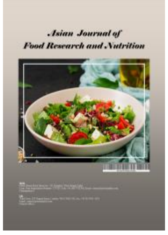

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Oxidative Stress Markers and Toxic Metals Assessment in Albino Wistar Rat fed with *Vigna unguiculata* Expose to Biopesticides (*Bacillus thuringiensis,* Neem *Azadirachta*) and Agrochemical

Oguh Collins Egwu [a*], Alexander Ikechukwu Ajai [b], Osuji Chigoziri Akudo [c], Ugwu Chukwuebuka Victor [d], Adinnu Chiamaka Maria-Goretti [e], Okeke Chioma Blessing [f], Ugwu Obiora Celestine [g], Obasi Glory Otuomasirichi [d], Umezinwa Ogochukwu Jennifer [h], Ugoeze Ucheoma Elele [i], Dickson Achimugu Musa [j] and Makun Husseini Anthony [k]

[a] Department of Subnatinal, Nigeria Center for Disease Control and Prevention, Abuja, Nigeria.
[b] Department of Chemistry, Federal University of Technology Minna, Niger State, Nigeria.
[c] Department of Biochemistry, Gregory University Uturu Abia State, Nigeria.
[d] Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.
[e] Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
[f] Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria.
[g] Department of Pharmacology, Enugu State University of Science and Technology, Enugu State, Nigeria.
[h] Department of Science Laboratory Technology, University of Nigeria, Nsukka, Enugu State, Nigeria.
[i] Department of Chemistry, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria.
[j] Department of Biohemistry, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria.
[k] Department of Biochemistry, Federal University of Technology Minna, Niger State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author OCE involved in the planting, and the writing of the manuscript. Authors AIA and OCA contributed to the manuscript. Authors UCV and ACM contributed in the literature review. Author OCB involve in the sampling and preparation of reagent. Authors UOC involve in the metal analysis. Authors OGO involve in the statistical analyses. Authors UOJ involves in the estimations and calculations. Authors UUE and MHA read and edit the article before publication. All authors read and approved the final manuscript.

**Corresponding author: E-mail: collinsoguh@gmail.com;*

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ABSTRACT

The study aimed to determine the Oxidative Stress markers and Toxic metals risk assessment in Albino Wistar rat fed with *Vigna unguiculata* expose to biopesticides *Bacillus thuringiensis* (*Bt*), Neem seed oil *Azadirachta* (*Aza*) and agrochemical (Lambda_cyhalothrin 15g/l and Dimethoate 300g/l) use for pest control. Dried mature *Vigna unguiculata* seed was randomly collected from four different field locations where Biopesticide (*Bt,* and *Aza*), agrochemical, and control were used to manage pest. Standard procedures were used to determine the physicochemical parameters of the soil samples and oxidative stress on the Albino Wistar rat fed. Phytate and oxalate contents were evaluated using the titrimetric method, while cyanogenic glycoside, tannin, and alkaloid concentrations were determined using the Pearson method. Atomic Absorption Spectrophotometry was used to determine the concentrations of toxic metals. Standard formulas were used to estimate the health risk assessment. The results shows that agrochemical led to a significant ($P< 0.05$) increased in lipid peroxidation in the rat blood sample, antinutrient factors, heavy metals and a significant decrease in the activities of the antioxidants enzymes: Superoxide dismutase, catalase and xanthine oxidase activities in the blood and cowpea seed compared to the biopesticides. Heavy metal contamination in seeds of cowpea controlled with agrochemicals had a hazard quotient and Hazard Index greater than 1, which indicates unsafety especially to children. The study concludes that biopesticides such as *Bt* and *Aza* have shown to be an alternative method in cowpea pest control with very less effect.

Keywords: Agrochemical; antinutrient; biopesticides; heavy metal; oxidative stress; risk assessment.

ABBREVIATIONS

LcD: Lambda_cyhalothrin 15g/l and Dimethoate 300g/l; BAF: Bioaccumulation factors; DIM: Daily intake of metal; ADDM: Average daily dose of metal; MC: Metal concentration; BW: Body weight; HQ: Hazard quotient; RFD: Reference oral dose; HI: Hazard index; CRD: completely randomized design; LP: Lipid peroxidation; SOD: superoxide dismutase; XO: xanthine oxidase; CAT: catalase; BHT: Butylated hydroxyl toluene; Bt: Bacillus thuringiensis; Aza: Azadirachta.

1. INTRODUCTION

Pests damage cause considerable crop losses and yield in recent years. To avoid these losses, farmers are now increasingly employing agropesticides in their agronomic practices to prevent losses and low yield. Pesticides are chemical substances that are used in agriculture to repel, prevent, and eradicate pests in order to increase yield. Agrochemicals have significantly increased agricultural productivity, but residual concentrations in the soil and potential ecosystem dangers are big concerns. Insects have a significant impact on African cowpea crop yields, influencing leaves, flower and stem component and also stage of development. The legume pod borer, Maruca vitrata, is the main preharvest pest of cowpeas [1]. The legume bug cause damage at all stages of development, more harm occurs during flowering [2]. Pesticides used to control these pests and prevent harm, especially those made of synthetic materials, and have a number of negative effects on humans and the environment [3].

