

Effects of *Megachile* sp, an insect flower visitor on nutrient content of progenies of cowpea [*Vigna unguiculata* (L.) Walp]

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Abstract: Though cowpea (*Vigna unguiculata* (L.)Walp) possess several nutritional values its mode of pollination is fraught with some forms of confusion. Attempt to ascertain the role of insect visitors on the plant led to collection of seeds from flowers visited by bee species known as *Megachile* (experimental plants) and flowers not visited by any organism (control plants). The seeds were re-sown to determine the influence of *Megachile* on nutrient contents of the seeds of the progenies. Nutrient analysis of the seeds from the progenies was done. Mean values were compared with paired t-test and Pearson correlation. Higher starch (carbohydrate) (362.6 ± 0.40 mg/Kg to 374.4 ± 0.28 mg/Kg) values were recorded for progenies of control than the experimental plants (340.4 ± 0.44 mg/Kg to 352.9 ± 0.27 mg/Kg). Differences between means were highly significant ($t = 16.91$; $P < 0.00$; $df = 17$). Mean calcium, phosphorus and protein values of seeds of the progenies of experimental plants were higher than that of the control plants. There was very strong positive correlation between protein and starch ($r = 0.727$; $p < 0.01$) for the progenies of control, but negative correlation between protein and starch ($r = -0.476$; $P < 0.05$) for experimental plants. In conclusion, visitation of *Megachile* to a cowpea flower may cause cross-pollination which may induce seeds of the progenies to develop higher calcium, phosphorus and protein values. It is recommended that cowpea farmers should adopt the best pest control measures in order to preserve useful insects to cause cross-pollination.

Keywords: *Megachile*, fibre, cross-pollination, nutrient, calcium, cowpea.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a leguminous crop that has established itself as very useful to human existence. Many parts of the crop serve various purposes. This can be attested to in several ways. For example, cultivated cowpea is the most important source of plant protein in the human diet in tropical Africa, particularly in the Sahel region (Marconi, Lombardi-Boccia, Camovale, & Ng, 1990). Singh, Mai-Koodomi and Terao (1995) also asserted that cowpea serves as a major source of protein in the daily diets of the rural and urban poor. In West and Central Africa, cowpea grains are primarily in demand for human consumption. They are used in over 50 different dishes in both whole grain and milled forms (Dovlo, Williams, & Zoaka, 1976). Boiled whole grains are sometimes eaten with oil and seasoning, but more common uses of whole grains are mixtures of cowpea and cereals (e.g. rice and beans) and as an ingredient in soups or stews. The most common use of milled cowpea grains in West Africa are fried cowpea balls called “akara”, “kosai”, “akla”, “accara”, and steamed cowpea cakes called “moin-moin”, “Ole-le”, or “tubani” (Dovlo *et al.*, 1976).

Cowpea can be cooked as leafy vegetable, or as green pods, green seeds or dry seeds cooked and mixed with maize or sorghum (Saxena & Kidiavai, 1997). According to Singh *et al.* (1995) the tender leaves of cowpea are eaten as spinach-like vegetable, while it’s immature pods and seeds are also consumed as vegetables. Farmers in the dry savanna use cowpea

haulms as a nutritious fodder for their livestock. The plant's ability to fix atmospheric nitrogen helps to maintain soil fertility while its tolerance to drought extends as adaptation to drier areas considered marginal for most other crops (Singh *et al.*, 1995). Ahenkora, Adu Dapaah, and Agyeman (1998) also indicate that the young leaves of cowpea are consumed in several countries in Africa, Asia and the Pacific. Also, the leaves are among the top three or four leafy vegetables that are marketed and consumed in Africa (Imungi & Potter, 1983). Furthermore, the young tender leaves serve as relish along with the main staples, as the source of carbohydrates, proteins, fats, β carotene, and vitamins B and C (Enwere, McWatters & Phillips, 1998; Mamiro, Mbwaga, Mamiro, Mwanri & Kinabo, 2011). Mamiro *et al.* (2011) observed that other vegetative parts of cowpea plants are used as feed, forage hay and silage for livestock after removal of the grain. Vurukonda, Vardharajula, Shrivastava and Skz (2016) also reported that the leaves of cowpea can be used as green manure and organic material for soil amendment and fertilisation.

