

# Diversity of insect pollinators of sunflower (*Helianthus annus* L: Asteraceae) in response to host plant nutrient enhancement

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Behavioural interactions between insect pollinators and host plants increase pollinator efficiency, conservation and crop yield. This study analyzed the diversity and abundance of insect pollinators on sunflower varieties, Jos local and SAM-SUX-1 grown in soils treated with different application rates of NPK 15-15-15, namely 0, 30, 60 and 90 kg/ha. A field experiment was conducted on two sunflower varieties combined with four rates of NPK-15-15-15 fertilizer split applied at 2 and 4 weeks after planting and laid out in a randomized complete block design with three replicates. The nutritional content of the pollen grains, flavonoid, vitamin A and  $\beta$ -carotenoid contents of the flowers of the two sunflower varieties were analysed to determine treatment effect on nutritional quality. Data were collected on the abundance, diversity of insect pollinators' species and foraging response of honeybees. Descriptive and inferential statistics analysis of variance and the Student Neumann-Keuls test ( $\alpha=0.05$ ) were used as appropriate for data collected. NPK-15-15-15 fertilizer produced no significant effect ( $p>0.05$ ) on the flower external morphology of both varieties but increased pollen grain crude protein, fat, flavonoid contents, vitamin A and  $\beta$ -carotenoid contents of the flowers florets at the application rate of 90 kg/ha<sup>-1</sup>. Honeybees demonstrated distinct foraging preference for both sunflower varieties at 90 kg/ha<sup>-1</sup> NPK 15-15-15 ( $p\leq 0.05$ ). The two cultivated sunflower varieties attracted different assemblages of evenly distributed insect pollinators (90 – 92 %). The results showed that Jos local had the highest insect visitors (60.31%) while SAM-SUX-1 recorded 39.69% of insect pollinators visit. The study also revealed honeybees as second in abundance (37.75%) and foraging rate (2.17 s) on SAM-SUX-1 variety. The highest insect diversity was observed in Lepidoptera (Shannon's,  $H' = 2.40$ ), while Hymenoptera ( $H' = 2.38$ ) was the lowest. However, the highest insect dominance was observed in Hymenoptera (dominance,  $D = 0.099$ ) and on Jos local variety ( $D = 0.110$ ).

**Keywords:** Insect pollinators, diversity, sunflower, NPK 15-15-15 fertilizer

Insect pollinators provide a critical key of ecosystem services to both natural and agro-ecosystems. About 87% of the world major food crops depend on animal pollination for fruit, vegetable or seed production (Rostami et al. 2016). In 2005, the total economic value of insect pollinators were estimated to account for \$210 billion of worldwide agricultural production, the services of insect pollinators thereby are of pivotal significance in enhancing crop productivity in both natural and agro-ecosystems (Klein et al. 2007). A mutual ecological relationship of vital importance exists between the richness and abundance of floral resources of the host plant and animal pollinator diversity and activity (Steffan-Dewenter and Tscharntke 2000; Klein et al. 2003). The growth, survival, defense and reproduction of insects are

influenced by host plant nutrient concentrations and environmental factors such as temperature, moisture and habitat (Slansky and Rodriguez 1987; Fischer and Fiedler 2000). According to Jahn (2004) and Rostami et al. (2016), nitrogen, phosphorous and potassium are essential building blocks of the tissues of insects and greatly influence the production and quality of flowers (Mattson 1980). These major nutrients are reported to enhance vegetative growth and assist the plant to mobilize the process of flower opening during the blooming period. Nitrogen, phosphorus and potassium have significant effects on spike production and floret quality (Singh et al. 2004).

Several studies have indicated that excessive and/or inappropriate use of inorganic fertilizers can cause nutrient imbalances and lead to

decrease in the performance of insects that feed on those plant tissues. An increase in zinc fertilizer application has been reported to impart resistance against *Spodoptera litura* and *Scirpophaga* sp in rice cultivation (Muhammad 2011) while excess nitrogen and phosphorus fertilizer in maize cultivation increased the incidence of *Busseola fusca* (Wale et al. 2006). It is important to note that an increase in nitrogen fertilizer rate may trade off with pollinator attraction (Lu and Heong 2009). Flowering can be increased with increased levels of NPK application (Anamika and Lavania 1990). Increased flower productions, quality of flowers and pollen as well as perfection in the form of plants are important factors in pollination. Flowering plants synthesize compounds which emit complex odours and display wide spectra of colours in flowers to lure pollinators. Changes in plant nutrient affects the biosynthesis of these compounds and can therefore lead to modifications in the colour-odour combination in flowers and subsequently affect pollinator visitation pattern (Milet-Pinheiro et al. 2012; Dormont et al. 2014). Such insect pollinators could include bumblebees, honeybees, solitary bees, hoverflies, beetles, butterflies, wasps and other flies from both managed and wild populations; these provide an invaluable contribution to crop production. Bees are the most economically important group of insect pollinators of agricultural crops (Greenleaf and Kremen 2006). Over the past few years, services provided by wild pollinators are showing declining trends due to threats of diseases, attack by pests and pesticide misuse, among other factors (Berenbaum et al. 2006).

