

Research Article

Effect of *Callosobruchus maculatus* Infestation on the Nutrient-antinutrient Composition, Phenolic Composition and Antioxidant Activities of Some Varieties of Cowpeas (*Vigna unguiculata*)

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Abstract: Cowpea (*Vigna unguiculata* L. Walp.) is an important legume crops in most part of the world and is of health benefit in addition to the nutritional potential. However, this important nutrient and disease preventing crop, is prone to post harvest infestation damage, mostly by *Callosobruchus maculatus*. Therefore, the present study is to evaluate the antioxidant activities, nutrient composition, anti-nutrient content and phenolic composition of *Callosobruchus maculatus* infested and non-infested cowpeas commonly consumed in Nigeria. The result revealed that infestation brings about an increase in the evaluated anti-nutrients (phytate, oxalate and alkaloids) and a marked reduction in protein, carbohydrate content, whereas a marked increase was recorded for the fibre content. The result of the antioxidant activities revealed a significant reduction for total phenol, total flavonoids and reducing power and a slight reduction was recorded for the inhibitory action against lipid oxidation, hydroxyl radical scavenging, 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging and 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activities after infestation. The present investigations revealed that *Callosobruchus maculatus* infestation do not only affect the nutrient composition of the studied cowpeas by reducing the nutritional value, but would also have an adverse effect on their potential to prevent free radical mediated diseases.

Keywords: Anti-nutritional factors, antioxidant activities, *Callosobruchus maculatus*, cowpeas, nutrient composition, phenolics

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is the most important food legume crop in the world. The history of cowpea dates to ancient West African cereal farming, 5 to 6000 years ago, where it was closely associated with the cultivation of sorghum and pearl millet (Singh *et al.*, 2003). Cowpea is drought-tolerant and warm-weather crop, with the ability to fix atmospheric nitrogen through its root nodules (Singh *et al.*, 2003). It is the most versatile African crop; it feeds people and their livestock.

Cowpea is an important food legume indigenous to Africa and provides more than half the plant protein in human diets (Rachie, 1985; Philips and McWatters, 1991). It provides a significant amount of calories and is a good source of vitamins and minerals and provides a significant amount of dietary protein. In Nigeria, cowpea is commonly consumed amongst others; in the form of bean pudding, bean cake, baked beans, fried beans and bean soup. In addition to dietary fiber, cowpeas contain many health-promoting components such as vitamins, minerals and phytochemicals, which include phenolic compounds (Cai *et al.*, 2003; Granito

et al., 2005). Phenolic compounds are well-known antioxidants and have been shown to have the ability to prevent degenerative diseases (heart diseases and cancer) in which reactive oxygen species are involved (Harborne and Williams, 2000). The consumption of leguminous plant such as cowpeas has been linked to reduced risk of free radical mediated diseases such as diabetes, obesity and coronary heart diseases (Bazzano *et al.*, 2001).

However, the consumption of cowpeas, like that of other legumes, is limited by the presence of anti-nutritional factors which affect the digestibility and bioavailability of nutrients and thus cowpeas need to be processed to reduce or even remove these factors. Traditionally, legumes are processed in different forms before consumption and such processing enhances their nutritive value by reducing the content of anti-nutritional factors and improves the digestibility of carbohydrates and proteins (Bishnoi *et al.*, 1994).

Cowpea storage bruchid *Callosobruchus maculatus* is a cosmopolitan pest of cowpea in the tropics and subtropics of the world (Booth *et al.*, 1990; Bagheri, 1996). It is commonly referred to as cowpea weevil and

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well known to deteriorate stored cowpeas, its larva infest other grains such as chickpea, bambara groundnut, green gram, lentil, broad bean and green pea and subsequently resulted in physical and nutritional losses. *Callosobrochus maculatus* infestation constitutes a serious threat to the cowpeas growing regions of the world. The infestation may start from the field and continue during storage (Osuji, 1985). In Nigeria, storage losses caused by *Callosobrochus maculatus* are estimated at 3.6 billion naira each year (Buckmire, 1978; Pierrard, 1986). Previous research also showed that insect pest can affect quality parameters of plant food and negatively alter the chemical composition (Evangelisti *et al.*, 1994) and thus, the antioxidant quality of the plant food (Tamendijari *et al.*, 2004; Spadafora *et al.*, 2008). The severity of the negative effects will depend on the stage of the development of the pest and the intensity of the attack.

The present study therefore is sought to investigate the effect of *Callosobrochus maculatus* infestation on the nutrient composition, anti-nutritional content and the antioxidant activities of some selected cowpeas commonly consumed in Nigeria.

MATERIALS AND METHODS

Materials: The different varieties of cowpeas in their dried state (the form in which it is commercially available in Nigeria) were bought from the King's market, Akure, Ondo State, Nigeria and was identified and authenticated in the Department of Crop, Soil and Pest Management, School of Agriculture, Federal University of Technology, Akure, Ondo State, Nigeria. These cowpeas include; Ife brown, Sokoto white and Oloyin brown. The chemicals were analytically graded, while the water used was glass distilled water.

Methods: The cowpeas were picked to separate the inedible from the edible portion. The edible portion was then divided into two groups. Group one (non-infested) and group two (infested). The selected cowpea varieties to be infested was artificially infested with *Callosobrochus maculatus* and stored in a jut bag at room temperature for ninety days. The non-infested cowpeas were also stored in a jut bag at room temperature, at the Department of Biology, Federal University of Technology, Akure, Ondo State, Nigeria for the same period.

