp. N, P	134	17822	06.07
Total Hymenoptera	2	6	1432	720024	64.91
Diptera	Calliphoridae	*Calliphora* sp. N	10	90	00.45
	Muscidae	*Muscus domestica*N	8	56	00.36
	Tephritidae	*Dacus bivitattus*N	6	30	00.27
Total Diptera	3	3	24	176	01.09
Lepidoptera	Pieridae	*Vanessa cardui*N	80	159	03.62
		*Catopsilia florella*N	68	4556	03.08
	Nymphalidae	*Hemiargus hanno*N	498	247506	22.57
		*Danaus chrysipus*N	6	30	00.27
		Nymphalidae sp. N	30	870	01.35
	Acraeidae	*Acraea acerata*N	62	3782	02.81
	Satyridae	*Lasiommata maera*N	6	30	00.27
Total Lepidoptera	5	7	750	256933	34.00
Total	10	16	2206	977133	100

N : nectar, P : pollen, *P (%)* : percentage of visit [$P = (n/2206) \times 100$].

The different families in the order of their importance were Megachilidae (47.51%), Nymphalidae (24.21%), Apidae (17.41%), Pieridae (06.71%), Acraeidae (02.81%), Calliphoridae (00.55%), Muscidae (00.36%) Tephritidae (00.27%) and Satyridae (00.28%). The diversity of the insects in the pigeon pea ecosystem estimated using the Simpson's diversity index indicated that, insect diversity was high in the pigeon pea ecosystem. The diversity index D = 0.2 (Simpson's Index) is closer to 0 than to 1 hence high insect diversity.

Floral products harvested and times of insects visit to Cajanus cajan flowers

Flowers of *C. cajan* were visited mainly by honey bees, similar to some Megachile (*Megachile bituberculata* and *Chalicodoma cincta cincta*) and xylocope (*Xylocopa* sp., *Xylocopa olivacea* and *Xylocopa torrida*) to collect nectar and pollen, and occasionally by Calliphoridae (*Calliphora* sp.), Muscidae (*Muscus domestica*) Tephritidae (*Dacus bivitattus*), Nymphalidae (*Danaus chrysipus*) and Satyridae (*Lasiommata maera*) to collect nectar. *Vanessa cardui, Catopsilia florella, Hemiargus hanno* and *Acraea acerata* visited flowers mainly to collect nectar exclusively. Unlimited visits implies that all this diversity of visitors was present. From Table 2, it appears that anthophilous insect foraged *C. cajan* flowers almost during the whole daily period.

Table 2. The foraging behaviour and times of insect visitations on *Cajanus cajan* flowers at Maroua.

Insects	Daily observations (hour)												
	7:00-8:00		9:00-10:00		11:00-12:00		13:00-14 :00		15:00-16:00		17:00-18:00		Total
	n	*P*(%)	*n*	*P*(%)	*n*	*P*(%)	*n*	*P*(%)	*n*	*P*(%)	*n*	*P*(%)	
M. bituberculata	16	02.05	86	11.05	428	55.01	156	20.05	84	10.79	8	01.02	778
A. m. adansonii	4	02.89	18	13.04	79	57.24	23	16.66	9	06.47	5	03.62	138
X. olivacea	-	-	10	17.85	18	32.14	18	32.14	8	14.28	2	03.57	56
X. torrida	2	03.57	6	10.71	22	39.28	10	17.85	12	21.42	4	07.14	56
Xylocopa sp.	12	08.95	44	32.83	42	31.34	22	16.41	8	05.97	6	04.47	134
C. c. cincta	6	02.22	26	09.62	101	37.40	88	32.59	47	17.40	2	00.74	270
Calliphora sp.	2	20.00	-	-	6	60.00	-	-	-	-	2	20.00	10
D. bivitattus	2	33.33	2	33.33	2	33.33	-	-	-	-	-	-	06
M. domestica	-	-	2	25.00	2	25.00	-	-	-	-	4	50.00	08
V. cardui	18	22.50	24	30.00	12	15.00	10	12.50	10	12.50	6	07.50	80
H. hanno	74	14.85	134	26.90	103	20.68	84	16.86	88	17.67	15	03.01	498
D. chrysipus	2	33.33	2	33.33	1	16.66	-	-	-	-	1	16.66	6
C. florella	22	32.35	18	26.47	12	17.64	6	08.82	8	11.76	2	02.94	68
A. acerata	18	29.03	28	45.16	-	-	-	-	14	22.58	2	03.22	62
L. maera	4	66.66	-	-	-	-	-	-	2	33.33	-	-	06
Nymphalidae sp.	6	20.00	6	20.00	10	33.33	4	13.33	-	-	4	13.33	30
Total	188	08.52	406	18.40	838	37.99*	421	19.08	290	13.15	63	02.86	2206

n : number of visit ; *P(%)* : percentage.