Sunflower, *Helianthus annuus* L. (Asteraceae) is an important oil seed crop cultivated widely for production of biodiesel and as an ornamental. The bright yellow ray floret of sunflower serves as a visual attraction for insects, especially pollinators (Stang et al. 2007). Sunflower is being used for food, therapy, cosmetics and in many ancient traditions. The morpho-physiological disagreement of the stamens and pistils breeds self-incompatibility,

thus making the pollen not well adapted to be transported by wind (anemophily). However, the floral arrangement and sequence of flower opening allow them to be assisted when visited by pollinating insects (Free 1993). Floral cues such as colour, odour, shape and arrangement are important in helping to attract pollinators (Berjano et al. 2009). According to Morgado et al. (2002), bees are the most important insects in sunflower pollination process. The nutrient statuses of host plants is important in biological research especially in species management, understanding of foraging theory and pollination ecology (Kendal et al. 2013). The aim of this study was to investigate the effect of NPK 15-15-15 rates of application on plant-pollinator interaction by assessing the diversity of visiting pollinators and foraging behaviour of honeybee on the sunflower varieties used. Effects of NPK 15-15-15 application rate on floral traits and quality of sunflower were also examined.

## Materials and methods

The field experiment was conducted during the rainy season (June to September 2019) at the Arboretum of the Forestry Research Institute of Nigeria, Jericho-Hills, Ibadan, Oyo State, Nigeria. Two sunflower varieties (Jos local and SAM-SUX-1) were treated with NPK fertilizer application at 2 and 4 weeks after planting (WAP) at four rates – 0, 30, 60 and 90 kg/ha on each application. The experiment was a 4 × 2 factorial randomized complete block design with three replicates on a 300m<sup>2</sup> plot of land. Two or three sunflower seeds were sown directly on the 3 m × 2 m treatment plots with a planting distance of 50 cm × 50 cm. Borders of 75 cm between blocks and 50 cm between plots were maintained.

### *Effects of NPK 15-15-15 fertilizer on floral traits and quality in sunflower*

To measure the effects of NPK 15-15-15 application at different rates on the floral traits and sunflower varieties used, three flowers

were purposively selected per treatment plot at 25 days after flowering (DAF) and the following parameters were measured on the flowers: capitulum diameter, flower height, number of fillaries, flower weight, flower pollen weight, number of ray florets, disc diameter, ray floret length and disc floret length (Cardoza et al. 2012).

#### *Observation of pollinator diversity in relation to NPK 15-15-15 fertilizer rate on two sunflower varieties*

Determination of the spectrum of pollinator insects commenced at the start of the flowering stage (7 WAP) using a hand sweep net (38 cm rim diameter, 50 cm bag depth and 60 cm handle length) throughout the flowering period on a daily basis between 8.30 am and 12.30 pm. The total number of insect pollinators found at the time of visit per plots was recorded visually per plot using a hand tally counter. The collected insects were preserved in 70% ethanol inside a 2×4 McCartney bottle on the field and thereafter, pinned, dried and kept in an insect box for subsequent identification. Specimens were pre-identified using pictures and descriptions as well as existing analytical keys. Detailed identification of the insect specimens was based on comparison with specimens in the Insect Museum of Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan (Kevan 1999).

#### *Observation of honeybee visitation in relation to NPK 15-15-15 fertilizer rate effect on two sunflower varieties*

A chronometer (stopwatch) with an accuracy of 0.01 second was used to record the time spent by an individual honeybee per flower during foraging. The observations were recorded per plot from flower initiation till 95% of the flower faded. The numbers of bees and their visit length (minutes) were recorded for each of the treatments between 8.30 am to 12.30 pm when the insects were relatively active (Cardoza et al. 2012).