Sample treatment and preparation: The infested cowpeas were picked to separate the pest and excretory materials and subsequently pulverized and stored in a sealed polythene bag prior analysis. The non-infested cowpea was also milled to powder.

Proximate analysis: Proximate composition (moisture, proteins, fat, carbohydrates and ash) of infested and non infested cowpeas were determined by the standard

(AOAC, 1990) methods. The crude protein content ($N \times 6.25$) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at $600 \pm 15^\circ\text{C}$. Total carbohydrates were calculated by difference.

Determination of mineral elements: Sample product (2 g) was ashed in a muffle furnace. Fifteen milliliter of 20% (v/v) of nitric acid solution was ashed in the crucible to break up the ash. This was boiled and filtered and acid-washed through Whatman paper. The residue and the paper were washed 3 times with de-ionized water into a standard 100 mL volumetric flask, then diluted to 100 mL with de-ionized water. Atomic absorption spectrophotometer (Pye Unicam sp. 9 AAS) was used for determination of calcium, magnesium, iron, sodium, manganese, phosphorous and potassium.

Determination of phytate: Four grams of the sample was soaked in 100 mL of 2% HCl for 3 h and then filtered through a No 1 Whatman filter paper. Twenty five milliliter was taken out of the filtrate and placed inside a conical flask and 5 mL of 0.3% of ammonium thiocyanate solution was added as indicator. After which 53.5 mL of distilled water was added to give it the proper acidity and was titrated against iron (iii) chloride solution that contains about 0.00195 g of iron per milliliter until a brownish yellow colouration persist for 5 min as described by Day and Underwood (1986) with slight modifications.

Determination of oxalate: This was determined by soaking 1 g of the sample in 75 mL of 1.5 N. H_2SO_4 for 1 h and then filtered through a No 1 Whatman filter paper. Twenty five milliliter will be taken out of the filtrate and placed inside a conical flask and was titrated hot (about $80-90^\circ\text{C}$) against 0.1 m KMnO_4 until a pink color that persist for 15 sec described by Day and Underwood (1986) with slight modifications.

Determination of alkaloids: Five grams of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and allowed to stand for 4 min, this was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide is added drop wise to the extract until precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed and is as described by Harbone (1973) with slight modifications.

Extraction of sample for antioxidant assay and phenolic identification: Finely grinded samples of the cowpeas (0.3 g) were extracted with 30 mL acidified methanol (1% conc. HCl in methanol) in three phases

as follows: 10 mL solvent was added to the flour sample in a conical flask and completely covered with aluminum foil. The sample was stirred magnetically (magnetic stirred) for 2 h, centrifuged in a 40 mL plastic centrifuged tube at 1900 rpm for 10 min (25°C) and decanted, Keeping the supernatant, the residue was then extracted again in 10 mL of the solvent for 20 min, centrifuged and decanted keeping the supernatant and this process was repeated the third time. The supernatant was then combined and stored in a amber glass bottle and kept in refrigerator prior to analysis.

Total phenolic content: The total phenolic content of the ethanol/water extracts was determined by the Folin-Ciocalteu assay of as described by Waterman and Mole (1994). The hydro-alcoholic extract (0.25 mL), was placed in a 25 mL volumetric flask and 5 mL distilled water was added. Folin-Ciocalteu's phenol reagent (1.25 mL) was added and mixed. After 2 min, 3.75 mL 20% (w/v) sodium carbonate solution was added. The contents were mixed and distilled water was added to volume and mixed. The mixture was left to stand for 2 h after addition of the sodium carbonate after which the absorbance of the mixture was measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA). The standard used was tannic acid and the results were expressed as mg tannic acid equivalents per g of the sample.

Total flavonoid content: The total flavonoid content of the extract will be determined using a slightly modified method reported by Meda *et al.* (2005). Briefly, 0.5 mL of appropriately diluted sample will be mixed with 0.5 mL methanol, 50 μ L of 10% AlCl_3 , 50 μ L of 1 mol/L potassium acetate and 1.4 mL water and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture will subsequently be measured at 415 nm. The total flavonoid will be calculated using quercetin as standard by using a seven point standard curve (0-40 or 0-100 μ g/mL), the total flavonoids content of samples was determined in triplicates and the results were expressed as mg quercetin/g of sample.

Reducing antioxidant power: The reducing power of the extracts was determined by assessing the ability of the extract to reduce FeCl_3 solution as described by Oyaizu (1986). Briefly, appropriate dilution of the extract (2.5 mL) was mixed with 2.5 mL 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 2.5 mL 10% trichloroacetic acid was added. This mixture was centrifuged at 353 \times g for 10 min. Five millilitres of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant power was

expressed as mg ascorbic acid equivalent/mL of the sample.

DPPH antiradical assay: The DPPH assay was done according to the method of Brand-Williams *et al.* (1995) with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and then stored at -20°C until needed. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1 units at 515 nm using the spectrophotometer. Phenolic extracts (300 mL) were allowed to react with 2700 mL of the DPPH solution for 6 h in the dark. Then the absorbance was taken at 515 nm. Results are expressed in μ MTE/g sample. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve.