An imbalance between free radicals and antioxidants in the body causes oxidative stress. Free radicals are oxygen-containing molecules with an uneven number of electrons. They can easily interact with other molecules because of their unequal quantity. Lipid peroxidation (LP) precedes oxidative damage in plants and animals. Antioxidant defense mechanisms, on the other hand, are found in living organism. Antioxidant defense mechanisms include enzymes like superoxide dismutase (SOD), xanthine oxidase (XO) and catalase (CAT), as well as non-enzymes like ascorbic acid. Oxidative stress is measured by changes in the levels of these antioxidants. Furthermore, the activity of xanthine oxidase is a measure of oxidative stress as well as a defense mechanism [4].

Long-term use of synthetic pesticides in agriculture has resulted in the accumulation of pesticidal residues in the environment as a result of run-off, and also heavy metals which are not biodegradable, has led to a variety of chronic illnesses and non-target organism toxicity. Heavy metals also transported to humans through the food chain, where they may cause variety of human health issues [5, 6]. Synthetic pesticides have shown to be effective in pest management but increasing focus is being placed on the creation of ecologically friendly pesticides that will aid in the efficient management of pests while also reducing chronic health issues [7]. One of the most important alternative strategies is the use of Biopesticides (*Bt* and *Aza*) [8].

Bacillus thuringiensis (Bt) has been employed in agriculture because of its insecticidal proteins, making it an environmentally friendly biopesticide. The presence of -endotoxins, particularly cry protein, is what gives the bacteria its insecticidal properties. Its application, however, is not limited to only insecticidal property but also a biofertilizer for boosting plant growth, the generation of transgenic plants, and other applications has been demonstrated in previous studies [9, 10]. Neem oil has parasitic, insecticidal spermicidal properties, killing a wide variety of organisms, including pests [11]. Neem's constituent phytochemicals have been discovered to have a wide range of therapeutic benefits [12]. Azadirachtin is the most active complex secondary metabolite identified in neem seeds, which has long been known as an important insecticidal component. In insects, it acts as an antifeedant, and repellant that is Neem prevent insects from feeding [13, 14].

Anti-nutritional factors are compounds present in food that interfere with beneficial nutrients, minerals, and metabolic processes from being absorbed, as well as reducing the bioavailability of nutrients from plants or plant products used as human diets. When antinutrients such as cynogenic glycoside and alkaloid are consumed in high concentrations, they hinder cells from utilising oxygen, which can lead to infertility, cancer, gastrointestinal and neurological disorders [15, 16]. Phytate, oxalate, and tannins reduce the bioavailability of proteins, carbohydrates, and essential minerals like calcium, magnesium, zinc, iron, and phosphorus by forming insoluble complexes that aren't easily absorbed by the gastrointestinal tract, resulting in health problems like oxalemia [17 - 20].

Cowpea are mostly damage by insects, so synthetic chemicals is regularly use to control pest and for the millions of people that consume them, this is a huge health risk. Agrochemicals are quick and easy way to eliminate pests in the field which increases long-term toxicity risks to people and other ecosystem biota. Nonetheless, biopesticides properties have been discovered in neem plant and in *Bt* as an alternative to agrochemicals. Hence, this study aimed to determine the Oxidative Stress markers and Toxic metals risk assessment in Albino Wistar rat fed with *Vigna unguiculata* expose to biopesticides *Bacillus thuringiensis* (*Bt*), Neem seed oil *Azadirachta indica* (*Aza*) and agrochemical (Lambda_cyhalothrin 15g/l and Dimethoate 300g/l) use for pest control.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Biochemistry Department Federal University of Technology Minna Niger State, Bosso Campus. Bosso is situated at 9º65’ North latitude, 6º52’ East longitude, with an area of 72km^2.

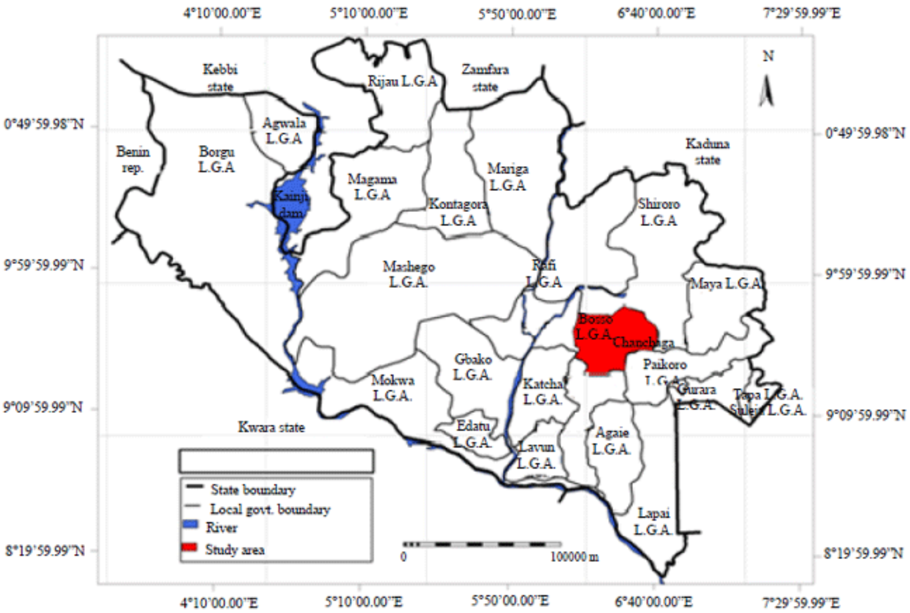

Fig. 1. Map of Niger State Showing study area in red spot

2.2 Collection of Experimental Rats, Soil and Biopesticides

Top soil sample about seven hundred grams use for planting was collected 6 inches (15cm) below the surface with a sterile hand shovel from a fallow land from Agriculture department Federal University of Technology, Minna and was used to test different soil physicochemical properties. Biopesticide *Bt* and neem oil was purchase from Konga online and the instruction for use were strictly followed. Twelve mature experimental male AlbinoWistar rats weighing 192 -200g were purchase from animal house Federal University of Technology Minna.

