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SYNERGISTIC INTERACTIONS BETWEEN HONEYBEE *Apis mellifera* L. AND FLOWER COLOUR OF SUNFLOWER IN RESPONSE TO NPK FERTILIZER APPLICATION

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Abstract

Plant colour contrast as perceived by insect pollinators is important in our understanding of pollination ecology, foraging theory and conservation of plant species. Field experiment was conducted on the interactions between honeybee Apis mellifera L. and flower colour contrast of sunflower as influenced by different levels of NPK 15-15-15 fertilizer application at Forestry Research Institute of Nigeria, Jericho-Hills Ibadan. Two sunflower varieties combined with four levels of NPK-15-15-15 fertilizer were laid out in Randomized Complete Block Design with three replicates and data were collected on honeybee abundance, sunflower ray florets colour contrast, vitamin A and β-carotenoid contents of the ray florets, crude protein, fats and flavonoid contents of pollen grain and foraging response of honeybees to the treatment. The degree of association between honeybee length of visit and ray florets colour contrast was also investigated. Analysis of Variance, SNK test, and Pearson's correlation (α=0.05) were used for data analysis. Results indicated that NPK-15-15-15 fertilizer significantly (p>0.05) increased pollen grain crude protein, fat, flavonoid contents, vitamin A and β-carotenoid contents of the flowers florets of the sunflower varieties used in the study at 90 kgha^{-1}. Honeybees also demonstrated distinct foraging preference for both sunflower varieties at 90 kgha^{-1} NPK 15-15-15 (p<0.05) corresponding to golden yellow in Jos Local and titanium lemon yellow in SAM-SUX-1 variety, relative to other yellow colour tints.

Key Words: *Honeybee, Interactions, Flower colour, Sunflower, NPK fertilizer*

Introduction

Honeybees (*Apis mellifera* L.) play an important ecological role as pollinators of many plant species thereby enhancing agricultural productivity and biodiversity conservation (Britain *et al.*, 2013). Several studies conducted on insect-plant interactions shows that while interesting specialized relationships exist between host-plants and their insect, many factors including the nutrient status of the host plant affect the physiology of foraging insect pollinators (Leiling, 2013). *Apis mellifera* L. especially uses pheromones, chemical signals, dance language and clues which are extremely important for their survival to locate or detect resources. They actively seek out flowers with pollen with higher nutritive values and nectars having sugar content and pollen. Honeybees have trichromatic colour vision that can distinguish color, shape and symmetry through thousands of ommatidia present in their compound eyes while the ocelli only provide information about light intensity (Jha and Vandermeer, 2009). The behaviour and life parameters of *A. mellifera* are influenced by environmental factors, such as temperature, moisture, habitat morphological and chemical components of host-plants (such as nitrogen, sugars, amino acids, semio-chemicals in host plants and the plant water content) (Slansky and Rodriguez, 1987; Fischer and Fiedler, 2000). Honeybees have ability to learn and remember the color, shape, and fragrance of flowers that are bountiful in these nutrients. According to Rostami *et al*. (2016), increased levels of NPK fertilizer application can cause a significant effect on spike and flower production as well as floret quality of the host-plant. Changes in the biosynthesis of floral pigments compounds such as anthocyanins, carotenoids, and betalains as a result of changes in the plant nutrient can lead to modification in the colour-odour combination in flowers and subsequently affect pollinator visitation pattern (Milet-Pinheiro *et al.,* 2012; Dormont *et al.,* 2014). Colour acts as a long distance cue and location signal for insect visitors. In sunflower for instance, the presence of bright yellow ray florets serves as a visual attraction for insects especially pollinators (Thapa, 2006, Stang *et al*., 2007). The floral arrangement and sequence of flower opening allows them to be assisted when visited by honeybee during sunflower pollination process (Morgado *et al.*, 2002). Although honeybees can fairly perceive a broad color range, they strongly differentiate yellow, blue-green, blue, violet, ultraviolet, bee purple (a mixture of yellow and ultraviolet) colours but sees red poorly (Celli and Maccagnani, 2003).

