Effect of insect pollinators on crop yield and quality of cowpea (*Vigna unguiculata* (L.) Walp.) in Wukari, Nigeria

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ABSTRACT

Investigations were conducted to determine the impact of insect pollinators on the yield and quality of cowpea plant (*Vigna unguiculata* (L.) Walp.). The experiment was carried out at the Biological Garden of Federal University Wukari, Nigeria within the 2023 wet season. Two treatments were evaluated: T_1: Insect pollination inclusion/opened plants (crops allowed to have access to self, wind, and insect pollination) and T_2: Insect pollination exclusion/netted plants (plots were netted with synthetic nylon netting and crops restricted from insect pollination). The treatments were randomly assigned to plots in 3 replicates. Yield and seed quality data were collected. Statistical comparisons were made between the treatments using Student's t-test. The most common insect associated with cowpea flower was observed to be *Apis mellifera* L. with average number of 6.00/plot. This was followed by *Danaus plexippus* L. and *Eurema lisa* Bois & Lec. each with an average count of 4.00/plot. The least abundant, which may be adjudged accidental visitors, were *Libellula luctuosa* Bur and *Aesha grandis* L. with average count of 1.00/plot. While we observed no colour variation between the harvested seeds of opened and netted plants; we found out that the absence of insect pollinators largely and significantly ($t\alpha<0.05$) impacted negatively on the yield and the other qualitative parameters assessed. Marginal but significant differences were observed in the proximate compositions of the grains retrieved from the opened vis-à-vis netted plants. Crude fibre (4.56±0.37%), moisture (9.90±0.30%), and ash content (3.42±0.02%) were significantly ($t\alpha<0.05$) higher in the opened crops as detected by student's t-test; while crude protein (20.68±0.72%), lipids (1.79±0.01%), and carbohydrate (57.90±0.57%) were significantly ($t\alpha<0.05$) higher in the netted plants. The study's findings indicate that insect pollination significantly benefits the yield of cowpea grown in Wukari, Nigeria. Results of this study reinforce knowledge on the importance of pollination services in crop production by showing the significance of insect pollinators in crop yield and quality.

Key words: *Apis mellifera*, Cowpea, *Danaus plexippus*, Flower visiting insects, Entomophily, Plant-pollinator relationship, Pollination exclusion, Pollination inclusion

INTRODUCTION

Research evidences have shown that, ecological services such as pollination enhance crop production (Pablo et al., 2021). Global food production is largely pollinator-dependent as > 70% of crop plants depend largely on entomophily (Klein et al., 2007; Aizen et al., 2008; Okrikata et al., 2019). Some crops depend wholly on insect pollinators to set fruit, while many others produce well over 90% of their potential yield without animal pollinators (Klein et al., 2007). Pollinators are important in improving the growth and yield of crops, including cowpea (Lindström et al., 2016). Insect pollinators not only help maintain yields but also promote genetic variability and reduce inbreeding depression in crops, as noted by Stein et al. (2017). Pollination is required to produce quantitative food in agricultural crops. Both abiotic and biotic factors are responsible for pollination. Abiotic pollination occurs with the involvement of non-living agents like wind and rain/water. On the other hand, biotic pollination occurs through the involvement of living agents such as insects (entomophily) and birds (ornithophily). Among animals, insects play a critical role in pollination (Shivanna, 2015). Declining pollinators can reduce global food production. Crops are pollinated more efficiently by wild insects (Garibaldi et al., 2013). In some crops, the order Lepidoptera (comprising mainly Nymphalidae and Pieridae) has been reported as the most prominent insect pollinators, followed by Hymenoptera. Diptera and Coleoptera were reported as less prominent (Das et al., 2018). Given that bees visit more than 90% of staple crops worldwide, they can be adjudged the main contributors to pollination (Klein et al., 2007; Khalifa et al., 2021).