#### *Data analysis*

Insect population of count was transformed using square root model  $\sqrt{(x+0.5)}$  then subjected to two way analysis of variance (ANOVA) to determine the interactions between the treatments using SAS 9.1 software. Where significance was detected, means were then compared using the Student-Newman-Keuls (SNK) test ( $p \leq 0.05$ ). The effects of independent variables (rate of NPK 15-15-15 application) on the dependent variable (various floral traits) were measured by linear regression analysis in the following form:  $y = a + bx + \varepsilon$  using SAS 9.1 software.

Where:

- y = dependent variable (various floral traits),
- x = independent variable (rate of NPK 15-15-15 application),
- a = intercept,
- b = slope of the line,
- $\varepsilon$  = residual error.

Correlation coefficient of determination ( $R^2$ ) was used to estimate the relationship between the independent variables and responses. The diversity indices: Shannon Wiener species diversity index ( $H'$ ), Margalef diversity index and Jaccard similarity index (J) were used to compute the diversity of insect pollinators in relation to different NPK 15-15-15 fertilizer rates and two varieties of sunflower (Samways et al. 2010).

#### **Results**

##### *Effect of NPK 15-15-15 fertilizer on floral traits and quality in sunflower*

The results showed that there was no significant relationship between the rate of NPK 15-15-15 application and the various sunflower varieties floral traits measured in the study ( $p > 0.05$ ) (Table 1). The nutritional composition of pollen grains, vitamin A and  $\beta$ -carotenoid concentrations of the ray florets of the two sunflower varieties varied significantly ( $p \leq$

0.05) among the levels of fertilizer. There was a significant increase in the protein and flavonoid contents in pollen grains of sunflower varieties reaching the highest concentration at 90kg/ha NPK 15-15-15 fertilizer (Table 2). The ray florets of Jos local variety had the highest concentration of vitamin A ( $0.80 \pm 0.00 \mu\text{gg}^{-1}$ )

and  $\beta$ -carotenoid ( $56.07 \pm 0.51 \mu\text{gg}^{-1}$ ) recorded at 90kg/ha while the ray florets in the control plots had the lowest value  $p \leq 0.05$ . The highest value of  $0.67 \pm 0.0 \mu\text{gg}^{-1}$  was also obtained for vitamin A and  $86.90 \pm 0.04 \mu\text{gg}^{-1}$  for  $\beta$ -carotenoid at 90 kg/ha in ray florets of SAM-SUX-1 variety ( $p \leq 0.05$ ) (Table 2).

Table 1: Relationship between NPK 15-15-15 fertilizer application and flower traits of two sunflower varieties

Flower traits	Jos Local variety			SAM-SUX-1 variety		
	a	b	R <sup>2</sup>	a	b	R <sup>2</sup>
Capitulum diameter (cm)	3.35	0.48	0.15	9.82	-0.22	0.05
Flower height (cm)	4.20	0.40	0.03	8.55	-0.72	0.05
Flower weight (g)	4.56	0.02	0.04	8.19	-0.04	0.12
Number of fillaries	5.84	0.00	0.00	12.21	-0.11	0.18
Flower pollen weight(g)	5.34	20.25	0.09	6.19	-44.68	0.05
Number of ray florets	24.85	-0.54	0.64	8.36	-0.06	0.04
Disc diameter (cm)	12.01	-0.77	0.09	12.01	-0.77	0.09
Ray floret length (cm)	4.68	0.09	0.01	-3.30	1.37	0.19
Disc floret length (cm)	5.98	6.90	0.11	-4.99	5.73	0.10

a= Intercept    b= Slope of the line    R<sup>2</sup>= Coefficient of determination

Table 2: Nutritional composition  $\pm$  SE of pollen grains and ray florets of two sunflower varieties grown in soil treated with different NPK 15-15-15 level