The individual insect foraging activity was higher between 11:00 and 12:00 a.m. For Hymenoptera, besides *Xylocopa* sp. which higher foraged between 09:00 and 10:00 a.m all the others were the same that individual insect behaviour. For non-bees, besides *Calliphora* sp., *Muscus domestica* and Nymphalidae sp. which higher foraged was after noon, all the other species were prominent in the morning before 11:00 am. After 2:00 p.m., the anthophilous insect activity decreased due undoubtedly to the temperature raising.

Impact of insect activity on pollination and yield of Cajanus cajan

Table 3, documented the high fruiting rate or pod formation during unlimited visits (where high diversity of insects were observed) compared with bagged flowers.

The fruiting rate ranged from 43.91% in treatment 1 to 12.45% in treatment 2. The comparison of the fruiting rate showed that the differences observed were highly significant between treatments 1 and 2 (χ^2 = 304.74, df = 1; P < 0.01).

The percentage of the fruiting rate due to insect activity was 71.64%. The percentage of normal seed was 93.02% and 84.55% in treatments 1 and 2 respectively. The difference between treatments 1 and

2 (χ^2 = 25.09, *df* = 1; *P* < 0.01) was significant. Consequently, the percentage of normal seeds of floral access to insect (treatment 1) was higher than that of flowers bagged during their opening period (treatment 2). This may show high pollination deficit on the crop, indicating need for insect management to increase developed seeds. The contribution of insect to the increment of the percentage of normal seed was 09.11%. The mean numbers of seeds per pod were 2.75 and 2.09 in treatments 1 and 2 respectively. The difference was significant between treatments 1 and 2 (t = 4.18, *df*= 204; *P*< 0.05). Consequently, the number of seed yields per pod of flowers unprotected and visited by insect (treatment 1) was higher than that of flowers bagged during their flowering period (treatment 2). The contribution of insect to the increment of the number of seeds per pod was 24.00%.

Table 3. *Cajanus cajan* yields under pollination treatments.

Parameter	Treatment 1	Treatment 2	Comparison of treatments
Fruiting rate (%)	43.91	12.45	χ^2 = 304.74 ; *df* = 1 ; *P* < 0.01
% of normal seed (%)	93.02	84,55	χ^2 = 25.09 ; *df* = 1 ; *P* < 0.01
Seeds/pod	2.75 (*n* = 103; *s* = 0.75)	2.09 (*n* = 103; *s* = 1.01)	*t* = 4.18 ; *df* = 204 ; *P* < 0.05
Seeds weight/pod (mg)	0.47 (*n* = 103; *s* = 0.12)	0.36 (*n* = 103; *s* = 0,17)	*t* = 4.03 ; *df* = 204 ; *P* <0.05

The mean weight of seeds per pod was 0.47 mg and 0.36 mg in treatments 1 and 2 respectively. The difference between these mean was significant (*t* = 4.03, *df* = 204; *P* <0.05) and the percentage of weight seeds due to the foraging activity of insects was 23.40%.

Fig. 1. Unprotected inflorescences of *Cajanus cajan*.

pigeon pea flowers with bees forming more than 50%. High floral rewards from the pigeon pea plants might have attracted these bees to the pigeon pea flowers. Even though butterflies and flies respectively constitute non-bee flower visitors that were identified to frequent the pigeon pea crop, their numbers were different from each other (*P* ≤ 0.01).

Fig. 2. Bagged inflorescences of *Cajanus cajan*.

Discussion

Earlier studies on the flower visitors of pigeon pea suggested insects as the most frequent (Heard, 1999; Otieno, 2013; Tchuenguem *et al.*, 2014). The present assessment of the flower visitors of pigeon pea confirmed insects as the principal visitors of the

The non-bee visitors may however not be recommended as possible pollinators since their body examination after floral visit does not show the evidence of pollen. In other parts of the world such as in Indonesia (Heard, 1999), Tanzania (Martins,

2008) and Kenya (Otieno, 2013), only bees have been reported as the floral visitors of this crop. *Megachilebi tuberculata* was the most abundant bee visitor of *C. cajan* flowers followed by C. *c. cincta*.