Ghanaians generally prepare and eat cowpea as a whole or as part of a meal. Meals like 'koose' (cowpea fritters) and 'gari' and beans (roasted graded fermented cassava and cooked beans) have cowpea as the main raw material (Asare, Agbemafle, Adukpo, Diabor & Adamtey, 2013). Further, it is used in Ghana for preparing soup and stew (Appiah, Asibuo & Kumah, 2011). However, in developed countries, cowpea is processed into flours and used as protein concentrate and isolates and animal feed formulation (Chinma, Alomede & Emelife, 2008).

Some health benefits of cowpea have been reported. For example, cowpeas are known to be high in polyphenols, compounds that act as antioxidants in the body to prevent cell damage and protect against disease (Link, 2020). Other health benefits include benefits such as toning the spleen, stomach and pancreas; helps induce urination and relieves damp conditions like leucorrhoea (Link, 2020). It is also reported to boost weight loss, reduces level of hunger, lowers risk of increased belly fat, lowers risk of obesity, helps reduce body fat percentage, prevents digestive disorders, stimulates the growth of the beneficial bacteria in the gut to help foster a healthy microbiome to reduce inflammation, enhanced immune function, and reduced cholesterol levels; helps to keep the heart healthy and strong (Link, 2020).

Iqbal, Khalil, Ateeq and Sayyar Khan (2006), Clifton (2010) and the United Nations (UN, 2014) documented similar things when they indicated that health organizations around the world recommend consuming legumes as part of a healthy diet, because they have an important role in the control and prevention of chronic non-communicable diseases (NCDs) such as diabetes, cardiovascular diseases and cancer. The legumes also favour the control of body weight, since they give greater satiety, prevent the accumulation of fat at the abdominal level and regulate blood sugar levels (Tharanathan & Mahadevamma, 2003; Williams, Grafenauer & Shea, 2008; Clifton, 2010).

Apart from the above, various parts of cowpea are reported to cure a number of diseases (Awurum, 2000; Okwu & Njoku, 2009) against snake bite (HBT, 2016); as tonics, appetizers, stimulants, aphrodisiacs and anthelmintic (FAO, 2016); and fighting malnutrition (Alemu, Asfew, Woldu, Fanta & Medvecky, 2016; Food and Agriculture Organization (FAO, 2016).

All the above benefits of cowpea make the crop very appealing to a large segment of society including the rich and poor. Similarly, pollination biologists have also become attracted to this wonderful crop. To satisfy his curiosity about cowpea pollination, the researcher undertook a study to ascertain whether cowpea is wholly self-pollinated or both self- and cross-pollinated. In that study the researcher studied probable insect pollinators of cowpea. It was realized that a bee species known as *Megachile* visited some flowers. Results suggested that *Megachile* sp might be a probable pollination agent of cowpea. However, it was not clear whether the probable pollination phenomenon would have any effect on the progenies, especially nutrient values of seeds. Therefore seeds harvested from pods developed from flowers not visited by any organism (control group) and those visited by *Megachile* sp (Experimental group) were re-sown. In order to determine the probable effects of *Megachile* sp on the nutrient contents of the seeds from progenies this study was conceived. Hence, the main aim of the study was to ascertain the effects of *Megachile* sp as probable pollinator of cowpea on the seeds of progenies of flowers visited by the bee.

Therefore, the null hypothesis is that there is no statistically significant difference between the mean nutrient contents of the seeds from progenies of the experimental and control groups. The alternate hypothesis is that there is statistically significant difference between the mean nutrient contents of the seeds of the experimental and control groups. Another null hypothesis is that there is no relationship between the mean nutrient contents of the seeds of the progenies of control

plants and between mean nutrient contents of the seeds of experimental plants. The alternate is that there is a relationship between the mean nutrient contents of the seeds of the progenies of control plants and between mean nutrient contents of the seeds of experimental plants.