ABTS antiradical assay: Antioxidant activity of the extracts was determined using the 2, 2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS antiradical assay (Awika *et al.*, 2003). The $\text{ABTS}^{+\cdot}$ (mother solution) was prepared by mixing equal volumes of 8 mM ABTS and 3 mM potassium persulphate ($\text{K}_2\text{S}_2\text{O}_8$) (both prepared using distilled water) in a volumetric flask, which was wrapped in foil and allowed to react for a minimum of 12 h in a dark place. The working solution was prepared by mixing 5 mL of the mother solution with 145 mL phosphate buffer (pH 7.4). A range of trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-carboxylic acid) standard solutions (100-1000 μ M) were prepared in acidified methanol. The working solution (2.9 mL) was added to the methanolic extracts (0.1 mL) or Trolox standard (0.1 mL) in a test tube and mixed with a vortex. The test tubes were allowed to stand for exactly 30 min. The absorbance of the standards and samples was measured at 734 nm with a Lambda EZ150 spectrophotometer. The results were expressed as μ M Trolox equivalents/g sample, on dry weight basis.

OH radical scavenging ability: The ability of the extract to prevent $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ induced decomposition of deoxyribose will be carried out using the method of Halliwell and Gutteridge (1981). Briefly, freshly prepared extract (0-100 μ L) will be added to a reaction mixture containing 120 μ L, 20 mM deoxyribose, 400 μ L, 0.1 M phosphate buffer pH 7.4, 40 μ L, 20 mM hydrogen peroxide and 40 μ L, 500 mM FeSO_4 and the volume will be made to 800 μ L with distilled water. The reaction mixture will be incubated at 37°C for 30 min and the reaction will be stopped by the addition of 0.5 mL of 2.8% TCA, this will be followed by the addition of 0.4 mL of 0.6% TBA solution. The tubes will subsequently be incubated in boiling water for 20 min.

The absorbance will be measured at 532 nm in spectrophotometer.

Quantification of phenolic compounds using HPLC:

Chromatographic equipment consisted of a Hewlett-Packard (Avondale, PA) liquid chromatography model 1090 equipped with a diode array Ultraviolet (UV) detector. A TSK-GEL Super-ODS (Supelco, Bellefonte, PA) column was used. The absorbance of the effluent was monitored at 254 and 238 nm. The mobile phase consisted of solvents A-C using three pumps equipped with the chromatograph. Solvent A was 0.1% trifluoroacetic acid in acetonitrile, solvent B, 0.1% trifluoroacetic acid in HPLC grade water and solvent C 100% methanol. Flow rate was set at 1.0 mL/min and column temperature was maintained at 37°C throughout the test. The initial solvent condition was 100% solvent B. A linear gradient was used to increase solvent A from 0 to 10% within 7 min. This solvent composition was maintained at an isocratic flow for 3 min. Solvent A was then increased from 10 to 40% using a 20-min linear gradient. This composition was then maintained for 2 min and returned to the initial condition in 3 min. Solvent C was used for column washings between and after runs. Samples were injected into the HPLC for analyses and the concentrations of phenolic compounds were calculated from standard curves calibrated using the 16 phenolic standards. The phenolic contents were expressed as milligrams per hundred grams of cowpea flours.

Lipid oxidation assay using egg yolk homogenate lipid rich media:

Egg homogenate (0.5 mL, 10% v/v) and 0.1 mL of each extract were added to a test tube made up to 1 mL with distilled water (Ruberto *et al.*, 2000). (0.05 mL) FeSO₄ (0.07M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 mL of 0.8% (w/v) TBA in 1.1% sodium deodecyl sulphate and 20% TCA were added and the resulting mixtures were vortexed and then heated at 95°C for 60 min. After cooling, 5.0 mL of butan-1-ol was added to each tube and centrifuge at 300 rpm for 10 min. The absorbance of the organic layer was measure at 532 nm. The percentage inhibition of lipid peroxide formation by the extract was calculated.

Statistical analysis: All experiments were performed in triplicates. Analysis at every time point from each

experiment was carried out in triplicate. Means, standard errors and standard deviations were calculated from replicates within the experiments and analyzed using Microsoft Excel XP.

RESULTS AND DISCUSSION

The results of the proximate composition of the different varieties of unripe plantain-sweet potato blends are as shown in Table 1. The result showed that the protein content decreases with respect to infestation: Ife brown from 28.4-24.7%; Oloyin brown: 25.5-23.4%; and Sokoto white: 21.7-19.5%. The decrease in the protein content could be attributed to the utilization of these nutrients by the infesting *Callosobruchus maculatus* (Ojimelekw, 2002), the result also agrees with the earlier reports (Mbah and Silas, 2007; Ojimelekw, 2002; Owolabi *et al.*, 2012). A decreasing value was also noticed in the carbohydrate content after *Callosobruchus maculatus* infestation with Sokoto white decreasing from 66.0-60.5%; Oloyin brown: 59.2-54.2%; Ife brown 57.4-51.9%. The decreasing value of the carbohydrate content may be attributed to the feeding activities of the insect (Owolabi *et al.*, 2012). There was no appreciable difference in the fibre content of the infested and non-infested cowpeas varieties: Sokoto white (non-infested: 1.1%; infested: 1.0); Ife brown (non-infested: 1.2%; infested: 1.1%); and Oloyin brown with a fibre content of 1.1% for infested and non-infested cowpeas. These results obtained for the fibre content after infestation is similar to previous report (Ojimelekw, 2002).The results showed an increasing value in the moisture, fat and ash content as a result of pest infestation, the moisture content increased in Ife brown from 9.5-13.7%; Oloyin brown: 8.5-12.5%; Sokoto white: 6.6-10.4%. The increase in moisture content observed as a result of infestation could be ascribed to the humidity of the storage facility that promotes infestation. Storage conditions, variety, geographical location and time of the year affect moisture content (Mbah and Silas, 2007). The fat content increased from 2.5-4.3% in oloyin brown; 2.3-4.5% in sokoto white and 2.1-5.2% in ife brown, this may be as a result of excretion and other secretion by the insect, This observation is in agreement with earlier report (Owolabi *et al.*, 2012; Mbah and Silas, 2007). There is an increase in the ash

Table 1: Proximate composition (%) of some selected infested and non-infested cowpeas (Mean±S.D.)