Honeybee-host-plant flower colour (especially colour contrasts) interaction have been reported to be important in foraging studies and predictor of detectability in biological research especially in species management, understanding of foraging theory and pollination ecology (Kendal *et al*., 2013). To measure plant colour traits in biodiversity and conservation management applications, the use of digital cameras with internet web software is gradually gaining attention as it overcomes the short comings of spectradiometer and the imperfection of human measurement (Dominy *et al*., 2001; Mausfeld, 2003). Examples are the RGB analysis software and the image colour summarizers which are based on an RGB (red colour, green colour and blue colour) and HSL (hue, saturation and lightness) colour system (Stevens *et al.*, 2007). An understanding of honeybee – flower colour contrast interaction due to host-plant nutrient status is of immense benefit to agriculture, food security and ecosystem conservation in Nigeria ecosystem.

This study was carried out to investigate the flower colour response of sunflower to different rates of NPK 15-15-15 fertilizer and its interaction with honeybee in the southwestern part of Nigeria.

Materials and Methods

Experimental Site and Planting Materials

The study was carried out at the Arboretum of Forestry Research Institute of Nigeria, Jericho-Hills, Ibadan, Oyo State, Nigeria (7° 22' 34.608" N 3° 50' 37.572" E) between May to September, 2017. Seeds of two sunflower varieties (Jos Local and SAM-SUX-1) were sown directly on a 6 m^2 plot with spacing of 50 cm × 50 cm in a 2 × 4 factorial fitted into Randomized Complete Block Design (RCBD) with 3 replicates on a 300 m^2 experimental field. N.P.K. 15-15-15 fertilizer was split applied at 2 - and 4 weeks after planting (WAP) at the rate of 0 $kgha^{-1}$, 30 $kgha^{-1}$, 60 $kgha^{-1}$ and 90 $kgha^{-1}$.

Effect of NPK 15-15-15 fertilizer on flowers of two sunflower varieties

At one week after the onset of flowering, pollen grains from twenty randomly selected flowers in each plot were dusted and analyzed for crude protein, fat and flavonoid contents, the vitamin A and β carotenoid contents of the ray florets were also analysed using spectrophotometer (Cardoza *et al*., 2012). Six random sunflower stands were tagged per plots for colour contrast quantitative examination. The ray florets of these flowers were photographed with Sony digital video camera (DCR-SX22E) and the images uploaded unto HP 655 laptop for analysis using Image Color Summarizer (ICS) 2019 software (Mausfeld, 2003; Stevens *et al*., 2007)

Responses of honeybee to effect of NPK 15-15-15 fertilizer on flowers of two sunflower varieties

The number of honeybee and foraging time (min) was recorded daily for each of the treatments from one week after flowering till seed maturity between 8.30 am and 12.30 pm. These observations were recorded using a hand tally counter and chronometer (stopwatch) with an accuracy of 0.01 second to record the time spent per flower and the number of honeybees visiting at the time of study (Cardoza *et al*., 2012).

Data Analysis

The numbers of honeybees collected were transformed using square root model ($\sqrt{(X+0.5)}$), the relative densities and foraging time of honeybees collected in the different treatment plots were compared using ANOVA ($p<0.05$) and where there were significant differences, Studentized Newman-Keuls (SNK) test were used for means separation. Correlation matrix was carried out to study the influence of ray floret colour contrast of the two sunflower varieties on honeybee foraging time.

Results

Effects of different levels of NPK 15-15-15 fertilizer on pollen and ray florets quality of two sunflower varieties

Significant variations was recorded in the concentrations of crude protein, fat, flavonoid, vitamin A and β-carotenoid in both the pollen grain and ray floret of the sunflower varieties used for the study under the influence of different application of the NPK (Table 1). There was significant increase in the protein and flavonoid contents of the pollen grains of the Jos Local sunflower varieties used in the study with the highest ($p < 0.05$) concentration at 90 kg/ha NPK 15-15-15 fertilizer while crude protein content was statistically similar at 30 and 60 kg/ha NPK 15-15-15 and fat content did not differ at 60 and 90 kg/ha NPK 15-15-15 (Table 1). The highest concentrations of vitamin A (0.80 ± 0.00) and flavonoid (56.07 ± 0.51) were found at 90 kg/ha NPK 15-15-15 fertilizer application in Jos Local.