2. METHODOLOGY

This study was undertaken at Ekwamkrom in the Gomoa Central District of the Central Region of Ghana. Seeds obtained from pods developed from flowers visited by *Megachile* sp (experimental group) and unexposed flowers (control group) in an earlier experimental farm were sown again. Seeds were sown on a plot of land as described by Hordzi (2018). When the plants fruited and matured, seeds were removed from harvested pods and used for nutrient analysis. Seeds were collected from pods grown on various blocks.

Seeds of the progenies of the control and experimental groups were oven-dried and ground. Zero point two grammes (0.2 g) of each ground specimen was weighed into a digestion tube. After that, 4.4ml of digestion mixture was added and digested for 2 hours. The digest was diluted into a 100ml volumetric flask. The solution was then used for the determination of nitrogen (protein), calcium and phosphorus.

Protein (Kjeldahl method)

An aliquot of the diluted digest was taken and distilled with alkali mixture for nitrogen using boric acid indicator. The distillate was titrated with 1 / 140 M HCl. If T ml of 1 / 140 M HCl were required for the titration, then:

$$\% N = \frac{T \text{ (ml)} \times \text{Solution volume}}{10^2 \times \text{aliquot} \times \text{sample}}$$

Therefore % protein = % N x 6.25 (Allen, Grinshaw & Quarmby, 1974). The values obtained were multiplied by 10,000 to convert them into milligrams per kilogramme (mg / Kg)

Calcium, using the flame photometer

A stock solution (1000 ug/ml Ca) was prepared by dissolving 2.4973g of dry CaCO₃ in 200 ml water containing 5ml concentrated HCl and made up to 1 litre. Out of this, solutions with the concentrations 0, 10, 20, 30 40, and 50 ug/ml Ca were made. The digest that was prepared for the protein determination was then used for the determination of calcium also. The flame photometer was switched on and warmed for 15 minutes. The calcium filter was then selected. The system was flushed with distilled water. The standards were then aspirated and their emissions recorded. The samples were also aspirated and their emissions recorded. With the concentrations and emissions of the standards a calibration curve was plotted. Using the emissions of the samples, the concentrations (c ug/ml) of calcium in the samples were extrapolated from the calibration curve. (Allen *et al.*, 1974).

$$\text{Therefore \% Ca} = \frac{c \text{ (ug/ml)} \times \text{Solution volume}}{10^4 \times \text{Sample weight}}$$

The values obtained were converted into mg/Kg by multiplying with 10, 000.

Phosphorus

A standard solution of phosphorus (P) was prepared in 25 ml volumetric flasks. Aliquot of dissolved digest was taken into 25 ml volumetric flasks. After that 4 ml of colour forming reagent was added to the standards and samples. More water was added to the 25 ml mark and the mixture shaken. Colour development was allowed over 15 minutes. The absorbances of the standards and samples were determined at 882nm using a spectrophotometer. A standard curve was drawn using the

concentration of the standards and their absorbances. The concentration of the aliquot was extrapolated with the absorbances of the samples in the 25 ml from the standard curve. The values were converted into mg/Kg. (Allen *et al.*, 1974).

Soluble carbohydrate (Starch)

Extraction:- Fifty milligrammes (50 mg) of ground sample was weighed into 100 ml conical flask and then 30 ml of distilled water was added and simmered gently on a hot plate for 2 hours. The mixture was allowed to cool slightly and filtered through number 44 whatman paper into 50ml volumetric flask. The paper was washed and the mixture diluted to 50ml mark when cooled.

Colour development: Two millilitres (2 ml) of each standard solution was pipetted into a set of boiling water tubes. Then, 2 ml of the extract was pipetted into boiling tubes and 10 ml of anthrone reagent was rapidly added to both standards and samples. The tubes were immersed in running cold water (Or ice bath). The tubes were placed in boiling water in a darkened fume cupboard and boiled for 10 minutes. The tubes were then placed in cold water and allowed to cool. The optical density was then measured at 625 nm. A calibration graph was prepared from the standard and used to obtain mg glucose in the sample aliquot.

$$\text{Therefore \% Soluble Carbohydrate} = \frac{C \text{ (mg)} \times \text{extract volume}}{10 \times \text{aliquot} \times \text{sample weight}}$$

The values obtained were converted into mg/Kg (Allen *et al.*, 1974)

Crude fibre

Acid Hydrolysis:- One gramme (1 g) of ground sample was weighed into 600 ml Pyrex beaker. After that 100 ml of boiling 1.25% Sulphuric acid (H₂SO₄) was added. It was covered with a watch glass and boiled gently on a hot plate for 30 minutes. The acid was removed by suction. The mixture was washed three times with 50 ml boiling water.