Sample	MC	Ash	Fat	Fibre	Crude protein	CHO
IBI	13.7±0.81	5.2±0.20	3.4±0.13	1.1±0.03	24.7±0.09	51.9±0.77
OBI	12.5±0.29	4.5±0.27	4.3±0.30	1.1±0.02	23.4±0.35	54.2±0.56
SWI	10.4±0.30	4.2±0.04	4.5±0.10	1.0±0.00	19.5±0.00	60.5±0.08
IBN	9.5±0.14	1.5±0.02	2.1±0.09	1.1±0.01	28.4±0.39	57.4±0.53
OBN	8.5±0.34	3.1±0.12	2.5±0.06	1.2±0.00	25.5±0.00	59.2±0.29
SWN	6.6±0.31	2.3±0.12	2.3±0.02	1.1±0.00	21.7±0.00	66.0±0.18

Reported values are the means±S.D. (n = 3); MC: Moisture content; CHO: Carbohydrate; IBI: Ife brown infested; OBI: Oloyin brown infested; SWI: Sokoto white infested; IBN: Ife brown non-infested; OBN: Oloyin brown non-infested; SWN: Sokoto white non-infested

Table 2: Mineral composition (mg/kg) of some selected infested and non-infested cowpeas

Sample	Ca	Mg	Na	K	P	Cu	Zn	Fe
IBI	20198±45.0	16278±56.0	43962±5.5	48525±84.59	1323±44.5	0.02±0.00	34.10±0.81	53.2±0.15
OBI	19817±60.6	16116±4.7	43222±2.0	45308±3.50	1227±6.4	0.02±0.00	31.20±0.20	48.8±0.20
SWI	19029±6.1	14800±11.0	36969±6.0	43923±27.20	1164±52.3	0.02±0.00	25.48±0.03	41.4±0.05
IBN	22953±58.6	19373±1.2	51073±10.9	63014±2.00	1526±4.7	0.02±0.00	46.40±0.06	55.3±0.19
OBN	21697±51.4	18338±4.5	49540±55.5	55589±13.60	1306±3.8	0.02±0.00	45.80±0.30	53.4±0.20
SWN	19971±6.5	16319±4.0	40473±24.0	50734±46.10	1205±3.0	0.02±0.00	29.80±0.05	42.0±0.04

Reported values are the means±S.D. (n = 3); IBI: Ife brown infested; OBI: Oloyin brown infested; SWI: Sokoto white infested; IBN: Ife brown non-infested; OBN: Oloyin brown non-infested; SWN: Sokoto white non-infested

content in oloyin brown from 3.1-4.5%; sokoto white: 2.3-4.2% and ife brown: 1.5-5.2%. The increased value of the ash content of the infested cowpeas could be attributed to the feeding activities of *Callosobruchus maculatus* generating a lot of residue which amount to ash (Mbah and Silas, 2007) and also from the indigestible insect parts.

Mineral elements are considered essential to life and form the main electrolyte of the human body, maintain tissue homeostasis and at the same time form the main structural component of bones and teeth. A total of eight mineral elements, namely Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (K), Phosphorus (P), Copper (Cu), Zinc (Zn) and Iron (Fe) were analysed and detected in the three selected cowpea varieties. The result of the mineral composition (mg/kg) is given in Table 2. Similarly, *Callosobruchus maculatus* infestation also brought about a reduced concentration of all the mineral elements evaluated in the cowpeas. The only exception in the observed reduction of mineral content after infestation is copper that was found in trace amount in the studied infested and non-infested cowpea varieties. The marked reductions observed in the mineral content after infestation may be due to the feeding activities of the insect and the developmental stages of the insect (Ojimelekw, 2002), during which a high level of minerals will be utilized by the infesting pest.

Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrient. The result of the anti-nutrient (phytate, oxalate and Alkaloids), is as shown in Table 3. Phytate has a strong binding affinity to important minerals such as calcium, magnesium, iron and zinc. When a mineral binds to phytic acid; it becomes insoluble, precipitates and will be non-absorbable in the intestines. This process can therefore contribute to mineral deficiencies, it can also act as an acid, chelating the vitamin niacin, the deficiency of which is known as pellagra. In this study *Callosobruchus maculatus* infestation was observed to bring about an increase in the phytate content (mg/g) of the selected cowpea varieties from 10.4-19.2 for ife brown; 11.3-15.1 for oloyin brown and 8.9-12.9 for sokoto white. Alkaloids on the other hand cause gastrointestinal and neurological disorders (Aletor, 1993). The glycoalkaloids are haemolytically active and toxic to fungi and humans. Some of the toxicological manifestations of alkaloid involve gastrointestinal