Cowpea (*Vigna unguiculata* L.) is predominantly self-pollinated, but it maintains certain floral characteristics, such as extrafloral nectaries that attract insect pollinators (Purseglove, 1968). The flowers of cowpea are largely self-fertilizing before opening (cleistogamy) (Asiwe, 2009), and their morphology favors self-pollination as the anthers are in direct contact with the stigmas (Ige et al., 2011). However, some African countries have reported substantial cross-pollination (Fatokun and Ng, 2007; Kouam et al., 2012).

Sharp changes and decline in the assemblages of flower visiting insects is well reported globally (Bartomeus et al., 2014; Pablo et al., 2021). Hence, it is imperative to assess the impact of insect pollinators on crop yield and quality of crops. More so, though the importance of the diversity and abundance of pollinators on crop yield have been well established (Bartomeus et al., 2014; Dainese et al., 2019), the contribution of pollinator visits to cowpea yield and yield-related traits have been found to give dissimilar results in similar trials (Lazaridi et al., 2023). This further justifies the importance of assessing cowpea-pollinator relationship and impact separately in each environment. Furthermore, while the measurement of pollination dependence with adequate controls is necessary to properly account for the impact of entomophily on crop quality and yield, field studies aimed at manipulating flower visitation by insects to assess its qualitative and quantitative impact on crop performance, particularly in cowpea, are rare. This study therefore aims to fill these research gaps.

MATERIALS & METHODS

Study site and experimental description

The experiment was conducted at the Biological Garden, Federal University Wukari, Taraba State, Nigeria; located at 7.51°N 9.47°E longitude and 7.85°N 9.783°E latitude of the Southern Guinea Savannah Ecological Zone of Nigeria, within the rainy season of 2023. The study area experiences a warm tropical climate characterised by wet and dry season. The wet season starts in April and end in October with peak in June and September (Okrikata et al., 2019). The experiment however lasted within the months of May to August, 2023. A land was mapped out with a gross plot size 4.6m x 9.5m (43.7m²) and net plot size of 4.5m x 1.2m (5.4m²). The land was cleared, harrowed and ridged. The cowpea seeds (brown variety) were sown at inter-row spacing of 75cm and intra-row spacing of 20cm. Half a metre bund was placed between treatments.

Experimental design and treatments evaluated

Two (2) treatments were evaluated:

T_1: Insect pollination inclusion/opened plants (crops allowed to have access to self, wind and insect pollination).

T_2: Insect pollination exclusion/netted plants (plots were netted with synthetic nylon netting, and thus the crops were restricted from insect pollination).

The treatments were arranged in a completely randomized design (CRD) in 3 replicates in which weed control was carried out by hoe weeding till harvest and Laraforce (Lambda-Cyhalothrin 2.5% EC) insecticide was applied once a week at the rate of 1.2ml/L using a 2 litres handheld sprayer during the vegetative stage to control insect pests which were observed in the field. Harvesting was done when the crop attained maturity which is indicated by dried, yellow brown coloured pods. Pods from each plot were harvested into well labelled polyethene bags and were taken to the laboratory for further investigation.

Different methods were used to monitor and quantify the abundance and diversity of insect pollinators in the study plots. This includes sweep netting, visual observation and hand picking. Insects were sampled at the active flowering stage of cowpea (which lasted 2 weeks) within 06:30h - 09:30h. Specimens were identified using an insect identification App (Picture Insect) and were further confirmed using Seek app and Google Lens (Manderfield, 2022).

Crop yield measurement

The following quantitative data were collected: Number of pods/plot, number of pods/plant, and number of seeds/pod.

Quality assessment

The following qualitative data were collected:

1. Seed size [seed length (mm), and diameter (mm)]: Vernier calliper was used and the average record of 100 grains/plot was taken.

2. Seed colour: Hand lens, visual observation and microscope were used to assess the seed colour of 10 seeds/plot.

3. 100-grains weight (g): 100 grains were randomly selected from both open and netted plots. They were weighed and the average was recorded.

4. Grain weight (kg/ha): All the grains harvested from plots per treatments were weighed and the average was recorded and converted to kgha-1.