2.3 Experimental Design and Planting of Seed

The soil was divided into four groups and each group had twenty seven perforated polythene bags with 2kg of soil. Three seeds were planted in each test bag to an approximate depth of 2 cm. Biopesticides (*Bt* and *Aza*), agrochemical, and a control without treatment were used in groups A, B, C and D respectively. Biopesticides and Agrochemical were sprayed on the test crops from seven weeks of planting when blooming and flowering began to suppress insects, except on the control groups. And the number of spot on cowpea pods damage by pest was observed by circling the damaged spot with a permanent marker. To avoid counting the same spot twice, the counted spots were marked. Twelve mature male AlbinoWistar rats weighing 192 -200g were randomly assigned into four groups of three rats each. Groups 1, 2, 3 and 4 were fed with cowpea treated with Biopesticides (*Bt* and *Aza*), agrochemical and control for 12 weeks.

2.4 Sample Collection

Dried Seeds of cowpea from each group and their corresponding soil were randomly collected for analysis from each group were biopesticides (*Bt* and *Aza*), agrochemical was applied for pest control and each samples from four different location where use to feed rats. Animal blood was used to determine the level of oxidative stress. The soil used for planting was mixed, then air-dried for seven days at room temperature (27^{o}C) to stop all microbial activity in the soil. Using 2mm sieve mesh size, the air-dried soil samples were sieved and handpicked to remove trash and stones and was used to test different soil physicochemical properties before and after harvest.

2.5 Determination of the Physico-Chemical Parameters

A potentiometric meter and a digital pH meter were used to determine the pH of the soil samples. About 10 g of soil samples with 100 ml of distilled water using a glass rod to agitate, and pH of the suspension was determined. The physicochemical parameters of the soil were examined before and after treatment using [21]. The physicochemical variables tested were soil texture, pH, total organic carbon, organic matter, total nitrogen, total phosphorus, and exchangeable cation (sodium ion, magnessium and potassium ion) to determine the pesticide's biodegradability.

2.6 Preparation of Extracts for the Determination of Oxidative Stress Markers in Cowpea Seed

Three drops of butylated hydroxyl toluene (BHT) and 0.05 M phosphate buffer pH 7.5 were added to blood sample, and centrifuged at 5000 g for 10 min. The supernatant was used to determine oxidative stress indicators.

2.6.1 Determination of lipid peroxidation markers in cowpea seedlings

The assay is based on the reaction of malondialdehyde (MDA) with thiobabituric acid (TBA); forming a MDA-TBA2 adduct that absorbs strongly at 532 nm. Acetic acid (1.0 ml) was placed in a test tube and 1.0 ml of 10% TBA was added to the tube followed by 0.1 ml of the blood supernatant. The test tube was covered and immersed in boiling water for 15 min. After cooling the mixture, it was centrifuged at 5000 g for 10 minutes. The spectrophotometer was zeroed and absorbance of test sample was read at 532 nm against the reagent blank [4].

2.6.2 Determination of superoxide dismutase activity in cowpea seed

The process inhibits auto-oxidation of adrenaline from turning into adrenochrome. About 2.5 ml of a 0.05 M phosphate buffer with a pH of 7.4 were added to 2 ml of the homogenate. 0.5 ml of freshly made 0.3 nm epinephrine was added to the buffer-supernatant mixture to initiate the reaction. This was mixed by inversion.

Exact 2.5 ml of the buffer, 0.5 ml of epinephrine, and 2 ml of deionized water were contained in the reference cuvette. The rise in absorbance at 480 nm was monitored every second for 150 second. The amount of enzyme necessary to inhibit epinephrine's oxidation to adrenochrome by 50% at a rate of 480 nm per minute is known as one unit of superoxide dismutase activity [22, 23]. An Sp 1800 UV/VIS Spectrophptometer was used to assay the enzyme activity.

2.6.3 Determination of catalase activity in cowpea seedlings

Hydrogen peroxide is broken down by catalase to produce oxygen, which oxidizes potassium dichromate. A chromophore with a maximum absorption at 610 nm results from the oxidation of chromate. The reaction mixture contained 1 ml of 0.05 M phosphate buffer (pH 7.5), 0.5 ml of 0.2 M H_2O_2, and 0.4 ml H_2O. The enzyme extract (0.5 ml) was added to the reaction mixture, and the mixture was then incubated for different time periods, t1, t2, and t3, for 1 minute, 2 minutes, and 3 minutes, respectively. After each interval, the reaction was stopped by adding 2 ml of the acid reagent (dichromate/acetic acid mixture), which was made by combining glacial acetic acid and potassium dichromate at a 5% concentration (1:3 by volume). The enzyme was added to the control following the acid reagent. The absorbance was measured at 610 nm with a Sp 1800 UV/VIS Spectrophptometer after all the tubes had been boiled in boiling water for 10 minutes. Catalase activity was measured in moles of H_2O_2 used per minute [24].