Table 3: Anti-nutrients of some selected infested and non-infested cowpeas

Sample	Oxalate (Mg/g)	Phytate (Mg/g)	Alkaloids (%)
IBI	2.4±0.10	19.2±0.48	63.3±5.77
OBI	3.8±0.14	15.1±0.48	53.3±1.15
SWI	2.9±0.05	12.9±0.48	48.7±1.15
IBN	1.7±0.05	10.4±0.95	27.3±2.30
OBN	1.9±0.05	11.3±0.48	12.7±2.30
SWN	1.8±0.05	8.9±0.48	17.3±1.15

Reported values are the means±S.D. (n = 3); IBI: Ife brown infested; OBI: Oloyin brown infested; SWI: Sokoto white infested; IBN: Ife brown non-infested; OBN: Oloyin brown non-infested; SWN: Sokoto white non-infested

upsets and neurological disorders, especially in doses in excess. Infestation by *Callosobruchus maculatus* also resulted to an increased level of alkaloids content from 27.3-63% in ife brown; 12.7-53.3% in oloyin brown; and 17.3-48.7% in sokoto white.

Oxalatic acid in the body combines with divalent metallic cations such as calcium and iron to form crystals of the corresponding oxalates which are then excreted in urine as minute crystals. These oxalates can form larger kidney stones that can obstruct the kidney tubules. Recent studies have estimated that about 80% kidney stones are formed from calcium oxalate and the highly insoluble iron (ii) oxalate appears to play a major role in gout. The result of the oxalate content (mg/g) of the selected cowpeas after pest infestation revealed an increasing value compared with the non-infested samples; oloyin brown increased from 1.7-2.4; Sokoto white: 1.9-3.8; Ife brown: 1.8-2.9. The increase in all the evaluated anti-nutritional factors after storage and infestation may be related to the selective consumption of the endosperm by the insect. Such selective consumption by the infesting insect leaves behind portions rich in the studied anti-nutritional factors (Modgil and Mehta, 1997).

Phenolic constituents are very important in plants because of their scavenging ability due to presence of hydroxyl groups. A number of studies have focused on the biological activities of phenolic compound which are potential antioxidants and free radical scavengers (Lee *et al.*, 2003). The result of the total phenolic content (mg/g) of the methanolic extract of the cowpea varieties is as shown in Fig. 1 with Ife brown having the highest the total phenolic content (1.06), followed by Sokoto white (0.77) and the least was recorded for oloyin brown (0.48). This result of the total phenolic content is in agreement with the report obtained by

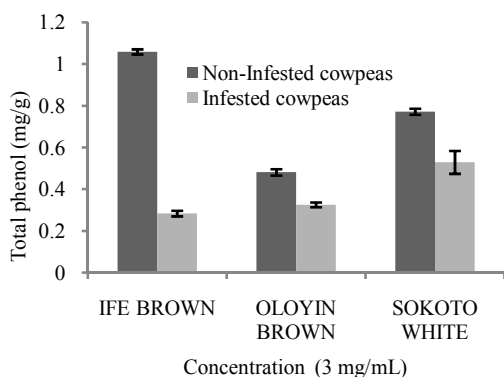


Fig. 1: Total phenolic content (mg/g ascorbic acid equiv.) of infested and non-infested cowpea (*Vigna unguiculata*) varieties. Values are given as mean±S.D. of independent experiment performed in triplicate; Error bars represent standard deviations

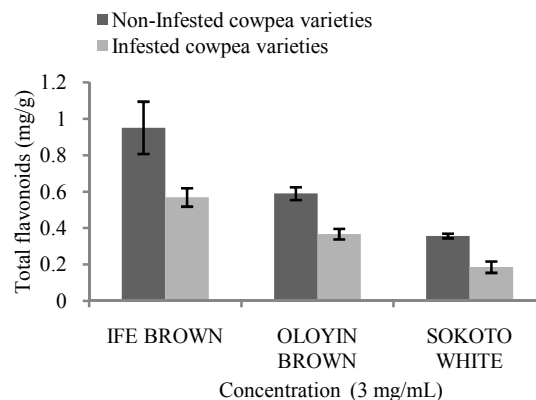


Fig. 3: Total flavonoid content (mg quercetin equivalent/g of sample) of infested and non-infested cowpea (*Vigna unguiculata*) varieties. Values are given as mean±S.D. of independent experiment performed in triplicate; Error bars represent standard deviations

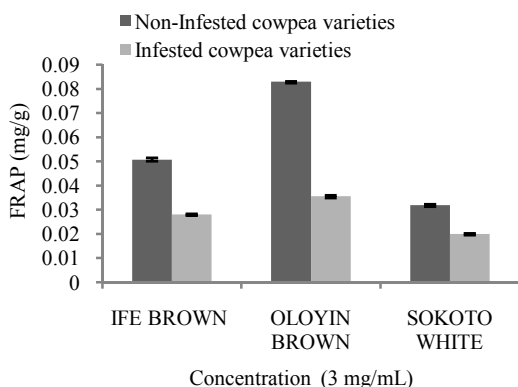


Fig. 2: Ferric reducing antioxidant power (mg ascorbic acid equivalent/g of sample) of infested and non-infested cowpea (*Vigna unguiculata*) varieties. Values are given as mean±S.D. of independent experiment performed in triplicate; Error bars represent standard deviations