5. Proximate composition: Moisture, ash, crude lipid, crude protein, crude fiber, and carbohydrate of samples of harvested seeds were assessed using standard analytical methods as described by Okwu and Morah (2004) and Okrikata et al. (2023).

Data analysis

All the collected data (except for the seed colour) were subjected to Student's t-test analysis to compare insect pollination inclusion from exclusion using SPSS version 2021, and the level of significance was pegged at p=0.05.

RESULTS

Insects associated with flowers of cowpea

Flowering commenced at week eight (8) after planting and actively lasted for 2 weeks. Thirteen (13) insect species were retrieved. In the 1st week of flowering, *Danaus plexippus* L., *Papilio demoleus* L., *Eurema lisa* Bois & Lec., *Cercyonis pegala* F., *Apis mellifera* L., *Eremnophila aureonotata* C., *Polistes carolina* L., *Meliponila ferruginea* Cock., *Harmonia axyridis* P., *Libellula luctuosa* Bur., and *Musca domestica* L. were observed. In the second week of flowering, *Halyomorpha haly* S., *Aeshna grandis* L. and *Camponotus* sp. were further observed in addition to the species recorded in week 1 (Table 1). The mean count shows that honey bee (*A. mellifera*) with average number of 6.00/plot was the most abundant insect associated with cowpea flower. This was followed by Monarch butterfly (*D. plexippus*) and Little yellow butterfly (*E. lisa*) with average number of 4.00/plot. The least abundant, which apparently are accidental visitors, were the Brown hawker (*A. grandis*) and Window skimmer (*L. luctuosa*) each with a weekly mean count of 1.00/plot (Table 1).

Cowpea yield from open and netted plants

Student's t-test detected significant differences in yield parameters between the opened and netted plants. Number of pods per plot, number of pods per plant, number of seeds per pod, seed weight, and seed weight (kgha^{-1}) were significantly (tα<0.05) higher in insect pollinated plants (Table 2).

Comparison of cowpea seed quality between opened and netted crops

Though no variation was observed in the seed colour of the seeds harvested from plants in opened and netted plots; student's t-test showed differences between the opened and netted plants with respect to the seed parameters assessed with the insect pollinated plants being significantly (ta<0.05) superior (Table 3).

Quality assessment in terms of proximate composition of cowpea seeds obtained from open and netted plants

While the differences in proximate contents between the opened and netted plants were largely marginal; student's t-test detected significant differences between them. Insect pollination resulted in significantly (tα<0.05) higher crude fibre, moisture, and ash content. The netted plants had significantly (tα<0.05) higher crude protein, lipids, and carbohydrate (Table 4).

DISCUSSION

Active flowering commenced at week eight (8) after planting and lasted for 2 weeks. Insect pollinators play a crucial role in the production of plants, including cowpea (*V. unguiculata*). Cowpea is primarily a self-pollinating crop, but it can also benefit from insect pollination. Thirteen (13) insect species were found to be associated with cowpea flowers in the study location. Some of which are *D. plexippus*, *P. demoleus*, *A. mellifera*, *E. aureonotata* C., *P. carolina*, *M. ferruginea*, *H. axyridis*., *L. luctuosa*, *E. aureonotata*, *L. luctuosa*, *A. grandis*, *E. lisa* and *C. pegala*. The most common insect associated with cowpea flower was honey bee (*A. mellifera*) with average number of 6.00/plot. Findings from the study are in consonance with the report of Singh (1979) in India who reported honey bees among the most important flower visitors of strawberry. However, Hooper (1932) reported that pollination is carried mainly by insects other than bees and especially by Dipterans when it is cold. In our study, the least abundant insects which visited the cowpea flowers, which apparently were accidental visitors, were widow skimmer (*L. luctuosa*) and brown hawker (*A. grandis*) with average number of 1.00/plot. Insect pollinators are reported to vary from crop to crop, one location to another, and during different parts of the year. However, research evidence are increasingly suggesting that honeybees are not always the most efficient or effective pollinators (Grass et al., 2018), including in legume crops where they are among the most frequent flower visitors (Marzinzig et al., 2018).