Increased fertilizer level to soils of SAM-SUX-1 sunflower variety significantly increases the flavonoid (42 ± 0.00), vitamin A (0.67 ± 0.00) and β-carotenoid (86.90 ± 0.04) content of both pollen grain and ray florets with the highest concentrations recorded at 90 kg/ha, while the control plots recorded the lowest (Table 1). At 60-90 kg/ha NPK 15-15-15, the concentrations of crude protein and fat in the pollen grains of SAM-SUX-1 sunflower variety were statistically similar (Table 1).

Effects of different levels of NPK 15-15-15 fertilizer on yellow contrast of ray florets of two sunflower varieties

Yellow contrasts variation was observed in the colours of ray florets of the two sunflower varieties used in the study at different concentrations of NPK 15-15-15 fertilizer (Plate 1). The in-situ ray florets digital images further processed by Image Color summarizer Software (2019) revealed that the mean Red, Green, Blue (RGB) and Hue, Saturation, Luminosity (HSL) intensity values of both sunflower varieties varied significantly along the fertilizer rates (Tables 2 and 3). There were no significant differences in red reflectance spectra of the ray florets of Jos local at 30, 60 and 90kg/ha as well as its blue attribute at 60 and 90kg/ha while at 0 and 30 kg/ha blue attribute were statistically similar (Table 2). For ray florets of SAM-SUX-1 at 60 and 90 kg/ha, red attributed were statistically similar, while green and blue reflectance spectra at all the NPK 15-15-15 rates were not affected at all (Table 3). While the highest colour saturation of 97% was observed in Jos Local variety at 90 kg/ha, it was also observed that the higher the fertilizer rate, the lower hue intensity (61 - 51%) (Table 2). Although, there were no consistent differences in the mean values of the saturation intensity of SAM-SUX-1 ray florets at the different fertilizer rates, the luminosity increases (79 - 89%) as the fertilizer levels increased (Table 3). Furthermore, as the level of fertilizer applied increases, the brighter the simulated images of the ray florets in both sunflower varieties used (Plates 2 and 3).

Honeybee responses to different levels of NPK 15-15-15 fertilizer on two sunflower varieties

On Jos Local variety flowers, honeybees abundance (61.97± 10.99) and length of visit (1.5 ± 0.18 sec) were significantly higher at 90 kg/ha compared to the control in which the lowest mean values of 7.03± 2.53 and 0.47± 0.12 sec, respectively, were recorded (Table 4). Similarly, highest number ($p < 0.05$) of honeybees and the length of visit time were observed at 90 kg/ha which was statistically similar to 60 kg/ha on SAM-SUX-1 flowers.

Table 1: Pollen (Crude Protein, Fat and Flavonoid) and ray floret (Vitamin A and β-carotenoid (μgg^{-1})) quality of sunflower varieties grown in soils treated with different NPK 15-15-15 levels.

Variety	NPK 15-15-15	Pollen quality			Ray floret quality	
		Crude Protein (%)	Fat (%)	Flavonoid (mg/100)	Vitamin A	β-carotenoid
Jos Local	0	0.04±0.00c	0.01±0.00c	0.02±0.00d	0.02 ± 0.0d	1.60 ± 0.34d
	30	0.07±0.00b	0.02±0.00b	0.24±0.00c	0.49±0.0c	9.88 ± 0.04c
	60	0.08±0.00b	0.03±0.00a	0.27±0.00b	0.74 ± 0.0b	17.74 ± 0.10b
	90	0.13±0.01a	0.03±0.00a	0.31±0.00a	0.80 ± 0.0a	56.07 ± 0.51a
SAMSUX-1						
	0	0.03±0.00c	0.01±0.00b	0.05±0.03c	0.01 ± 0.00d	1.28 ± 0.00d
	30	0.11±0.01b	0.04±0.00a	0.25±0.00b	0.50 ± 0.0c	53.30 ± 0.02c
	60	0.14±0.00a	0.04±0.01a	0.28±0.00b	0.59 ± 0.0b	82.44±0.55b
	90	0.16±0.01a	0.05±0.01a	0.42± 0.00a	0.67 ± 0.0a	86.90± 0.04a