2.6.4 Determination of xanthine oxidase (XO) activities in cowpea seedlings

Xanthine oxidase is an enzyme that catalyses the conversion of methylene blue to the reduced colorless forms. The reciprocal of the amount of time it takes for methylene blue to turn colorless is used to measure enzyme activity. A test tube rack was filled with two test tubes labeled "control" and "test," and one milliliter of neutral formaldehyde solution at 0.05% was pipetted into each test tube. In the test tube marked "test," the 0.02% methylene blue solution was added. Next, 1 ml of the blood supernatant was added to the corresponding test tube. 1 ml of distilled water was added to the control test tube, and in order to prevent air oxidation, 2 drops of liquid paraffin were also added to the both test tube [4].

2.7 Anti-nutrient Analysis

Titrimetric method of Association of official analytical chemist AOAC, [25], was used to estimate oxalate and phytate content while [26] method was use to estimate cyanogenic glycoside, tannin and Alkaloid content.

2.8 Determination of Heavy Metal

Blood, Soil and cowpea seed samples (1ml: 1.00:0.1g each) were placed in separate 100ml beakers and given 15ml of a tri-acid mixture (70 percent high purity HNO_3, 65% $HClO_4$, and 70 percent H_2S0_4 in a 5:1:1 ratio). The solution was digested at 800°C till it became transparent. The resultant solution was filtered and dilute to 50mL with deionized water before being examined using atomic absorption spectrophotometry for As, Pb, Cr, Cd, Cu, and Hg [27].

2.9 Assessment of Human Health Risk

2.9.1 Bioaccumulation factor (BAF) estimation

The transfer coefficient (transfer or metal uptake from soil via cowpea seed) was calculated using [28].

$$BAF = C_{seed}/C_{soil} \quad (1)$$

C_{seed} = metal concentration in cowpea seed, mg/kg
C_{soil} = milligrams of metal per kilogram of dry weight of soil.
BAF greater than 1 signifies that the cowpea enriched metal from the soil.
BAF less than 1 indicates that the cowpea exclude metals from the soil

2.9.2 Estimation of the daily intake of metal (DIM)

The following formula was used to calculate the daily metal intake [29].

$$ADDM = DI \times MC_{seed}/BW \quad (2)$$

Where;

ADDM = indicates average daily dose of metal (mg,kg/d).
DI = Cowpea seed daily intake (0.83 kg/d for adults, 0.88 kg/d for children).
MC_{seed} = is the metal concentration in the seed (mg/kg)
BW = Indicate the body weight of average individual 55.7kg for adults and 14.2kg for Children).

2.9.3 Estimation of hazard quotient HQ

The Hazard Quotient (HQ) assess the possible risks to human health associated with consumption of these cowpea grown in pesticide-contaminated soil using the following equation [30].

HQ is the ratio between exposure and the reference oral dose (RFD)

Ratio lower than one 1, means no obvious risk.

$$HQ = ADDM/RFDM \quad (3)$$

Where;

ADDM = The average daily dose (mg,kg/d) of the metal
RFDM = The reference dose of metal (mg,kg/d) which is the maximum tolerable daily intake of metal with no adverse effect

2.9.4 Estimation of Hazard Index (HI)

The HI assess the total risk of heavy metal exposure from consuming a particular cowpea [31]. The value of the hazard index is proportional to the level of toxicity in the cowpea consumed. If the HI value is more than one, the anticipated exposure is likely to cause health problems.

$$HI = \sum HQ_{As} + HQ_{Cu} + HQ_{Pb} + HQ_{Cd} + HQ_{Cr} + HQ_{Hg} \quad (4)$$

2.9.5 Analytical statistics

The data was analyzed using IBM Statistical Product and Service Solution (SPSS) version 20 and Microsoft Excel 2013. The information was presented in the form of a mean and standard deviation (SD). One-way analysis of variance (ANOVA) was use for significant different. Duncan's multiple range test (DMRT) was used to compare mean values across test groups and controls, as well as between test group means.

3. RESULTS

3.1 Physicochemical Properties of Soil Samples before and After Planting

Table 1 summarizes the physicochemical properties of soil samples. The pH of the soil was 6.91, 6.51, 6.42, 4.25 and 6.57 before planting (control soil with no pesticide), after planting (control soil with no pesticide), soil with *Aza* solution, soil with synthetic agrochemical, and *Bt* soil respectively.

3.2 Physical Observation on the Number of Spot on *Vigna unguiculata* Pod Damage by Pest

Table 2 shows the total number of injured pods in the test samples and the control. The observations continued for another five weeks after the seventh week of planting. Pod of cowpea was randomly peak from each field and the group that got no treatment had the maximum damage on the pods, with 55 places of damage, while *Vigna u* treated with *Aza, Bt* and agrochemical had 13, 10 and 9 spots of damage, respectively.