Alkali extraction: - Hundred millilitres (100 ml) of boiling 1.25% Sodium hydroxide (NaOH) was added to the mixture. The set up was covered with watch glass and boiled gently on hot plate for 30 minutes. The alkali was removed through suction. The mixture was then washed with 25 ml boiling 1.25% H₂SO₄. It was then washed two times with 50 ml boiling water. The contents of the beaker were washed into a weighed glass crucible using 50 ml boiling water. It was further washed with 30 ml ethanol. The crucible and its contents were dried for three hours at 105°C. It was then cooled and weighed. The weight difference gave the fibre content. (Allen *et al.*, 1974).

3. DATA ANALYSIS

Means of the values were determined and compared using paired t-test. Also, Pearson Correlation between the mean values of progenies of control plants as well as between mean values of progenies of the experimental plants were done.

4. RESULTS AND DISCUSSION

The mean values of starch, calcium and phosphorus obtained from seeds of progenies of control and experimental groups have been presented in Table 1. Similarly, the t-test results of all the nutrients have been presented in Table 2.

It is scientifically proven that consumption of cowpea supplies most of the macro nutrients which includes total carbohydrate, total fat, protein and alcohol and are primary contributors to energy intakes as well as micronutrients including minerals, vitamins and dietary fibre of the diet (Bialostosky, Wright, Kennedy-Stephenson, McDowell & Johnson, 2002). A study involving 30 newly developed Brazilian cowpea genotypes obtained by conventional plant breeding among other minerals revealed 9570–12,510 ppm potassium and 290–440 ppm calcium (da Silva, da Costa, Teixeira Junior, da Silva, dos Santos & Siviero, 2018). Snow, Spira, Simpson and Kilips (1996) also observed that if pollinators differing in foraging pattern visit self-compatible plants, their differential effect on host plant fitness would mainly appear in later, post-dispersal life cycle stages, causing the plant to produce low-quality seeds after pollination.

In this study, results of nutrient analysis showed that progenies of control group generally contained higher levels of starch (carbohydrate) ($362.6 \pm 0.40 \text{ mg/Kg}$ to $374.4 \pm 0.28 \text{ mg/Kg}$) than progenies of the experimental group ($340.4 \pm 0.44 \text{ mg/Kg}$ to $352.9 \pm 0.27 \text{ mg/Kg}$). The differences between the means were highly significant (Table 2), ($t = 16.91$; $P < 0.00$; $df = 17$). This suggests that the differences between the means were not due to chance or experimental error. Photosynthesis produces carbohydrates. No doubt under this study, chlorophyll fluorescence induction kinetics discussed earlier in Hordzi (2018) showed that photosynthesis was more efficient in the progenies of control group than the progenies of the experimental group. The results suggest that visitation of *Megachile* sp might have not induced any trait to cause higher levels of starch in the seeds of the progenies.

Perina and Muranoa (2017) and Sherrell (2022) intimate that starch is the main source of carbohydrates in human diet for many people. Sherrell (2022) further postulated that starch plays a crucial role in a nutritious, well-balanced diet, as it provides the body with glucose, which is the main energy source for every cell. It also provides a range of vitamins, minerals, fibre, and other nutrients. Eating starchy foods may help increase satiety, which is the feeling of being full, after eating (Sherrell, 2022). It is obvious that the human body needs energy, minerals and vitamins from a balanced diet and if this can be obtained from cowpea seeds which can easily be cultivated in poor developing countries the better for mankind. Here, it can be said that seeds from progenies of both control and experimental plants provided high levels of starch for the seeds. Therefore any of these seeds can serve as useful source of starch for consumption. However, seeds developed from progenies of the control plants promised to provide higher levels of starch.