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

Jos Local 0kg/ha

Jos Local 30kg/ha

Jos Local 60kg/ha

Jos Local 90kg/ha

SAM-SUX-1 0kg/ha

SAM-SUX-1 30 kg/ha

SAM-SUX-1 60 kg/ha

SAM-SUX-1 90 kg/ha

Plate 1: *In situ* images of flowers of two varieties sunflower grown on soils treated with different NPK 15-15-15 levels

Table 2: Yellow attributes values from digital photo of ray floret of Jos Local sunflower variety grown in soils treated with different NPK 15-15-15 (Kg/ha) fertilizer levels

Treatment (Kg/ha)	Yellow tints	R (± SEM)	R %[a]	G (± SEM)	G %	B (± SEM)	B%	H %	S %	L
0	Strong Olive	173.50±0.50b	46.60	176.67±0.56c	47.50	21.83±1.66a	5.90	61:	87:	70
30	Mustard yellow	202.83±5.41a	48.40	184.83±5.40c	47.80	15.83±4.38a	3.80	59:	93:	79
60	Lemon yellow	216.83±8.80a	50.30	200.33±7.89ba	48.10	6.83±1.54b	1.60	57:	97:	80
90	Golden yellow	217.17±7.00a	53.30	207.17±6.63a	45.30	5.83±1.94b	1.40	51:	97:	76

Means followed by the same letter along the same row are not significantly different using Studentized Newman-Keuls (SNK) test ($p > 0.05$). N= 1,508cm^2 of pixel area were analyzed for each shade of yellow and the name of colours were provided by Image Colour Summarizer(ICS) 2019 web service. [a]= Percent red [R% = R/(R+G+B]; G% and B% were defined similarly. R: red, G: green, B: blue, Hue (H), Saturation (S) and Luminosity (L)

Table 3: Yellow attributes values from digital photo of ray floret of SAM-SUX-1 sunflower variety grown in soils treated with different NPK 15-15-15 (Kg/ha) fertilizer levels

Treatment (Kg/ha)	Yellow tints	R (± SEM)	R %[a]	G (± SEM)	G %	B (± SEM)	B%	H %	S %	L
0	Gold mustard	201.5±9.09b	47.90	202.83±10.94a	48.20	16.17±6.75a	3.80	60:	93:	79
30	Green lime	214.5±10.54ba	48.40	205.67±10.28a	46.40	22.83±11.69a	5.20	58:	90:	81
60	Bright Gold	227.67±5.17a	48.90	222.33±8.09a	47.80	15.17±6.47a	3.30	58:	94:	87
90	Titanium lemon yellow	235.33±4.59a	46.90	230.17±5.62a	45.90	36.5±17.94a	7.30	59:	85:	89

Means followed by the same letter along the same row are not significantly different using Studentized Newman-Keuls (SNK) test ($p > 0.05$). N= 1,508cm^2 of pixel area were analyzed for each shade of yellow and the name of colours were provided by Image Colour Summarizer (ICS) 2019 web service. [a]= Percent red [R% = R/(R+G+B]; G% and B% were defined similarly. R: red, G: green, B: blue, Hue (H), Saturation (S) and Luminosity (L) [a]= Percent red [R% = R/(R+G+B]; G% and B% were defined similarly