The seed yield data obtained revealed that seed set was significantly (tα<0.05) lower under insect exclusion. The mean 100-grains seed weight (g) was 22.10±0.50 and 18.10±0.50 in insect pollination inclusion and insect pollination exclusion treatments, respectively. Our assessment also revealed that grain weight (kg/ha) was significantly (tα<0.05) higher in the opened than in the netted cowpea plants. The comparatively lower yield performance in crops that were excluded from insect pollination can be attributed to lack of visitation by insects which are known to be largely efficient pollinators (Ibarra-Perez et al., 1999). Another explanation could be that common bean flowers do not activate well without insect visits. Therefore fewer pollen grains contact stigmas of self-pollinated flowers for fertilization. As the insects forage, they move/shake flowers which increases pollen-stigma contact and augment fertilization (Mainkete et al., 2019). While our results conform with the findings of Kumar and Jaiswal (2012) who opined that insect pollinators increases the yield performance of *Coriandrum sativum* L., it contrasts the findings of Free (1966) who showed that only moderate yield benefits is attributable to cowpea visited by insect pollinators.

Pollinators ensure pollen grain dispersal which is a key step of fruit and seed formation. The contact of pollen grains and stigma leads firstly to fertilization and then to seed formation; and auxins synthesized in seeds control cell division resulting in growth (Kumar et al., 2014). Bee species from various families are reported worldwide as cowpea major insect pollinators (Dingha et al., 2021). In addition, species of family Noctuidae, Pieridae and Vespidae have been mentioned as effective pollinators (Dingha et al., 2021). This is confirmed by our findings.

Junqueira and Augusto (2017) opined that poor seed quality could be as a result of pollination deficit. We recorded about 14% increase in the diameter of the cowpea seeds under insect pollination inclusion. More so, our assessment also revealed that seed length (mm) and 100 grains weight were significantly (tα<0.05) higher in the opened than in the netted cowpea plants. These increase in the qualities of cowpea seeds from opened crops is an indication of improved seed yield brought about by pollinating insects (Douka et al., 2018). Our observations in this respect are not in consonance with those of Bhowmik et al. (2017) at West Bengal which revealed that the diameter of *C. sativum* seed was increased in opened insect pollination. Our findings however, corroborates that of Paikara and Paikara (2021) who observed maximum seed size in total open, and minimum seed size in total closed treatment in their study on *C. sativum* at Chhattisgarh. While pod weight was not affected (Dingha et al., 2021), number of seeds per pod, weight of seeds per pod and pod length were reported to be reduced when crops were covered to prevent insect visitation (Fohouo et al., 2009; Musa et al., 2013). These findings are similar to ours.

Open pollinated crop varieties are known to be inherently genetically variable. Colour shades of fruits and seeds have been reported to vary at varying degrees in relation to whether a plant was exposed to pollination intervention by insects or not (Halder et al., 2018). In our study however, no such colour shade variation was detected. This perhaps, may be because cowpea is predominantly a self-pollinated crop.

While crude fibre, moisture, ash content were significantly ($t\alpha<0.05$) higher in the opened cowpea crops exposed to insect pollination, we observed crude protein, lipids, and carbohydrate to be significantly ($t\alpha<0.05$) higher in the netted plants which were excluded from insect pollination. However, it is interesting that the differences, although significant ($t\alpha<0.05$), were marginal. Findings from the present studies are not in consonance with observations of Toni et al. (2021), who reported high lipids composition in pollinator dependent crops. More so, the results of the present study also contradict the report of Wang and Ding (2012), who found that insect pollinators induce more than 80% of macronutrients such as protein and fibre. In entomophilous crops, adequate pollination often leads to produce with enhanced quality (Anderson et al., 2016). Absence of insect pollinators has generally been linked to decrease crop yield and, also quality

CONCLUSION

We assessed the impact of flower-visiting insects to yield and quality of cowpea. Findings from the present study revealed that insect pollination offers a significant benefit to the yield and quality of cowpeas grown in Wukari, Nigeria. The study suggests that sustainable crop yield is possible among smallholder farmers in the study area by maximizing pollination services, and conversely that income losses can be avoided by farming practices that reduce risk to pollinator populations, such as excessive spraying of pesticides. However, more information is needed on which species are the most important pollinator of cowpea crops and which specific field margin plants are more important in supporting them.