Pollen grain content	JOS local NPK Dosage				SAM-SUX-1 NPK Dosage			
	0	30	60	90	0	30	60	90
Crude protein (%)	0.04 <sup>c</sup> $\pm$ 0.00	0.07 <sup>b</sup> $\pm$ 0.00	0.08 <sup>b</sup> $\pm$ 0.00	0.13 <sup>a</sup> $\pm$ 0.01	0.03 <sup>c</sup> $\pm$ 0.00	0.11 <sup>b</sup> $\pm$ 0.01	0.14 <sup>a</sup> $\pm$ 0.00	0.16 <sup>a</sup> $\pm$ 0.01
CHO (NFE)	15.19 <sup>a</sup> $\pm$ 0.05	12.97 <sup>b</sup> $\pm$ 0.015	11.04 <sup>c</sup> $\pm$ 0.10	11.04 <sup>c</sup> $\pm$ 0.10	17.46 <sup>a</sup> $\pm$ 0.30	12.55 <sup>c</sup> $\pm$ 0.20	12.91 <sup>c</sup> $\pm$ 0.08	14.93 <sup>b</sup> $\pm$ 0.05
Crude fibre (%)	0.00 <sup>a</sup> $\pm$ 0.00	0.00 <sup>a</sup> $\pm$ 0.00	0.00 <sup>a</sup> $\pm$ 0.00	0.00 <sup>a</sup> $\pm$ 0.00	0.00 <sup>a</sup> $\pm$ 0.00	0.00 <sup>a</sup> $\pm$ 0.00	0.00 <sup>a</sup> $\pm$ 0.00	0.00 <sup>a</sup> $\pm$ 0.00
Fat (%)	0.01 <sup>c</sup> $\pm$ 0.00	0.02 <sup>b</sup> $\pm$ 0.00	0.03 <sup>a</sup> $\pm$ 0.00	0.03 <sup>a</sup> $\pm$ 0.00	0.01 <sup>b</sup> $\pm$ 0.00	0.04 <sup>a</sup> $\pm$ 0.00	0.04 <sup>a</sup> $\pm$ 0.01	0.05 <sup>a</sup> $\pm$ 0.01
Ash Content (%)	0.01 <sup>a</sup> $\pm$ 0.00	0.01 <sup>a</sup> $\pm$ 0.00	0.01 <sup>a</sup> $\pm$ 0.00	0.01 <sup>a</sup> $\pm$ 0.00	0.01 <sup>a</sup> $\pm$ 0.00	0.01 <sup>a</sup> $\pm$ 0.00	0.01 <sup>a</sup> $\pm$ 0.00	0.01 <sup>a</sup> $\pm$ 0.00
Moisture content (%)	84.75 <sup>d</sup> $\pm$ 0.05	86.93 <sup>c</sup> $\pm$ 0.02	88.8 <sup>a</sup> $\pm$ 0.10	87.95 <sup>b</sup> $\pm$ 0.03	82.80 <sup>c</sup> $\pm$ 0.01	87.3 $\pm$ 0.20	86.91 <sup>a</sup> $\pm$ 0.07	84.87 <sup>b</sup> $\pm$ 0.05
Flavonoid (mg/100g)	0.02 <sup>d</sup> $\pm$ 0.00	0.24 <sup>c</sup> $\pm$ 0.00	0.27 <sup>b</sup> $\pm$ 0.00	0.31 <sup>a</sup> $\pm$ 0.00	0.05 <sup>c</sup> $\pm$ 0.03	0.25 <sup>b</sup> $\pm$ 0.00	0.28 <sup>b</sup> $\pm$ 0.00	0.42 <sup>a</sup> $\pm$ 0.00
Vitamin A ( $\mu\text{gg}^{-1}$ )	0.02 <sup>d</sup> $\pm$ 0.00	0.49 <sup>c</sup> $\pm$ 0.00	0.74 <sup>b</sup> $\pm$ 0.00	0.80 <sup>a</sup> $\pm$ 0.00	0.01 <sup>d</sup> $\pm$ 0.00	0.50 <sup>c</sup> $\pm$ 0.00	0.59 <sup>b</sup> $\pm$ 0.00	0.67 <sup>a</sup> $\pm$ 0.00
$\beta$ -carotenoid ( $\mu\text{gg}^{-1}$ )	1.60 <sup>d</sup> $\pm$ 0.34	9.88 <sup>c</sup> $\pm$ 0.04	17.74 <sup>b</sup> $\pm$ 0.10	56.07 <sup>a</sup> $\pm$ 0.51	1.28 <sup>d</sup> $\pm$ 0.00	53.30 <sup>c</sup> $\pm$ 0.02	82.44 <sup>b</sup> $\pm$ 0.55	86.90 <sup>a</sup> $\pm$ 0.04

Means followed by the same letter along the same row are not significantly different using Student Newman-Keuls (SNK) test ( $p > 0.05$ ).