Table 1. Physicochemical properties of soil samples before and after planting

Soil properties	**(Before Planting)**	**Control soil***	***Aza* soil***	**Agro soil***	***Bt* soil***
Texture	**loamy**	**loamy**	**loamy**	**loamy**	**loamy**
pH	6.91 ± 0.03^a	6.51 ± 0.03^b	6.42 ± 0.03^c	4.25 ± 0.03^d	6.57 ± 0.03^b
Total N %	1.96 ± 0.04^a	1.92 ± 0.03^a	1.88 ± 0.03^b	1.52 ± 0.06^c	1.95 ± 0.03^a
Total P%	20.84 ± 0.19^a	20.78 ± 0.1^a	18.69 ± 0.07^b	18.47 ± 0.15^c	18.56 ± 0.07^b
OM %	3.78 ± 0.10^a	3.82 ± 0.01^a	3.66 ± 0.11^a	3.50 ± 0.06^b	3.73 ± 0.11^a
OC%	2.67 ± 0.06^b	2.64 ± 0.05^b	2.28 ± 0.03^c	2.75 ± 0.04^a	2.18 ± 0.03^d
K^+ meq/100g	1.99 ± 0.03^a	1.97 ± 0.04^a	1.76 ± 0.04^b	1.64 ± 0.04^c	1.52 ± 0.04^c
Mg^{2+} meq/100g	13.25 ± 0.02^a	13.21 ± 0.03^a	12.29 ± 0.08^b	11.50 ± 0.04^c	12.17 ± 0.08^b
Na^+ meq/100g	8.16 ± 0.06^a	8.13 ± 0.04^a	7.98 ± 0.03^b	7.86 ± 0.06^c	8.05 ± 0.03^b

*Results expressed as Mean ± SD. Mean values with same superscript letters on the rows are considered not significant (P>0.05). n=3 ** = After planting*

Table 2. Physical observation on the number of spot on *Vigna u.* damage by pest

Weeks	**Control**	***Vigna u.* with *Aza***	***Vigna u* with *Bt***	***Vigna u.* with Agro**
7	15	5	3	4
8	13	4	2	3
9	10	2	2	1
10	9	1	2	1
11	8	1	1	0
Total spot	55	13	10	9

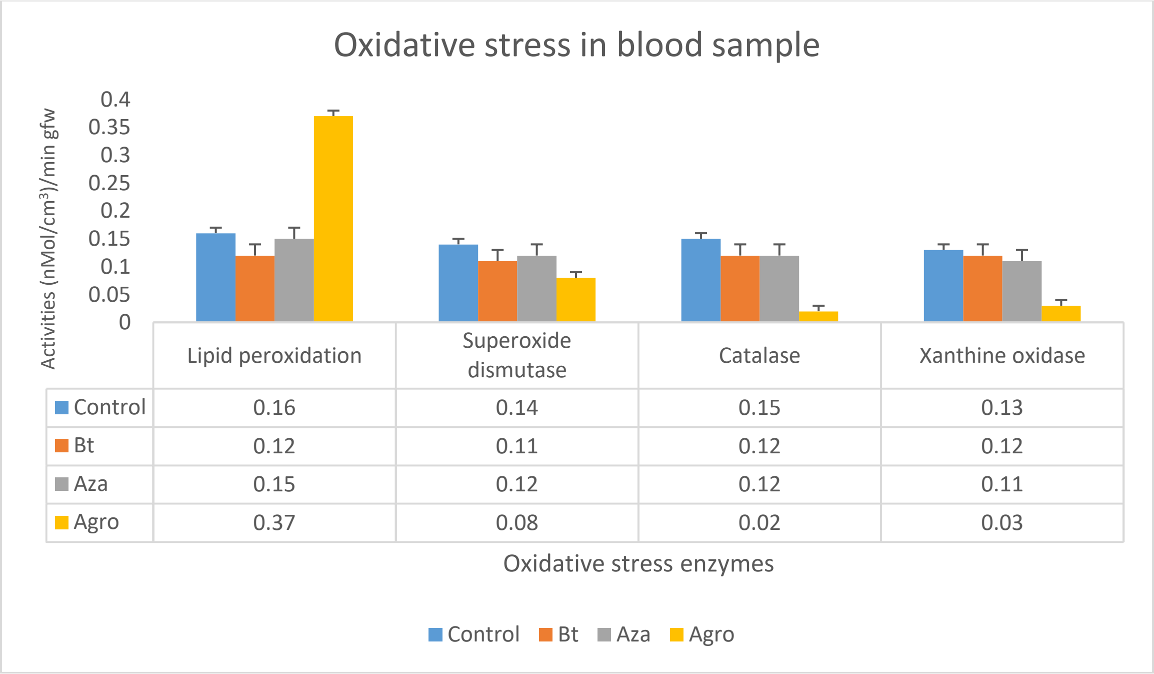

Fig. 2. Oxidative stress enzymes in blood

3.3 Oxidative Stress Markers in Blood Sample

Lipid peroxidation and antioxidant enzymes; superoxide dismutase, catalase and xanthine oxidase are shown in Fig. 2. Lipid peroxidation in rat blood fed with agro pesticides cowpea was found to increase significantly when compared with the control ($p < 0.05$). The value of LP in the control, *Bt, Aza* and agro were 0.16, 0.12, 0.15 and 0.37 respectively. The activities of SOD, CAT, and XO significant decrease ($p < 0.05$) in the blood of rat fed with cowpea grown with *Bt, Aza*, and Agro compared with the control.