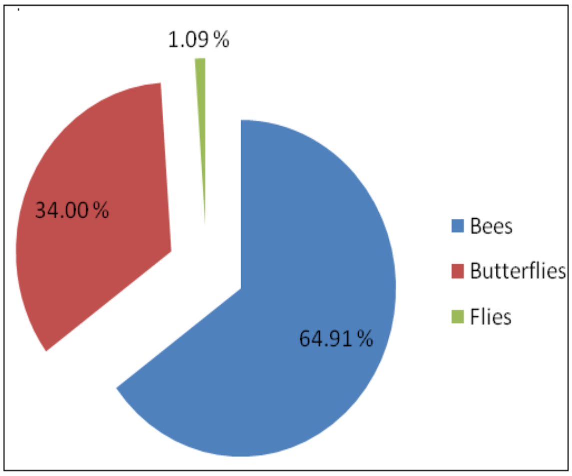

Fig. 3. Diversity and abundance of various insects that visit *Cajanus cajan* flowers.

These bees are able to press on the keel of the flowers to open and have access to the nectar and pollen under the weight of their bodies. Martins (2008) and Pando *et al.* (2011) observed that only Megachile bees and Xylocope bees could be responsible for out-crossing in pigeon pea which agrees with this current finding. Estimation of the diversity of the insect species in the pigeon pea ecosystem using the Simpson's diversity index showed that insect population is more diverse. The evidence of more diverse insect floral visitors coupled with pollen movement concerning bee suggests a possibility of cross pollination in the pigeon pea crop. It therefore implies that if indeed cross pollination could take place in pigeon pea, then bees may be the best insect groups that are responsible.

Field observations indicated that pollen and nectar were the resources collected by all the bees and non-bees flowers visitors collected exclusively nectar. Indeed, Reddy *et al.* (2004) reported that insects that visit flowers of various plant species mostly collect nectar and pollen. *Megachilebi tuberculata, C. c. cincta* and *Apis mellifera adansonii* per the resources they collected, their sizes, buzzing and abundance, may be considered as main agents of cross pollination in pigeon pea. During the collection of nectar and pollen on each flower, bee's foragers regularly come into contact with the stigma. They could enhance auto-pollination, which has been demonstrated in the past (Reddy *et al.,* 2004; Otieno *et al.,* 2011). Bees would provide allogamous pollination through carrying of pollen with their furs, legs and mouth accessories, which is consequently deposited on another flower belonging to different plant of same species.

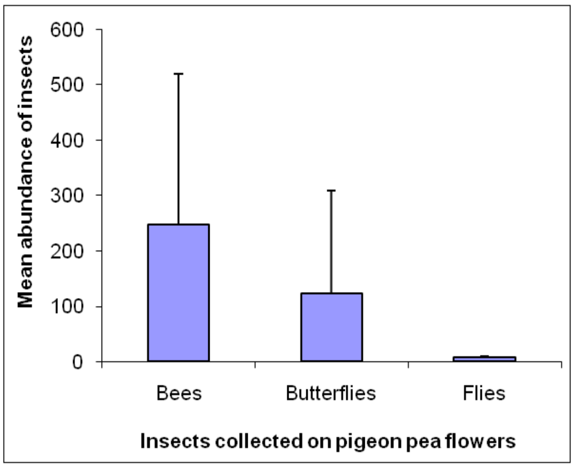

Fig. 4. Mean abundance flower visitors of *Cajanus cajan.*

This has also been observed by other studies (Martins, 2007; otieno *et al.*, 2011). As far back as 1990, Grewal *et al.* (1990) and Singh *et al.* (1990) observed Bumble bees as the primary pollinators of pigeon pea. Field observation also indicates that the pigeon pea flowers open and close at evening, confirming the studies by Tchuenguem *et al.* (2014) which showed that the flowers of the pigeon pea crop open early in the morning and close at evening of the same day.

The bee foragers had a high affinity with respect to *C. cajan* compared with the neighbouring plant species, indicating their faithfulness to this Fabaceae, a phenomenon known as 'floral constancy' (Basualdo *et al.*, 2000). This flower constancy could be partially due to the high sugar content of the nectar (52.05%: Pando, 2013), compared to range 15–75% in which most of the plant species fall (Proctor *et al.*, 1996).It was also observed that the opening and closing of the pigeon pea flowers coincided with the activity of flower visitors.