*Diversity of pollinator insects on sunflower*

A total of 14 pollinator insects species was recorded on *sunflower* belonging to the orders Hymenoptera and Lepidoptera (Plate 1). The Hymenopterans belonged to five families namely: Apidae, Halticidae, Anthophoridae, Megachilidae and Vespidae while the families Nymphalidae, Danaidae and Erebidae were the Lepidopterans found. The most common pollinator species were *Trigona* sp (61.00%), *Apis mellifera* (15.58%), *Dactylurina staudine*

(13.23%) and *Acrea zetes* (4.13%). The results showed that the order Hymenoptera (93.38%) were the most dominant insects in the study, followed by Lepidoptera (6.62%). The highest species diversity and variation were observed in the order Lepidoptera ( $H' = 2.40$ , Margalef = 1.80), while Hymenoptera had the lowest indices ( $H'=2.38$ , Margalef = 1.25). The Hymenoptera and Lepidoptera insects recorded in the study were evenly distributed within the sunflower experimental field with a value range of 0.90 - 0.96 (Table 3).



(i) *Apis mellifera* L.  
 Hym: Apidae (15.58%)



(ii) *Dactylurina staudingeri* G.  
 Hym: Apidae (13.23%)



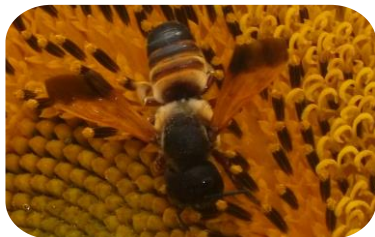
(iii) *Trigona* sp.  
 Hym: Apidae (60.99%)



(iv) *Meliponula bocandei* C.  
 Hym: Apidae (0.21%)



(v) *Nomia tridentata* S.  
 Hym: Halticidae (1.28%)



(vi) *Chalicodoma rufipes* F  
 Hym: Megachilidae (0.43%)



(vii) *Xylocopa* sp. L.  
 Hym: Anthophoridae (0.14%)

Plate 1 (i-vii): Pollinator insect species associated with sunflower in the experimental field with their orders, families and species composition.



(iix) *Xylocopa albiceps* F.  
Hym: Anthophoridae (0.49%)



(ix) *Xylocopa torrida* W.  
Hym: Anthophoridae (0.28%)



(x) *Ropalidia cincta* L.  
Hym: Vespidae (0.71%)



(xi) *Danaus chrysippus* L.  
Lep: Danaidae (0.99%)



(xii) *Acrea zetes* L.  
Lep: Nymphalidae (4.13%)



(xiii) *Precis oenone* S.  
Lep: Nymphalidae (0.28%)



(xiv) *Euchromia lethe* F.  
Lep: Erebidae (1.21%)

Plate 1 (iix-xiv): Pollinator insect species associated with sunflower in the experimental field with their orders, families and species composition.

Table 3: Diversity indices of the pollinator insects on sunflower varieties grown in soils treated with NPK 15-15-15 Fertilizer (0, 30, 60 and 90Kg/ha)

Order	Relative Abundance (%)	Dominance_D	Diversity Index (H')	Evenness_e^H/S	Margalef	Equitability_J
Hymenoptera	93.38	0.10	2.38	0.90	1.25	0.96
Lepidoptera	6.62	0.09	2.40	0.92	1.80	0.97
Sunflower varieties						
Jos local	60.31	0.11	2.32	0.85	1.32	0.94
<i>Fisher-alpha</i> ( $\alpha$ ) = 1.511						
SAMSUX-1	39.69	0.08	2.44	0.97	1.39	0.99
<i>Fisher-alpha</i> ( $\alpha$ ) = 1.609						

*Effect of NPK 15-15-15 fertilizer on pollinator diversity responses to two sunflower varieties*

The abundance of the pollinators varied significantly ( $p \leq 0.05$ ) within the different levels of rates of NPK 15-15-15 applied. Four major insect pollinator species: *Trigona* sp (stingless bees), *Apis mellifera* (honeybees), *Dactylurina staudine* (stingless bees) and *Acraea zetes* (large spotted Acraea) were highly significant in abundance in both sunflower varieties used for the study ( $p \leq 0.05$ ). As the level of applied NPK 15-15-15 increased, the density of *Trigona* sp ( $178.58 \pm 26.55$ ) was significantly higher than *Apis mellifera* ( $67.50 \pm 21.04$ ) followed by *Dactylurina staudine* ( $53.42 \pm 8.79$ ) and *Acraea zetes* ( $21.67 \pm 2.83$ ),

compared to other insect pollinators which were statistically similar on the Jos local sunflower variety. Similarly, at 90kg/ha NPK 15-15-15 rate, significantly higher ( $p \leq 0.05$ ) numbers of *Trigona* sp ( $178.25 \pm 10.75$ ), *Apis mellifera* ( $37.75 \pm 5.93$ ), *Dactylurina staudine* ( $9.92 \pm 2.73$ ) and *Acraea zetes* ( $2.50 \pm 1.15$ ) were recorded on SAM-SUX-1 sunflower variety, while other insect pollinator species were not significantly different from one another (F value,  $p > 0.05$ ) (Tables 4 and 5).