3.4 Antinutrient Factors in *Vigna unguiculata*

The cyanogenic and oxalate levels from Agro chemical are 2.56 mg/100g and 25.32 mg/g^{-1} and are within the limits in cowpea 0.5 - 3.5 mg/kg and 500 mg/100g respectively. Phytate (12.39), Alkaloid (1.37) and tannin (1.46) contents in Cowpea seeds cultivated with agrochemical were above the threshold in cowpea 0.035 %, 0.02 % and 0.25 g/l respectively.

3.5 Heavy Metal Concentration in Soils

Heavy metals As, Pb, Cu, Cd, Cr, and Hg concentrations in soil with Aza, Bt, agrochemical, and no pesticide were (2.08, 2.54, 2.81, 0.52, 3.55, 0.38), (2.10, 2.58, 2.89, 0.75, 3.87, 0.52), (5.78, 8.89, 4.52, 3.74, 8.46, 4.48), and (2.03, 2.35, 0.72, 0.39, 3.46, 0.36 mg/kg), respectively. According to the study, the concentrations of the heavy were significantly ($p<0.05$) higher in the soil with agrochemical pesticide than other tested samples (Table 4). In the soil with the *Aza and Bt*, the majority of the metals analyzed were identified in the lowest quantities.

3.6 Heavy Metal Level in *Vigna u* Controlled with Neem, Bt and Agro Pesticide

Vigna u with *Bt, Aza*, agrochemical, and control without pesticide mean levels of heavy metals are summarized in Table 5. In *Vigna u*, the concentrations of As, Pb, Cu, Cd, Cr, and Hg in *Bt* (0.12, 0.14, 0.91, 0.03, 0.08, 0.18), *Aza* (0.32, 0.57, 2.78, 0.09, 0.30, 0.27), agrochemical (4.06, 7.41, 3.53, 2.04, 4.86, 3.04), and without pesticide (0.02, 0.51, 1.33, 0.21, 0.29, 0.17 mg/kg). All metal levels in *Vigna u* with agrochemicals were greater than the FAO/WHO limit of metal in cowpea 0.5, 2.0, 0.04, 0.5, 0.3, and 0.1 mg/kg for As, Pb, Cu, Cd, Cr, and Hg, respectively. The metal Cu and Hg contents in cowpeas with Agro chemical were more (2.78 and 0.27 mg/kg, respectively), which exceeds the permissible limits (0.04 and 0.1 mg/kg).

Table 3. Antinutrient factors in cowpea controlled with agrochemical and neem solution

Antinutrient factors (mg/100g)	**Agro**	**Cowpea samples**		**Control**	**Limit**	**Source**
		Aza	***Bt***			
Cyanogenic	2.56 ± 0.03^{a}	0.56 ± 0.01^{b}	0.54 ± 0.01^{b}	0.23 ± 0.08^{c}	0.5 – 3.5	[32]
Phytate(g/100g)	12.39 ± 0.23^{a}	4. 12 ± 0.23^{b}	4. 18 ± 0.23^{b}	2. 4 ± 0.03^{c}	0.035 %	[33]
Oxalate (mg/g^{-1})	25.32 ± 0.81^{a}	9.29 ± 0.15^{b}	9.32 ± 0.15^{b}	5.71 ± 0.13^{c}	200–500	[34]
Alkaloid %	1.37 ± 0.01^{a}	0.02 ± 0.01^{b}	0.01 ± 0.01^{c}	0.02 ± 0.01^{b}	0.02 %	[35]
Tannin g/l	1.46 ± 0.04^{a}	0.19 ± 0.03^{b}	0.20 ± 0.03^{b}	0.12 ± 0.05^{c}	0.25 g/l	[36]

Table 4. Heavy metal concentration in soils with *Aza, Bt* and Agro pesticide

Heavy metals (mg/kg)	**Samples**				
	Aza	***Bt***	**Agro**	**Control**	**PL(mg/kg) in soil FAO/WHO [37, 38]**
As	2.08 ± 0.04^{b}	2.10 ± 0.04^{b}	5.78 ± 0.12^{a}	2.03 ± 0.03^{b}	20
Pb	2.54 ± 0.10^{c}	2.58 ± 0.10^{c}	8.89 ± 0.04^{a}	2.35 ± 0.13^{b}	50
Cu	2.81 ± 0.09^{b}	2.89 ± 0.09^{b}	4.52 ± 0.10^{a}	0.72 ± 0.18^{b}	100
Cd	0.52 ± 0.06^{c}	0.75 ± 0.06^{b}	3.74 ± 0.04^{a}	0.39 ± 0.03^{d}	3.0
Cr	3.55 ± 0.03^{c}	3.87 ± 0.03^{b}	8.46 ± 0.07^{a}	3.46 ± 0.37^{c}	100
Hg	0.38 ± 0.07^{c}	0.52 ± 0.07^{b}	4.48 ± 0.04^{a}	0.36 ± 0.09^{c}	2.0

Mean values with same superscript letters on the rows are considered not significant ($P>0.05$). PL= Permissible limit

Table 5. Heavy Metal Concentration in *Vigna u.* treated with *Bt, Aza* and agrochemical pesticide