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TABLES

Common name*	Scientific name	Order	Family	Number/plot
Monarch butterfly	*Danaus plexippus* L.	Lepidoptera	Nymphalidae	4.00
Lime butterfly	*Papilio demoleus* L.	Lepidoptera	Papilionidae	1.50
Little yellow butterfly	*Eurema lisa* Bois & Lec.	Lepidoptera	Pieridae	4.00
Common wood nymph	*Cercyonis pegala* F.	Lepidoptera	Nymphalidae	3.50
Honey bee	*Apis mellifera* L.	Hymenoptera	Apidae	6.00
Thread-waist wasp	*Eremnophila aureonotata* C.	Hymenoptera	Sphecidae	3.50
Red paper wasp	*Polistes carolina* L.	Hymenoptera	Vespidae	3.00
Stingless bee	*Meliponila ferruginea* Cock.	Hymenoptera	Apidae	3.50
Lady beetle	*Harmonia axyridis* P.	Coleoptera	Coccinellidae	2.50
Widow skimmer	*Libellula luctuosa* Bur.	Odonata	Libelludidae	1.00
Housefly	*Musca domestica* L.	Diptera	Muscidae	3.50
Brown hawker	*Aeshna grandis* L.	Odonata	Aeshnidae	1.00
Carpenter ant	*Camponotus* sp.	Hymenoptera	Formicidae	3.00

Table 1. Insects associated with cowpea in the study area [* = Insects observed/retrieved from opened plots during the 2 weeks active flowering period of cowpea].

Variables	Opened plants	Netted plants	Mean difference	t-value	p-value
Number of pods per plot	536.67±7.54	465.00±4.86	71.67±2.68	17.53	0.001**
Number of pods per plants	21.00±1.15	17.00±0.56	4.00±0.59	7.22	0.001**
Number of seeds per pod	14.00±0.00	13.67±0.33	0.33±0.33	5.43	0.002**
Seed weight (kg/ha)	229.43±6.35	189.37±5.46	40.06±0.89	9.62	0.009**

Table 2. Yield comparison between opened and netted cowpea [Results presented as mean ± standard error; ** = Significantly different (P≤0.01)].

Parameter	Opened plants	Netted plants	Mean difference	t-value	p-value
100 grains weight (g)	22.10±0.50	18.10±0.50	4.00±0.00	6.51	0.001**
Seed length (mm)	8.98±0.50	8.75±0.10	0.23±0.40	3.54	0.001**
Diameter of seed (mm)	7.45±0.01	6.45±0.05	1.00±0.04	3.12	0.001**
Seed weight (kg/ha)	229.43±6.35	189.37±5.46	40.06±0.89	9.62	0.009**

Table 3. Comparative assessment of cowpea seed quality from opened and netted crops [Results presented as mean ± standard error; ** = Significantly different (P≤0.01)].

Treatment	Opened plants	Netted plants	Mean difference	t-value	p-value
Crude fibre (%)	4.56±0.37	3.79±0.10	0.77±0.27	15.32	0.001***
Moisture (%)	9.90±0.30	9.69±0.30	0.21±0.00	17.35	0.001***
Crude protein (%)	19.19±0.65	20.68±0.72	-1.49±0.12	-10.2	0.001***
Ash (%)	3.42±0.02	3.40±0.01	0.02±0.01	20.32	0.001***
Lipids (%)	1.38±0.00	1.79±0.01	-0.41±0.01	-6.55	0.001***
Carbohydrate (%)	54.75±0.95	57.90±0.57	-3.15±0.37	-4.62	0.001***

Table 4. Comparing the proximate composition of cowpea seeds obtained from opened and netted plants [Results presented as mean ± standard error; *** = Significantly different (P≤0.001)].