Oboh (2006). *Callosobruchus maculatus* infestation reduced the total phenolic content to 0.28 in Ife brown, 0.33 in oloyin brown and 0.53 mg/g in Sokoto white. The notable loss in the total phenolic content could probably be due to polyphenoloxidase activity that catalysed the changes caused by larval damage to tissues and by the presence of exit holes which exposed the cowpeas to oxygen and environmental factors (Tamendijari *et al.*, 2004; Spadafora *et al.*, 2008).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir *et al.*, 1995). Figure 2 shows the result of the ferric reducing power (mg/g) of the studied cowpea varieties. It was observed that oloyin brown have higher reducing ability (0.082), followed by Ife brown (0.05) and the least was recorded for Sokoto white (0.03). *Callobruchus maculatus* infestation decreased the

reducing capacity of the infested cowpea varieties to 0.035 in oloyin brown, 0.02 in Ife brown and 0.019 in Sokoto white. The decrease observed may be attributed to the activities of the insect and by the presence of exit holes which exposed the cowpeas to oxygen and environmental factors (Tamendijari *et al.*, 2004). Different studies have indicated that the reducing power of bioactive compound is associated with antioxidant activity. The reducing capacity of the cowpea varieties might be due to their hydrogen-donating ability. Therefore, cowpeas might contain reducing compounds, which can react with free radicals to stabilize and terminate radical chain reactions.

Flavonoids are commonly found in both edible and non-edible plants and they have been reported to have multiple biological effects including antioxidant activity and have been suggested to play a preventive role in the development of cancer and heart disease (Hollman *et al.*, 1996). Figure 3 shows the result of the total flavonoid content (mg/g) and it was observed that Ife brown has the highest flavonoid content (0.95), followed by oloyin brown (0.59), with sokoto white having the lowest flavonoid content (0.36). *Callosoroehus maculatus* infested cowpeas was observed to cause a decrease in the total flavonoid content of the three cowpea varieties to 0.56 in ife brown, 0.36 in oloyin brown and 0.19 in sokoto white, respectively. The decreasing level of flavonoid may be as a result of the presence of larvae in the cowpeas which use cowpeas for feeding to complete their development and enzymatic reaction that plays a key role in the defence response against insect injuries (Tamendijari *et al.*, 2004).

The hydroxyl radicals generated by the Fenton reaction in the system were scavenged by the cowpea varieties extract. The hydroxyl radical scavenging

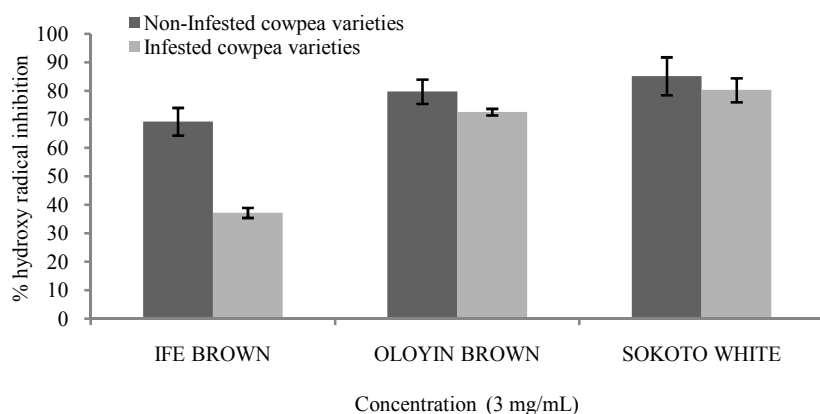


Fig. 4: Hydroxyl radical scavenging ability (OH* scavenging) of infested and non-infested cowpea (*Vigna unguiculata*) varieties. Values are given as mean±S.D. of independent experiment performed in triplicate; Error bars represent standard deviations

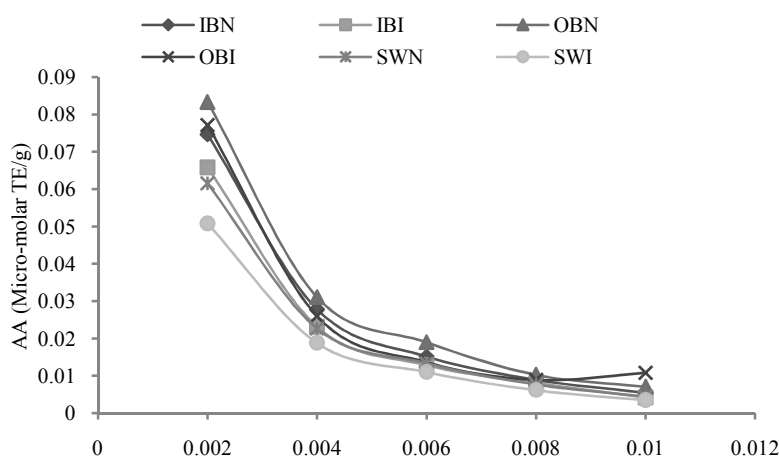


Fig. 5: DPPH radical scavenging activity (μM trolox equivalent/g of sample) of infested and non-infested cowpea (*Vigna unguiculata*) varieties. Values are given as mean±S.D. of independent experiment performed in triplicate; Error bars represent standard deviations