Calcium values of the seeds of progenies of control groups ranged between $2.1 \pm 0.21 \text{ mg/Kg}$ and $2.4 \pm 0.17 \text{ mg/Kg}$ while that of the experimental group ranged between $2.4 \pm 0.09 \text{ mg/Kg}$ and $2.6 \pm 0.26 \text{ mg/Kg}$. This shows that progenies of the seeds of the experimental group generally contained slightly more calcium than progenies of the control plants. The differences between the means were significant ($t = 2.19$; $P < 0.04$; $df = 17$). This also suggests that the differences were real but not due to chance. The results can be interpreted to mean that *Megachile* sp that visited flowers earlier on might have induced the flowers with some traits that influenced higher levels of calcium in seeds of the progenies of the flowers that they visited.

Tandogan and Ulusu (2005) as well as Pravina, Sayaji and Avinash (2013) chronicled some uses and health benefits of calcium such as essential in muscle contraction, oocyte activation, building strong bones and teeth, blood clotting, nerve impulse, transmission, regulating heart beat and fluid balance within cells. The requirements are greatest during the period of growth such as childhood, during pregnancy, when breast feeding (Pravina, Sayaji & Avinash, 2013). Long term of calcium deficiency can lead to oestoporosis in which the bone deteriorates and there is an increased rise of fractures. Eating a well-balanced diet can provide all the necessary nutrients and help prevent calcium deficiency (Pravina, Sayaji & Avinash, 2013). Considering these beneficial effects of calcium on the human body, it is obvious to say that cowpea seeds can help to boost the health of humans. In this study the seeds of either of the progenies of the control or experimental plants can be useful. However, seeds from the progenies of the experimental plants show a slightly higher advantage when it comes to calcium. This suggests that if *Megachile* species pollinates cowpea flowers it is possible that seeds produced by progenies will form higher levels of calcium which is useful for humans, especially those who are vulnerable so far as calcium diet is concerned.

Table 1: Mean Starch, Calcium and Phosphorus contents of the seeds of progenies of control and experimental plants

Block	Starch (in Mm/Kg)		Calcium (in mg/Kg)		Phosphorus (in mg/Kg)	
	PC	PEG	PC	PEG	PC	PEG
1	367.3 ± 0.12	352.9 ± 0.27	2.2 ± 0.18	2.4 ± 0.17	5.3 ± 0.12	6.1 ± 0.18
2	372.9 ± 0.38	349.6 ± 0.43	2.3 ± 0.15	2.5 ± 0.21	5.5 ± 0.17	5.9 ± 0.24
3	362.6 ± 0.40	342.6 ± 0.46	2.2 ± 0.12	2.5 ± 0.26	5.5 ± 0.12	6.0 ± 0.19
4	374.4 ± 0.28	350.2 ± 0.52	2.4 ± 0.17	2.4 ± 0.26	5.2 ± 0.15	6.2 ± 0.18
5	372.6 ± 0.38	340.4 ± 0.44	2.1 ± 0.21	2.6 ± 0.26	5.3 ± 0.26	6.1 ± 0.38
6	369.2 ± 0.45	340.7 ± 0.91	2.2 ± 0.15	2.4 ± 0.09	5.5 ± 0.12	6.0 ± 0.21

PC = Progenies of control (Progenies of unexposed flowers)

PEG = Progenies of experimental group (Progenies of flowers visited by *Megachile* sp.)

Results of phosphorus content indicates 5.2 ± 0.15 to 5.5 ± 0.17 mg/Kg for progenies of control and 5.9 ± 0.24 to 6.2 ± 0.18 mg/Kg for experimental group. This means that higher phosphorus content was recorded for experimental group compared to the control. The mean differences between control and experimental groups were highly significant ($t = 22.83$; $P < 0.00$; $df = 17$). Thus, the differences between the means for control and experimental groups were not as a result of chance or error but real. Therefore, it can be suggested that the higher phosphorus content of seeds from progenies of flowers visited by *Megachile* sp might have been induced by traits transmitted to the flowers during the visitation. This is suggestive of cross-pollination that transmitted useful characteristics to the seeds of progenies.