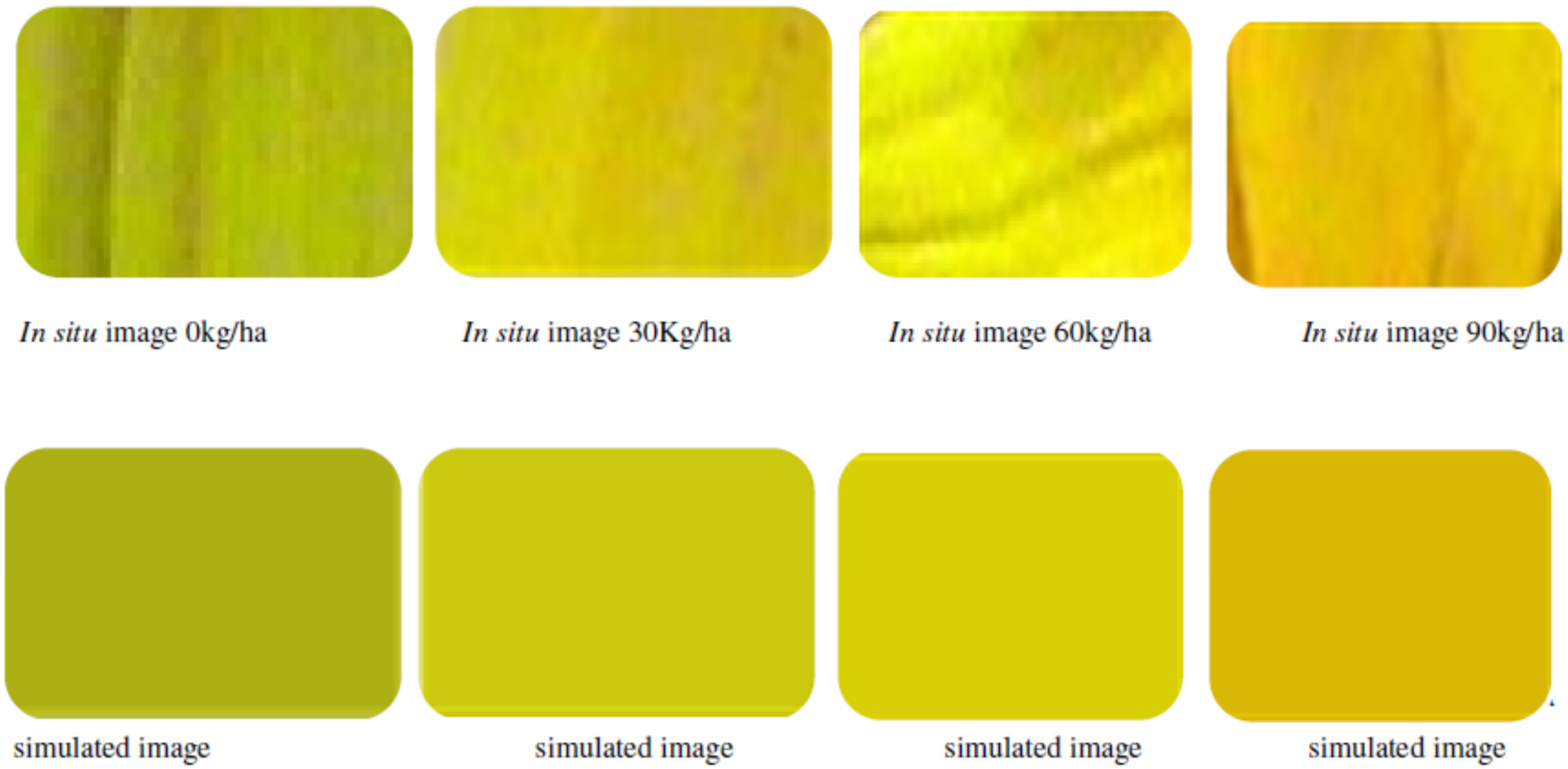

Plate 2: Yellow colour variants of Jos Local sunflower ray floret at different levels of fertilizer as capture by Image colour Sumarrizer and Colour Code Converter software

Plate 3: Yellow colour variants of SAM-SUX-1 sunflower ray floret at different levels of fertilizer as capture by Image colour Sumarrizer and Colour Code Converter software

Table 4: Honeybee abundance and visit length (secs) on two sunflower varieties grown in soil treated with different levels of NPK 15-15-15 fertilizer (Kg/ha)