The results also showed that Jos local sunflower variety had the highest abundance of insect species (60.31%) with  $D=0.11$  and Fisher-alpha index 1.51. In contrast, SAM-SUX-1 has lowest abundance (39.69%) and dominant (0.09) insects with highest Fisher-alpha index ( $\alpha = 1.61$ ) (Table 3).

Table 4: Abundance (mean  $\pm$  SE) of insect pollinator species associated with flowers of Jos local sunflower variety grown in soils treated with different levels of NPK 15-15-15 fertilizer

Insect order	Insect species	Fertilizer rate (Kg/ha)				Mean of pollinator
		0	30	60	90	
Hymenoptera	<i>Trigona</i> sp	57.70 <sup>e</sup> $\pm$ 1.20	143.30 <sup>c</sup> $\pm$ 1.67	220.00 <sup>b</sup> $\pm$ 7.64	293.30 <sup>a</sup> $\pm$ 8.82	178.58 <sup>a</sup> $\pm$ 26.55
		6.67 <sup>f</sup> $\pm$ 1.67	18.33 <sup>f</sup> $\pm$ 13.33	95.00 <sup>d</sup> $\pm$ 10.41	150 <sup>c</sup> $\pm$ 51.07	67.50 <sup>b</sup> $\pm$ 21.04
Hymenoptera	<i>Dactylurina staudine</i>	23.33 <sup>f</sup> $\pm$ 1.67	37.70 <sup>ef</sup> $\pm$ 1.86	54.30 <sup>e</sup> $\pm$ 2.96	98.3 <sup>d</sup> $\pm$ 10.14	53.42 <sup>c</sup> $\pm$ 8.79
		13.33 <sup>f</sup> $\pm$ 1.67	15.00 <sup>f</sup> $\pm$ 0.00	21.67 <sup>f</sup> $\pm$ 0.88	36.7 <sup>ef</sup> $\pm$ 1.67	21.67 <sup>d</sup> $\pm$ 2.83
Hymenoptera	<i>Acraea zetes</i>	3.33 <sup>f</sup> $\pm$ 1.67	5.00 <sup>f</sup> $\pm$ 0.00	5.00 <sup>f</sup> $\pm$ 0.00	13.33 <sup>f</sup> $\pm$ 1.67	6.67 <sup>e</sup> $\pm$ 3.09
		1.67 <sup>f</sup> $\pm$ 1.67	0.00 <sup>f</sup> $\pm$ 0.00	0.00 <sup>f</sup> $\pm$ 0.00	3.33 <sup>f</sup> $\pm$ 1.67	1.25 <sup>e</sup> $\pm$ 0.89
Hymenoptera	<i>Nomia tridentate</i>	1.67 <sup>f</sup> $\pm$ 1.67	1.67 <sup>f</sup> $\pm$ 1.67	0.00 <sup>f</sup> $\pm$ 0.00	0.00 <sup>f</sup> $\pm$ 0.00	0.83 <sup>e</sup> $\pm$ 0.56
		0.00 <sup>f</sup> $\pm$ 0.00	3.33 <sup>f</sup> $\pm$ 1.67	3.33 <sup>f</sup> $\pm$ 1.67	0.00 <sup>f</sup> $\pm$ 0.00	1.67 <sup>e</sup> $\pm$ 1.12
Hymenoptera	<i>Meliponula bocandei</i>	3.33 <sup>f</sup> $\pm$ 1.67	0.00 <sup>f</sup> $\pm$ 0.00	0.00 <sup>f</sup> $\pm$ 0.00	8.33 <sup>f</sup> $\pm$ 4.41	2.92 <sup>e</sup> $\pm$ 1.44
		0.00 <sup>f</sup> $\pm$ 0.00	0.00 <sup>f</sup> $\pm$ 0.00	16.67 <sup>f</sup> $\pm$ 12.02	1.67 <sup>f</sup> $\pm$ 1.67	5.83 <sup>e</sup> $\pm$ 3.42
Hymenoptera	<i>Xylocopa torrida</i>	3.33 <sup>f</sup> $\pm$ 1.67	0.00 <sup>f</sup> $\pm$ 0.00	11.67 <sup>f</sup> $\pm$ 9.28	1.67 <sup>f</sup> $\pm$ 1.67	4.17 <sup>e</sup> $\pm$ 2.45
		0.00 <sup>f</sup> $\pm$ 0.00	0.00 <sup>f</sup> $\pm$ 0.00	0.00 <sup>f</sup> $\pm$ 0.00	10.00 <sup>f</sup> $\pm$ 7.64	2.50 <sup>e</sup> $\pm$ 2.09
Hymenoptera	<i>Ropalidia cincta</i>	0.00 <sup>f</sup> $\pm$ 0.00	6.70 <sup>f</sup> $\pm$ 1.67	3.33 <sup>f</sup> $\pm$ 1.67	13.30 <sup>f</sup> $\pm$ 7.26	5.83 <sup>e</sup> $\pm$ 2.29
		0.00 <sup>f</sup> $\pm$ 0.00	0.00	0.00	0.00	0.00