Scientifically, phosphorus forms bones and teeth, as well as repairing bones (Gal & Dahl- <https://edis.ifas.ufl.edu/pdf/FS/FS237/FS237-D3zzyqryma.pdf>). The known health benefits of phosphorus includes, keeping the bones and teeth strong, helping the muscles contract, aiding muscle recovery after exercise, filtering and removing waste from the kidneys, promoting healthy nerve conduction throughout the body, making DNA and RNA and managing the body’s energy usage and storage (Fletcher, 2019). Fletcher (2019) further intimates that phosphorus contributes to bodily functions that involve the nervous system, kidney function, muscle contraction and heartbeat regulation. Considering these health benefits of phosphorus in the human body, it will not be out of place to recommend eating cowpea seeds by individuals. This also means that eating boiled cowpea seeds from progenies cross-pollinated by *Megachile* sp may provide the human body more phosphorus in the diet. Since phosphorus aids muscle recovery after exercise, it is equally important to suggest that sport men and women eat cowpea seeds.

Table 2: Paired t-test results of the nutrients

Nutrient	t-value	Degree of freedom	Significance (2 tailed)
Starch PC – Starch PEG	16.91	17	.000
Calcium PC – Calcium PEG	-2.19	17	.043
Phosphorus PC – Phosphorus PEG	22.83	17	.000
Fibre PC – Fibre PEG	7.68	17	.000
Protein PC – Protein PEG	-22.60	17	.000

PC = Progenies of control (Progenies of unexposed flowers)

PEG = Progenies of experimental group (Progenies of flowers visited by *Megachile* sp.)

The mean values for fibre and protein contents of the seeds of the progenies of control and experimental plants have been presented in Table 3.

The seeds of cowpea are incredibly nutrient-dense, packing plenty of fibre and protein into each serving (Link, 2020). They carry good amounts of dietary fibre; provide 10.6 g or 28% of fibre per 100 grams (Rudrappa, 2009). Results from this study show that there were higher fibre contents for the control (26.5 ± 0.26 to 32.1 ± 0.26 mg/Kg) than in the experimental groups (23.9 ± 0.20 to 26.3 ± 0.35 mg/Kg). Comparison using t-test shows that there were high significant differences between the means for control and experimental groups ($t = 7.68$, $P < 0.00$; $df = 17$). So here also, the differences showing that control groups’ seeds had higher fibre contents than the experimental seeds were real. The implication here is that a visitation by *Megachile* sp. might have not influenced higher fibre content of the seeds from progenies.

Link (2020) observes that soluble fibre forms a gel-like consistency and moves through the digestive tract slowly to help keep one feeling full between meals. Increasing intake of soluble fibre helps to promote regularity and increase stool frequency in those with constipation thereby helping in digestive health. Thus, the fibre could help prevent digestive disorders, such as acid reflux, hemorrhoids, and stomach ulcers. Dietary fibre is reported to prevent the risks of chronic diseases such as cancer, cardiovascular disease (CVD), and diabetes mellitus (Roberfroid, 1997). Other health benefits include benefits such as toning the spleen, stomach and pancreas; helps induce urination and relieves damp conditions like leucorrhoea (Link, 2020). These submissions are in favour of seeds developed from progenies of self-pollinated cowpeas, in this case the control group because they produced more seeds with higher fibre contents than progenies of flowers seemed to be cross-pollinated by *Megachile* sp.

Sherrell (2022) observes that foods rich in starch are abundant fibre sources. No doubt that the seeds of the progenies of control plants contained higher levels of both starch and fibre. Chambers, Guess, Viardot and Frost (2011) also stated that

dietary starch and fibre, particularly the fermentable content, may be important for long-term weight loss and the prevention and management of Type 2 diabetes. Therefore, eating cowpea seeds is a useful source of weight loss. In this experiment it can be said that seeds of the progenies of both control and experimental plants may be useful for long-term weight loss and the prevention and management of Type 2 diabetes. However, seeds from progenies of the control plants may be better. This suggests that when it comes to starch and fibre contents of cowpea seeds self-pollination may produce better results than probable cross-pollination by *Megachile* sp.