Heavy metals (mg/kg)	Samples				
	Vigna u* with *Bt	***Vigna u* with *Aza***	***Vigna u* with Agro**	**Control pesticide**	**PL (mg/kg) in *Vigna u* FAO/WHO, [39*,40]****
As	0.12 ± 0.04^{c}	0.32 ± 0.04^{b}	4.06 ± 0.04^{a}	0.02 ± 0.02^{d}	0.5*
Pb	0.14 ± 0.04^{c}	0.57 ± 0.04^{b}	7.41 ± 0.02^{a}	0.51 ± 0.13^{b}	2.0*
Cu	0.91 ± 0.03^{c}	2.78 ± 0.03^{b}	3.78 ± 0.11^{a}	0.33 ± 0.02^{c}	0.04**
Cd	0.03 ± 0.01^{c}	0.09 ± 0.01^{b}	2.04 ± 0.02^{a}	0.21 ± 0.01^{c}	0.5*
Cr	0.08 ± 0.05^{c}	0.30 ± 0.05^{b}	4.86 ± 0.03^{a}	0.29 ± 0.02^{b}	0.3*
Hg	0.18 ± 0.01^{b}	0.27 ± 0.01^{b}	3.04 ± 0.02^{a}	0.17 ± 0.01^{b}	0.1*

Mean values with same superscript letters on the rows are considered not significant (P>0.05)

Table 6. Heavy Metal level in blood of rat fed with *Vigna u*

Heavy metals (µg/L)	Samples				
	Vigna u* with *Bt	***Vigna u* with *Aza***	***Vigna u* with Agro**	**Control pesticide**	**Limit (µg/L) in blood metal**
As	0.49 ± 0.08^{c}	1. 34 ± 0.05^{b}	6.72 ± 0.07^{a}	0.45 ± 0.07^{c}	3.12 [41]
Pb	0.94 ± 0.02^{b}	0.97 ± 0.03^{b}	9.41 ± 0.09^{a}	0.34 ± 0.10^{c}	2.0 [42]
Cu	2.73 ± 0.06^{c}	3.65 ± 0.07^{b}	3.82 ± 0.92^{a}	0.67 ± 0.07^{c}	1495 [41]
Cd	0.08 ± 0.05^{c}	0.10 ± 0.08^{b}	1.21 ± 0.08^{a}	0.06 ± 0.01^{c}	0.15 [42]
Cr	0.04 ± 0.05^{c}	0.22 ± 0.05^{b}	7.95 ± 1.10^{a}	0.31 ± 0.06^{b}	1.86 [41]
Hg	0.06 ± 0.02^{b}	0.09 ± 0.02^{b}	2.07 ± 0.02^{a}	0.08 ± 0.01^{b}	0.1 [39]

Mean values with same superscript letters on the rows are considered not significant (P>0.05)

Table 7. Estimation of bioaccumulation factor (BAF)

Heavy metals (mg/kg)	BAF			
	Bt	**Aza**	**Agrochemical**	**Control**
As	0.06	0.15	0.70	0.01
Pb	0.05	0.22	0.83	0.21
Cu	0.31	0.98	0.83	0.45
Cd	0.04	0.17	0.54	0.53
Cr	0.02	0.08	0.57	0.08
Hg	0.35	0.71	0.67	0.47

3.7 Heavy Metal Level in Rat Blood Samples fed with *Vigna u* with Neem, Bt and agro Pesticide

Blood sample of rat fed with expose *Vigna u* with *Bt, Aza*, agrochemical, and control mean levels of heavy metals are summarized in Table 6. All metal levels in blood sample of *Vigna u* with agrochemicals were greater than the limit of metal in blood 3.12, 2.0, 1495, 0.15, 1.86, and 0.1 µg/L for As, Pb, Cu, Cd, Cr, and Hg, respectively.

3.8 Estimation of Bioaccumulation Factor (BAF)

Shows the bioaccumulation factor (BAF) of heavy metals from soil to cowpea plants, which is the ratio of metal concentration in cowpea to total soil concentration. In *vigna u* treated with *Bt, Aza*, agrochemical, and no pesticide, the bioaccumulation factors of metals As, Pb, Cu, Cd, Cr, and Hg were (0.06, 0.05, 0.31, 0.04, 0.02 and 0.35), (0.15, 0.22, 0.98, 0.17, 0.08 and 0.71), (0.70, 0.83, 0.83, 0.54, 0.57 and 0.67), and (0.01, 0.21, 0.45, 0.53, 0.08 and 0.47), respectively.

3.9 Daily Intake and Potential Hazard (Hazard Quotient) of Metal in Human

Daily intake and hazard quotient that will be derived from trace metal consumption in *Vigna u* for both adults and children are shown in Table 8. The estimated daily intake of heavy metals (DIM) was calculated using the average cowpea consumption for both adults and children. The HQ of heavy metal detect a significant quantity of Cu in adults (1.04 and 1.41) and children (4.31 and 5.86) in *Vigna u* treated with *Aza* and agropesticide (4.31 and 5.86). A high amount of Cr (1.00) and Hg (2.78) HQ was found in *Vigna u* with agropesticide for children.