Means followed by the same letter along the same row and column are not significantly different using Student Newman-Keuls (SNK) test ( $p > 0.05$ )

Table 5: Abundance (mean + SE) of insect pollinator species associated with flowers of SAM-SUX 1 variety grown in soils treated with different levels of NPK 15-15-15 fertilizer

Insect order	Insect species	Fertilizer rate (Kg/ha)				Mean of pollinator
		0	30	60	90	
Hymenoptera	<i>Trigona sp</i>	123.33 <sup>d</sup> ±	180.00 <sup>c</sup> ±	192.33 <sup>b</sup> ± 3.33b	217.33 <sup>a</sup> ±	178.25 <sup>a</sup> ±
		11.67	2.89		3.71	10.75
Hymenoptera	<i>Apis mellifera</i>	11.67 <sup>i</sup> ± 6.00	33.33 <sup>g</sup> ±	43.33 <sup>f</sup> ± 4.41	62.67 <sup>e</sup> ±	37.75 <sup>b</sup> ± 5.93
			1.67		6.22	
Hymenoptera	<i>Dactylurina staudine</i>	2.33 <sup>i</sup> ± 1.20	5.67 <sup>i</sup> ±	8.33 <sup>i</sup> ± 1.67	23.33 <sup>h</sup> ±	9.92 <sup>c</sup> ± 2.73
			3.48		4.41	
Lepidoptera	<i>Acraea zetes</i>	1.67 <sup>i</sup> ± 1.67	3.33 <sup>i</sup> ±	1.67 <sup>i</sup> ± 1.67	3.33 <sup>i</sup> ±	2.50 <sup>d</sup> ± 1.15
			1.67		1.67	
Hymenoptera	<i>Nomia tridentate</i>	3.33 <sup>i</sup> ± 1.67	0.00 <sup>i</sup> ±	0.00 <sup>i</sup> ± 0.00	0.00 <sup>i</sup> ±	0.83 <sup>d</sup> ± 0.56
			0.00		0.00	
Lepidoptera	<i>Euchromia lethe</i>	3.33 <sup>i</sup> ± 1.67	0.00 <sup>i</sup> ±	1.67 <sup>i</sup> ± 1.67	0.00 <sup>i</sup> ±	1.25 <sup>d</sup> ± 0.89
			0.00		0.00	
Lepidoptera	<i>Precis oenone</i>	5.00 <sup>i</sup> ± 0.00	0.00 <sup>i</sup> ±	0.00 <sup>i</sup> ± 0.00	1.67 <sup>i</sup> ±	1.67 <sup>d</sup> ± 1.28
			0.00		1.67	

Means followed by the same letter along the same row and column are not significantly different using Student Newman-Keuls (SNK) test ( $p > 0.05$ ).

*Honeybee intensity and length of foraging time on sunflower treated with different levels of NPK 15-15-15*

The abundance ( $61.97 \pm 10.99$ ) of honeybees and their length of visitation ( $1.5 \pm 0.18$  sec) were significantly higher ( $p \leq 0.05$ ) at 90 kg/ha NPK 15-15-15 on Jos local variety flowers,

compared to the control, in which the lowest values of  $7.03 \pm 2.53$  and  $0.47 \pm 0.12$  seconds, respectively, were recorded (Table 6). The highest number ( $101.07 \pm 19.86$ ) ( $p \leq 0.05$ ) of honeybees and longest visiting time ( $2.17 \pm 0.25$  sec) were also observed at 90 kg/ha NPK 15-15-15 which was statistically similar at 60 kg/ha NPK 15-15-15 rate on SAM-SUX-1 variety flowers (Table 6).