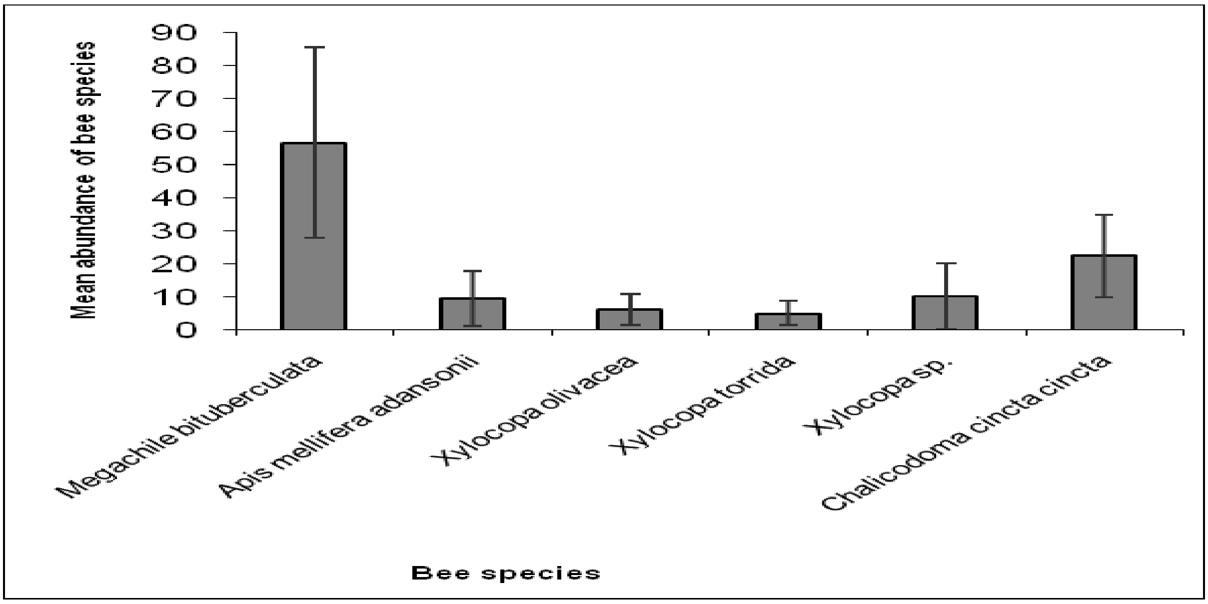

Fig. 5. Abundance and diversity of bee species visiting *Cajanus cajan* flowers.

This suggests that effective spraying to reduce pest infestation can be timed to coincide with low pollinator activity when flowers are closed to avoid poisoning or killing pollinators. The most significant yields (pods, seeds, percentage of normal seeds in pods seed weight) recorded during unlimited visits can be attributed to the important role played by the pollinating insects. A similar result has been reported by Pando *et al.* (2011). The flowers that were exposed to pollinators produced more pods per inflorescence, more seeds per pod, heavier seeds and seeds having better shape compared with the bagged flowers, in agreement with previous results reported for *P. coccineus* (Pando, 2013) and *V. unguiculata* (Pando *et al.,* 2013). That the higher productivity of flowers left unprotected for unlimited visits compared with that of the bagged flowers indicates that insects' visits were effective at increasing cross-pollination. Our results confirm those of Martins (2008), Pando *et al.* (2011) and Otieno (2013) that *C. cajan* flowers set few pods in the absence of insect pollinators (Table 3).

Conclusion

In conclusion, bees are the most dominant insects that frequent the pigeon pea flowers alongside insects such as butterflies and flies. *Megachilebi tuberculata* is the most dominant insect flower visitor of pigeon pea followed by *Chalicodoma cincta cincta* whose activities are likely to influence cross pollination of pigeon pea since pollen was found on their bodies. The comparison of pods and seeds set of bagged flowers with that of flowers visited by insect underscores the value of the insects in increasing pods and seed yields as well as seed quality. The visitation of all the bee species coincided with that of the opening and closing of the pigeon pea flowers. Among the pigeon pea floral visitors collected, bees are the most diverse.

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