Table 3: Mean fibre and protein contents of the seeds of progenies of control and experimental plants

Block	Fibre (in mg/Kg)		Protein (in mg/Kg)	
	PC	PEG	PC	PEG
1	26.5±0.26	25.2±0.37	232.7±0.25	249.6±0.38
2	32.1±0.26	25.1±0.28	235.6±0.19	248.9±0.20
3	28.4±0.31	26.3±0.35	230.8±0.17	253.9±0.21
4	29.6±0.21	25.4±0.27	232.9±0.15	249.8±0.35
5	27.4±0.21	23.9±0.20	234.3±0.19	249.0±0.36
6	27.7±0.23	24.6±0.25	234.0±0.21	252.1±0.15

PC = Progenies of control (Progenies of unexposed flowers)

PEG = Progenies of experimental group (Progenies of flowers visited by *Megachile* sp.)

Cowpea seeds are a cheap source of protein (Rocha, Ma, Vosátka, Freitas & Oliveira, 2019). Analysis of the improved cowpea varieties by Mamiro, Mbwaga, Mamiro, Mwanri and Kinabo1 (2011) showed relatively higher protein content ranging from 24- 26% than local varieties. Similar results were obtained from this study where seeds of progenies of control groups recorded lower levels of protein (230.8±0.17 to 235.6±0.19mg/Kg) than the experimental groups (248.9±0.20 to 253.9±0.21mg/Kg). Thus, the seeds of progenies of experimental group contained higher protein values than the control. The differences between the means of the two groups were also highly significant ($t = 22.60$; $P < 0.00$; $df = 17$), (Table 2). This clearly shows that the differences were not as a result of experimental error but were actual. This also suggests that a visitation of *Megachile* sp. to a flower might have caused pollination which induced a genetic factor to the seeds of their progenies such that they would contain higher levels of protein than when *Megachile* sp. did not visit the flowers.

Getting enough protein can help one to build a healthy immune system, fight infection and recover from illness more quickly. Protein rich foods are a good source of calories which is an important part of maintaining body weight throughout treatment (Oncology Nutrition, BC Cancer Agency, 2012). According to Hermann (2019), protein is a nutrient that the body needs to grow and maintain itself. Other important parts of the body like hair, skin, eyes, and body organs are all made from protein. Many substances that control body functions, such as enzymes and hormones are also made from protein. Proteins are also important for formation of blood cells and making antibodies to protect us from illness and infections (Hermann, 2019). Here, also it is clear that eating cowpea seeds can help the human body to benefit from all the usefulness of proteins. Meanwhile, in this study, indications are that seeds obtained from progenies of experimental plants may be better in achieving the discussed benefits of proteins because they contained higher levels of proteins. Thus, it will not be out of place to posit that probable cross-pollination of cowpea by *Megachile* sp may produce healthier seeds from progenies in terms of protein benefits.

Correlation of nutrients

For the progenies of controls, there was a very strong positive correlation between protein and starch ($r = 0.727$; $p < 0.01$). This suggests that there was some form of relationship between protein and starch contents of the progenies of the control. Thus, an increase in protein content produces a corresponding increase in the starch or the other way round. For the rest of the nutrients, there was no form of correlation relationship between them (Table 4). A similar study by Padhi, Bartwal, John, Tripathi, Gupta, Wankhede, *et al.* (2022) reported a highly significant and negative correlation between starch and protein content ($r = -0.63$, $p < 0.001$).

From Table 5, the least protein value of 230.8±0.17mg/Kg with corresponding starch value of 362.6±0.40mg/Kg was recorded in Block 3. Apart from the mean protein and starch values in block 6, an increase in mean protein value showed corresponding increased values for starch in all other blocks. This means that an increase in protein value corresponds in an increase in starch value. Hence, the positive relationship between protein and starch.

Table 4: Correlations (r) of nutrients of control group progenies (N=18)

Variable	Starch	Calcium	Phosphorus	Fibre
Calcium	0.055			
Phosphorus	-0.239	0.160		
Fibre	0.421	0.232	0.152	
Protein	.727**	-0.029	0.049	0.449

** . Correlation is significant at the 0.01 level (2-tailed)

Table 5: Mean Protein and starch contents of the seeds of progenies of control plants in mg/Kg

Block	Proteins	Starch
3	230.8±0.17	362.6±0.40
1	232.7±0.25	367.3±0.12
4	232.9±0.15	374.4±0.28
6	234.0±0.21	369.2±0.45
5	234.3±0.19	372.6±0.38
2	235.6±0.19	372.9±0.38

Considering the progenies of experimental groups in Table 6, there was a negative correlation between protein and starch ($r = -0.476$; $P < .05$) whereas there was a positive correlation between protein and fibre ($r = 0.530$; $P < .05$). Here also, the implication is that while the value of protein is increasing the value of starch is reducing or the other way round. The positive relation between protein and fibre also implies that while protein values increase, fibre values also increase or the other way round.