Response variables	Sunflower	NPK 15-15-15 (Kg/ha)			
		0	30	60	90
Honeybees abundance	Jos Local	7.03 ± 2.53c	27.63 ± 4.49b	56.3 ± 14.89ab	61.97 ± 10.99a
Visit length (sec)		0.47 ± 0.12d	0.9 ± 0.12c	1.3 ± 0.20b	1.5 ± 0.18a
Honeybees abundance	SAM-SUX-1	22.33 ± 9.15c	62.63 ± 23.40b	93.43 ± 10.30a	101.07 ± 19.86a
Visit length (sec)		0.60 ± 0.14d	1.13 ± 0.25c	2.07 ± 0.32b	2.17 ± 0.25a

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

Honeybee responses to different yellow colour contrasts of ray florets of two sunflower varieties grown in soils enhanced with different levels of NPK 15-15-15. Furthermore, on Jos local variety, golden yellow was the most preferred colour for foraging by honeybee with the highest visiting time observed at 90 kg/ha, while strong olive recorded in the control was the least preferred (Figure 1). Similarly, on SAM-SUX-1 variety, titanium yellow was the most preferred for foraging by honeybees with the highest visiting time observed at 90 kg/ha, while gold mustard recorded in the control had the lowest visiting time (Figures 2).

Discussion

Increased rates of NPK 15-15-15 irrespective of variety, increased protein, fat, flavonoids, vitamin A and beta-carotene contents of the pollen grain, which are important in the body metabolism of honeybee workers as well as providing clues to help the bees in identifying its host plant. Many past studies have indicated that soil nutrient enhancements can affect insect-plant interactions (Cardoza, 2011; Cardoza *et al*., 2011). However, this study further stressed Cardoza *et al*. (2012) that application of soil amendment can impact plant interactions with beneficial arthropods especially the pollinators.

The simulated images generated by the image colour summarizer (ICS) and Colour Code Converter Software (CCCS) from the captured sunflower images revealed that as the concentrations of NPK 15-15-15 in the two varieties increased the brighter the yellow colour of the ray florets. Increased levels of NPK application has been reported to increase flower production, flower quality and floral colour variation in flowering plants (Anamika and Lavania, 1990).

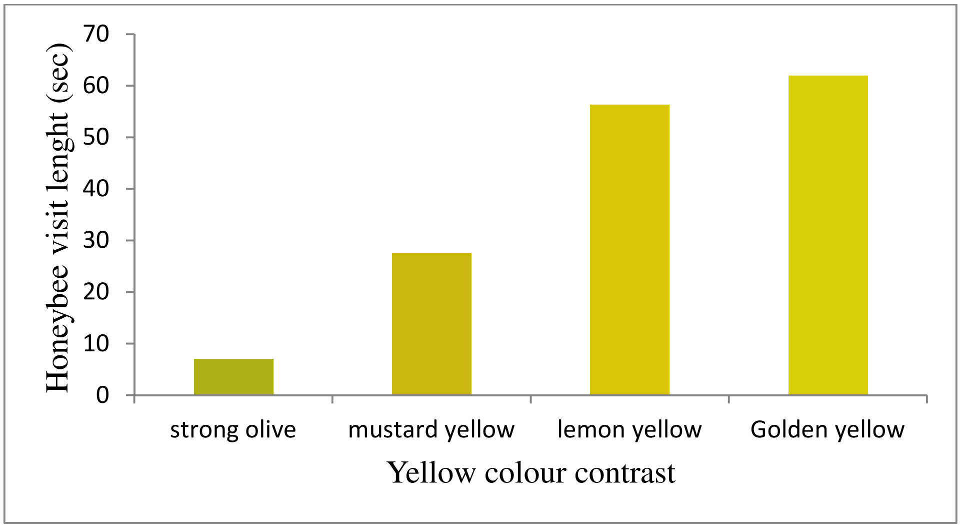

Fig. 1: Ray floret yellow colour contrast of Jos Local sunflower variety grown in soils treated with different NPK 15-15-15 (Kg/ha) levels and the visit length (secs) of honeybee. Strong olive= 0 kg/ha, Mustard yellow= 30 kg/ha, Lemon yellow= 60 kg/ha, Golden yellow= 90 kg/ha