effect of the infested and non-infested cowpea varieties is as shown in Fig. 4. Among the three varieties, sokoto white exhibited the strongest hydroxyl radical scavenging activity (85.20%), followed by oloyin brown (79.78%) and the least was recorded for ife brown (69.26%). Pest infestation was observed to reduce the hydroxyl radical scavenging activity in the infested cowpea varieties to 80.23% in sokoto white, 72.61% in oloyin brown and 37.21% in ife brown. The decrease could be attributed to the use of phenolic compounds by the pest as food necessary to maintain its life and to complete its larval development and metamorphosis (Mraicha *et al.*, 2010). Hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism among the free radicals, which can be formed from superoxide anion and hydrogen peroxide, in the presence of metal ions, such as copper or iron and cause the ageing of human body and some diseases, interact

with purine and pyrimidine bases of DNA as well as absorb hydrogen atoms from biological molecules, leading to the formation of sulphur radicals, a number of which damage biological molecule (Siddhuraju and Becker, 2007).

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The decrease in the absorbance of DPPH radical is caused by antioxidants, which react with the radical. The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared to other methods. The addition of the extracts to the DPPH solution causes a rapid decrease in the absorbance at 517 nm. The degree of discoloration indicates the scavenging capacity of the extract. Figure 5 shows the dose dependent effect of the various phenolic extracts of cowpea varieties on the DPPH

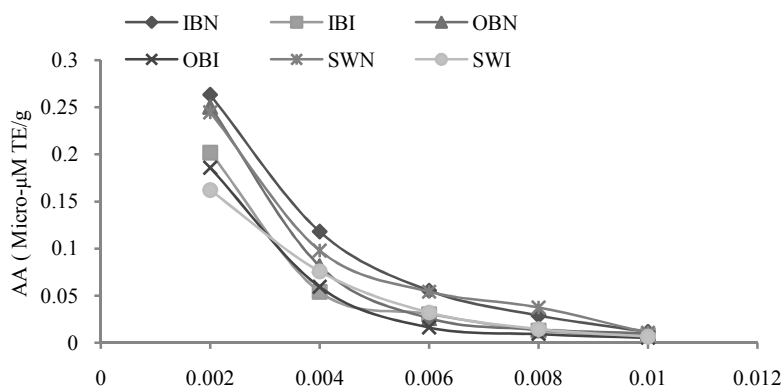


Fig. 6: ABTS antiradical activity (μM trolox equivalent/g of sample) of infested and non-infested cowpea (*Vigna unguiculata*) varieties
 Values are given as mean \pm S.D. of independent experiment performed in triplicate; Error bars represent standard deviations

radical scavenging activity. The DPPH radical scavenging activity of the cowpea extract reveals that Oloyin brown has the highest radical scavenging, followed by Ife brown, while the least was recorded for Sokoto white in the infested and non-infested cowpea varieties, with little or no difference in the activity after infestation. The scavenging activities of the infested and non-infested phenolic extracts showed a decreasing radical scavenging activity upon infestation and also a decreasing activity with increasing concentration of the phenolic extract.

The decolorization of ABTS^+ cation radical is an unambiguous way to measure the antioxidant activity of phenolic compounds. Awika *et al.* (2003) found positive correlations between the determination of phenolic antioxidant using the Oxygen Radical Absorbance Capacity (ORAC), ABTS and DPPH assays. Thus, monitoring the antioxidant activity of phenolic compounds by their ability to scavenge ABTS^+ radical was demonstrated to give good prediction. The results of the ABTS is represented in Fig. 6, it was observed that pest infestation by *Callosobruchus maculatus* brought about a decrease in the radical scavenging ability of all the studied cowpea varieties with Ife brown having the highest activity, followed by oloyin brown and the lowest was recorded for Sokoto white in both the infested and non-infested cowpea varieties. The ABTS method is widely employed for measuring the relative radical scavenging activity of hydrogen donating and chain breaking antioxidants in many plants extracts (Awika *et al.*, 2003).

A number of research investigation had establish a positive correlation between the radical scavenging activities and the phenolic content (Dlamini *et al.*, 2007; Siatka and Kašparová, 2010). Therefore, the observed reduction in the radical scavenging activities after infestation could be attributed to the use of

phenolic compounds as food necessary to maintain its life and to complete its larval development and metamorphosis (Mraicha *et al.*, 2010).

The quantitative estimate (mg/100 g) of phenolic compounds in the infested and non-infested cowpea varieties is as shown in Table 4. HPLC/DAD analyses of the phenolic compounds in the selected cowpeas revealed the presence of some phenolic acids and flavonoids. Protocatechuic acid was observed to be the highest phenolic acid in all the selected cowpea varieties both in the infested and non-infested, followed by kaemferol and other phenolic compounds which includes chlorogenic acid, p-hydroxybenzioc acid, caffeic acid, ferulic acid, catechin, cinnamic acid, vanillic acid and quercetin. The phenolic compounds identified in the non-infested cowpeas compare favorably with that of Cai *et al.* (2003), Duenas *et al.* (2005) and Sosulski and Dabrowski (1984). Similar to the folin-Ciocalteu assay, a higher total phenolic content was recored for ife brown (174.131) with no significant different in oloyin brown (150.32) and sokoto white (147.063) varieties. In the same vein, a decreasing total phenolic content was observed with the infested cowpea varieties.

Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. The damage has been implicated in the pathophysiology of various human diseases such as atherosclerosis, diabetes and cancer. The initiation of peroxidation sequence in membrane or polyunsaturated fatty acids is due to the abstraction of a hydrogen atom from the double bond in the fatty acids (Wagner *et al.*, 1994) with Malondialdehyde (MDA) as the major product of lipid peroxidation process. Incubation of egg yolk homogenates in the presence of FeSO_4 causes a significant increase in lipid oxidation. The ability of the phenolic extract of the cowpea varieties to inhibit lipid

Table 4: Quantitative estimates (mg/100 g) of phenolic compounds in infested and non-infested three cowpeas varieties (*Vigna unguiculata*)

Compounds	Rt (min)	SWN	SWI	IBN	IBI	OBN	OBI
Catechin	5.54	16.500	16.700	14.240	14.36	12.72	12.79
Cinnamic acid	8.22	7.040	7.000	8.670	5.64	6.39	6.44
Carvacrol	9.72	-	-	0.620	0.02	0.15	0.02
Protocatechuic acid	10.46	51.440	51.750	57.280	57.90	51.51	51.61
P-coumaric acid	11.25	4.760	3.610	7.480	3.01	5.66	4.48
Vnilllic acid	11.47	4.750	3.950	4.890	1.76	3.53	3.18
O-coumaric acid	11.60	0.180	0.100	0.770	0.39	0.70	0.38
p-hydroxybenzoic acid	12.68	-	-	8.760	4.75	6.77	3.45
Caffeic acid	14.17	6.660	6.670	6.280	6.13	5.57	5.40
Ferulic acid	14.84	5.310	4.630	5.190	2.99	4.50	3.79
Genistein	18.34	3.990	Trace	-	-	-	-
Kaempferol	21.44	28.340	27.910	30.940	31.14	33.04	33.39
Luteolin	21.85	0.083	0.067	0.041	0.11	0.05	0.03
Capsaicin	22.44	-	-	-	-	0.06	0.03
Gingerol	23.34	-	-	0.010	0.02	-	-
Delphinidin	22.43	1.870	Trace	2.020	Trace	2.51	Trace
Isorhamnetin	23.06	0.170	Trace	-	-	0.21	Trace
Malvidin	23.32	0.140	Trace	5.140	Trace	0.18	Trace
Petunidin	24.82	0.260	Trace	0.190	0.16	0.24	Trace
Chlorogenic acid	29.41	13.890	13.590	19.940	19.52	15.14	15.15
Quercetin	30.30	1.670	1.630	1.820	1.85	1.56	1.56
Total phenolics		147.063	137.217	174.131	149.19	150.32	136.30

Reported values are the means±S.D. (n = 3); IBI: Ife brown infested; OBI: Oloyin brown infested; SWI: Sokoto white infested; IBN: Ife brown non-infested; OBN: Oloyin brown non-infested; SWN: Sokoto white non-infested

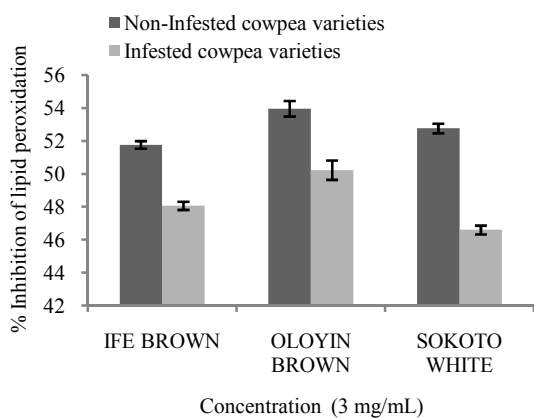


Fig. 7: The inhibitory effect of methanolic extract of infested and non-infested cowpea (*Vigna unguiculata*) varieties on Fe²⁺ induced lipid peroxidation using egg yolk homogenate as lipid source

Values are given as mean±S.D. of independent experiment performed in triplicate; Error bars represent standard deviations

peroxidation was tested using the method of Ruberto *et al.* (2000). Figure 7 shows that pest infestation by *Callosobruchus maculatus* brought about a decrease in the ability of the phenolic extracts of the studied cowpeas to inhibit lipid oxidation (Oloyin brown: 50.23%; Sokoto white: 46.60% and Ife brown: 48.06%). In the non-infested cowpeas, oloyin brown was seen to have the highest inhibition of lipid oxidation (53.97%), followed by Sokoto white (52.77%), with Ife brown having the least value (51.77%). Phenolic compounds have been reported to prevent the decomposition of hydrogen peroxide into free radicals (Maisuthisakul *et al.*, 2007). Hence, the observed inhibition of lipid oxidation of the cowpea varieties may be due to the presence of some phenolic

compounds in the cowpeas. However, the lower inhibitory action against lipid oxidation observed in the infested cowpeas may be due to the hydrolytic and oxidative processes which were favored by the exit holes by exposing the cowpeas to environmental influences (Mraicha *et al.*, 2010).

CONCLUSION

These studies showed that cowpeas possess some level of antioxidant properties and nutritive values and that *Callosobruchus maculatus* damage bring about a reduced nutritional quality and antioxidant potentials, with an increased level of the evaluated anti-nutrients. On the overall, this could be a threat to food security, the economic value and the utilization of the studied cowpea as functional food. It is therefore recommended that thorough pre-harvest cleaning of storage facilities, transport and harvesting equipment is critical for the management of bruchids. Grain residues in silos, harvesters, augers must also be eliminated as they can harbor *Callosobruchus maculatus* that can infest newly harvested grains.

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