A close observation of the mean values in Table 7 indicates that the lowest level of protein recorded in this study is 248.9 ± 0.20 mg/Kg with a corresponding starch level of 349.6 ± 0.43 mg/Kg. Meanwhile, in Blocks 4, 5 and 6 an increase in protein value causes a corresponding decrease in starch value. This shows that as protein values increase, starch values decrease indicating the opposite of what happened for the control group happening in the experimental group. This is suggestive that visits by *Megachile* sp to flowers lead to a decrease in starch level when protein level is high.

A further look at the mean values in Table 7 shows that the lowest fibre value for progenies of the experimental plants is 23.9 ± 0.20 mg/Kg with corresponding protein value of 249.0 ± 0.36 mg/Kg. Apart from mean values for fibre and protein in Block 2, all fibre values in all other blocks showed corresponding increase in protein values. The meaning is that an increase in fibre content leads to corresponding increase in protein value. This is not the case for control plants. Hence, it can be argued that visitation to the flowers by *Megachile* sp might have induced the plants with a trait that causes a corresponding increase in protein values as fibre values of the seed increase.

Table 6: Correlations (r) of nutrients of experimental group progenies (N=18)

Variable	Starch	Calcium	Phosphorus	Fibre
Calcium	-0.114			
Phosphorus	0.120	0.367		
Fibre	0.268	-0.022	-0.165	
Protein	-.476*	-0.096	-0.082	.530*

*. Correlation is significant at the 0.05 level (2-tailed).

Table 7: Mean protein, starch and fibre contents of the seeds of progenies of experimental plants in mg/Kg

Block	Proteins	Starch	Block	Fibre	Protein
2	248.9±0.20	349.6±0.43	5	23.9±0.20	249.0±0.36
5	249.0±0.36	340.4±0.44	6	24.6±0.25	252.1±0.15
1	249.6±0.38	352.9±0.27	2	25.1±0.28	248.9±0.20
4	249.8±0.35	350.2±0.52	1	25.2±0.37	249.6±0.38
6	252.1±0.15	340.7±0.91	4	25.4±0.27	249.8±0.35
3	253.9±0.21	342.6±0.46	3	26.3±0.35	253.9±0.21

5. CONCLUSIONS

From the findings of the study it can be concluded that there were significant differences between the mean values of the nutrients of the progenies of the control and experimental plants. Both starch and fibre values for progenies of control plants were higher than values for progenies of the experimental plants. On the other hand, mean calcium, phosphorus and protein values of the seeds of the progenies of experimental plants were higher than that of the control plants. Thus, it is suggestive that when the bee species known as *Megachile* visits a cowpea flower it is possible for cross-pollination to occur which may induce seeds of the progenies to develop higher calcium, phosphorus and protein values. It can further be said that there is some form of relationship between protein and fibre as well as protein and starch values of seeds of progenies of cowpea flowers visited by *Megachile* sp. Further, there is a strong positive relationship between the protein and starch values of the seeds of progenies of self-pollinated cowpea plants. The findings also further show that cowpea seeds are very useful nutritionally for different segments of mankind.

6. RECOMMENDATIONS

From all the nutrient parameters of the seeds of the progenies, it is hereby recommended that cowpea seeds should be consumed by everybody who does not react negatively to it so that they can derive the health benefits. Though, *Megachile* appeared to have caused cross-pollination in cowpea plants with a lot of positive nutrient results in the seeds of the progenies of the plants, there is the need for further study to confirm this. It is further recommended that cowpea farmers should be educated on benefits of insects such as *Megachile* so that they can adopt the best pest control measures on the plant in order to preserve these useful insects to cause cross-pollination.

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