Table 6: Honeybee abundance (Mean ± SE) and duration of visit (s) (Mean ± SE) on two sunflower varieties grown in soil treated with different levels of NPK 15-15-15 fertilizer (Kg/ha)

Response variables	Variety	Application rate (kg/ha)			
		0	30	60	90
Bee abundance	Jos local	7.03 <sup>c</sup> ± 2.53	27.63 <sup>b</sup> ± 4.49	56.30 <sup>a</sup> ±	61.97 <sup>a</sup> ±
				14.89	10.99
Duration of visit (s)	SAM-SUX-1	0.47 <sup>d</sup> ± 0.12	0.90 <sup>c</sup> ± 0.12	1.30 <sup>b</sup> ± 0.20	1.50 <sup>a</sup> ± 0.18
Bee abundance	SAM-SUX-1	22.33 <sup>c</sup> ± 9.15	62.63 <sup>b</sup> ±	93.43 <sup>a</sup> ±	101.07 <sup>a</sup> ±
			23.40	10.30	19.86
Duration of visit (s)	SAM-SUX-1	0.60 <sup>d</sup> ± 0.14	1.13 <sup>c</sup> ± 0.25	2.07 <sup>b</sup> ± 0.32	2.17 <sup>a</sup> ± 0.25

Means followed by the same letter along the same row are not significantly different using Student Newman-Keuls (SNK) test ( $p > 0.05$ )



## Discussion

The study revealed that several floral traits of the sunflower plant such as: capitulum diameter, flower height, flower weight, number of fillaries, pollen weight, number of ray florets, disc diameter, ray floret length and disc floret length, modulated by NPK 15-15-15 application, did not differ statistically as similarly observed and reported by Cardoza et al. (2012) on a study on cucumber plants. Increased rates of NPK 15-15-15 irrespective of sunflower variety, increased protein, fat, flavonoids, vitamin A and beta-carotene contents of the pollen grain, which are important in the body metabolism of pollinator insects as well as providing clues to help bee species identify a host plant (Atiyeh et al. 2002). The 14 insect pollinators observed on sunflower in the study area were distributed among two orders (Hymenoptera and Lepidoptera) and seven families among which the honeybees were ranked second in abundance. The result shows that bee species (92.43% of the total pollinator) were the main pollinators with honeybee ranking second in abundance on SAMSUX-1 variety. Atmowidi et al. (2007) reported that hymenopterans and lepidopterans were the most abundant and dominant insect pollinators of *Brassica rapa*, with honeybees playing a significant role in fruit set (92.72%). Honeybee pollination is important in the cultivation of sunflower where bees principally help in pollen transfer (Kumar et al. 2003; Pidek et al. 2004). The high diversity and evenness indices revealed that the insect species were closely related. Many past studies have indicated that soil nutrient enhancements can affect insect-plant interactions (Arancon et al. 2007; Cardoza 2011; Cardoza et al. 2011). In this study, increased rate of NPK 15-15-15 on the sunflower varieties significantly increased the densities of pollinator insects. Four major insect pollinator species (*Trigona* sp, *Apis mellifera*, *Dactylurina staudine*, *Acraea zetes*) were found to be highly abundant. This study

further stressed the findings of Cardoza et al. (2012) that the application of soil amendment can impact plant interactions with beneficial arthropods, especially pollinators. The increased rate of NPK on the sunflower varieties significantly increased the abundance of honeybees in the study and significantly increased the time spent on foraging flowers. These results suggested that soil quality improvement, can positively affect interactions with insect pollinators and thereby provide a higher quality food source for these insects (Arancon et al. 2007). SAM-SUX-1 variety in this study had increased number of *A. mellifera* visiting the flowers, suggesting that the cues produced by this sunflower variety are more appealing to the honeybees, than flowers of Jos local variety. This implies that SAM-SUX-1 variety might have produced more nutritious floral rewards for honeybee pollinators than Jos local variety (Dieringer et al. 1999).

In conclusion, the study revealed that increased application of NPK 15-15-15 fertilizer to sunflower plants increased their flower quality which subsequently attracted increased pollinator diversity. Insect pollinators do not only rely on flower physiometrics, but also use a variety of cues to identify high quality flowers. Also, NPK 15-15-15 application at 90 kg/ha attracted high densities of honeybee and visit length which are essential for the conservation of insect pollinators and to improve crop yield.

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