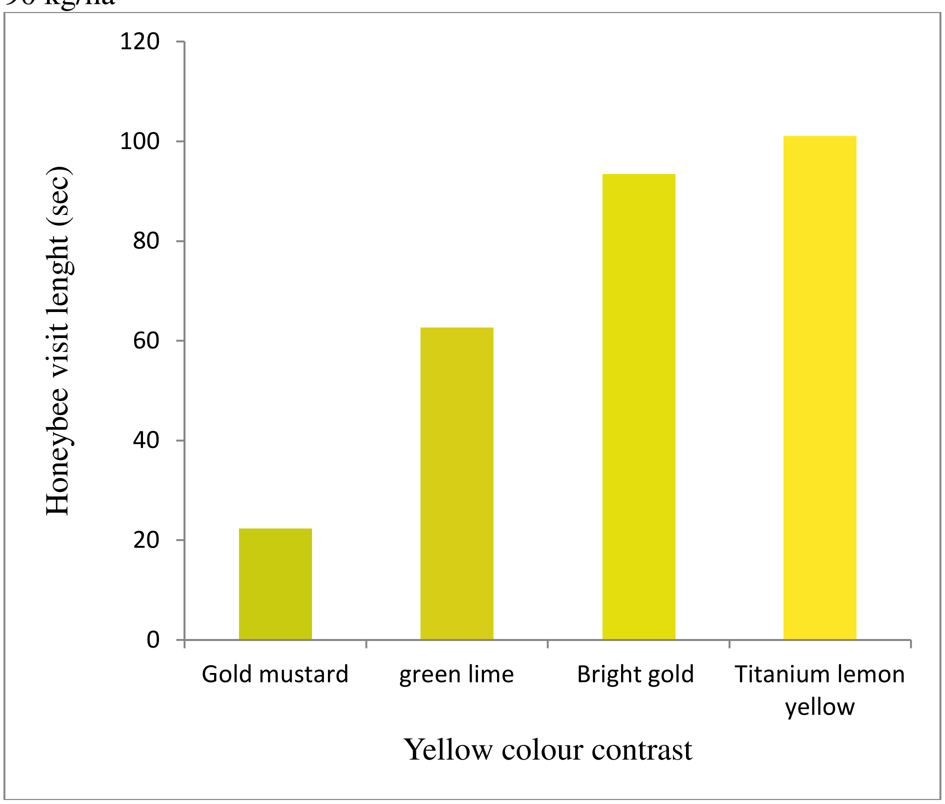

Fig. 2: Ray floret yellow colour contrast of SAM-SUX-1 sunflower variety grown in soils treated with different NPK 15-15-15 (Kg/ha) levels and the visit length (secs) of honeybee. Gold mustard= 0 kg/ha, Green lime = 30 kg/ha, Bright gold= 60 kg/ha, Titanium lemon yellow= 90 kg/ha

As recorded in this study, SAM-SUX-1 variety had increased number of A. mellifera visiting the flowers, suggesting that the cues produced by this sunflower variety is 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).

Colour contrasts between species have been reported to be important in foraging studies (Schmidt *et al*., 2004). Although, honeybee uses multisensory cues such as flower colour, odour, shape and other flower traits to direct them to potential sources of food in plant-pollinator interactions, one or both of these cues are altered by the soil amendments (Barber *et al*., 2011). Similar observation has been reported for Polemonium viscosum Nutt which has preference for flower colours varying from light blue, blue purple, to purple flowers and scent varying from "sweet" to "skunky" scent (Galen and Kevan 1980). McCall and Primack (1992) also found out that colouration influenced frequency of insect visitors; Apis mellifera recorded higher visits on yellow and mixed coloured flowers.

Conclusion

Based on the study, it can be concluded that increase in the soil nutrient with NPK 15-15-15 fertilizer increased the crude protein, fat, flavonoid content of pollen grain and vitamin A and β carotene content of ray florets of sunflower varieties used. This further increased the number of Apis mellifera adansonni visiting the flowers as well as the length of time spent to forage. Also, there was a strong correlation between variation in yellow tints of sunflower and its attractiveness to honeybees and the time they take to forage. They appear to like golden yellow from Jos local and titanium lemon yellow from SAM-SUX-1 sunflower varieties. Flower colour is therefore is essential in honeybee attraction for pollination and conservation ecology.

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