Table 8. Daily Intake and Potential Hazard (Hazard Quotient) of metal in human

Heavy metals	DIM and HQ for individuals					
	Individuals	Hazards	***Vigna u* with *Bt***	***Vigna u* with *Aza***	***Vigna u* with agrochemical**	**Control**
As	Adult	DIM	0.00	0.00	0.06	0.00
		HQ	0.00	0.01	0.12	0.00
	Children	DIM	0.00	0.02	0.25	0.00
		HQ	0.01	0.04	0.50	0.00
Pb	Adult	DIM	0.00	0.01	0.11	0.01
		HQ	0.00	0.00	0.06	0.00
	Children	DIM	0.00	0.04	0.46	0.03
		HQ	0.00	0.02	0.23	0.02
Cu	Adult	DIM	0.01	0.04	0.06	0.00
		HQ	0.33	1.04	1.41	0.12
	Children	DIM	0.01	0.17	0.23	0.02
		HQ	0.29	4.31	5.86	0.51
Cd	Adult	DIM	0.00	0.00	0.03	0.00
		HQ	0.00	0.00	0.06	0.01
	Children	DIM	0.00	0.01	0.13	0.01
		HQ	0.00	0.01	0.25	0.03
Cr	Adult	DIM	0.00	0.00	0.07	0.00
		HQ	0.00	0.01	0.24	0.01
	Children	DIM	0.00	0.02	0.30	0.02
		HQ	0.02	0.06	1.00	0.06
Hg	Adult	DIM	0.00	0.00	0.05	0.00
		HQ	0.03	0.04	0.45	0.03
	Children	DIM	0.01	0.02	0.23	0.01
		HQ	0.11	0.17	2.78	0.10

DIM = Daily intake of metal, HQ = Hazard quotient

Table 9. Estimation of hazard index (HI) of metal for adult and children

	HI for Individuals				
	Individuals	***Bt***	***Aza***	**Agrochemical**	**Control**
HI=∑HQ (HM)	Adult	0.36	1.1	2.34	0.17
	Children	0.44	4.61	10.62	0.72

HI = Hazard index. ∑ = Summation of the Hazard Quotient (HQ) arising from all the heavy metals (HM) examined

3.9.1 Estimation of hazard index (HI) of metal for individuals

Adult and children HIs in *vigna u* controlled with *Aza*, and agrochemical were all greater than 1, indicating toxicity, especially for the agrochemical pesticide, which had a 10.62 HI for children. *Bt* value is below 1, which indicate less toxicity to both adult and children. The findings revealed that children are more likely to be more affected when cowpea controlled with agrochemical pesticide are consume (Table 9).

4. DISCUSSION

The soil physiochemical analysis shows that the use of synthetic pesticides is most likely the cause of low pH (4.25) value in the soil with agrochemicals compared with the *Aza* and *Bt* solution. Agro pesticide soil pH was somewhat acidic, falling below the specified range (6.5-8.5) for agriculture farming [40], whereas the soil with neem solution, *Bt* and control soil was within the acceptable range (Table 1). There was a significant difference between soil applied with agrochemical and the soil applied with alternative methods of pest control (*Aza and Bt*) ($p<0.05$). The physicochemical properties of soil are altered by chemical application, especially in soils where agrochemical pesticides are applied for cowpea pest control. This leads to an increase in heavy metals in the soil, which is likely passed on to plants that grow on such soils, providing long-term toxicity risks to humans and other ecosystem biota when consume. The agrochemical pesticides did the least damage to cowpea leaves and bud, due to its effectiveness. There were significant variations between the pesticide and biopesticide use and control samples, due to their efficacy, which reduces pest effect on *Vigna.* The number of spots decreases over time, presumably due to the insecticide employed as well as the drying/hardening of the pods. Despite agrochemical effectiveness, it lead to the accumulation of harmful substances in the seed and soil, providing a health risk to humans (Table 2).

An earlier study showed that exposure to metal causes plants to produce reactive oxygen species [43, 44]. The current findings demonstrate that the amount of lipid peroxidation in the seeds of cowpea seedlings exposed to soil treated with agrochemical products increased as the quantity of agrochemical products in the soil rose (Fig. 2). Plants exposed to metal ions have been observed to have higher levels of lipid peroxidation [44-46]. High reactive oxygen species levels cause lipid peroxidation, which leads to oxidative stress [47]. It is important to note that agrochemical products may cause oxidative stress in exposed cowpea and decrease the level of antioxidants such superoxide dismutase, catalase, and xanthine oxidase activities as a result of increased generation of reactive oxygen species. Stress can increase the creation of reactive oxygen species, which can be dangerous to cells. However, plants have built-in defenses against reactive oxygen species. Superoxide dismutase (SOD) and catalase are two of the scavenging enzymes found in plants [48-50].

The antinutrients analysis showed that the cyanogenic glycoside, phytate, oxalate, alkaloid and tannin content in Cowpea samples that was controlled by agrochemical were higher than that of the biopesticides (*Aza* and *Bt*) and control sample which indicates that the cowpea controlled with agrochemical led to a significant ($p < 0.05$) increased of antinutrient factors in the cowpea seed. High concentration of cyanogenic glycoside stop cells from using oxygen and eventually causes heart, respiratory and central nervous problem [16]. Phytate, oxalate and tannins decreases the bioavailability of macromolecules (proteins, carbohydrate) and essential elements (calcium, magnesium, zinc, iron, and phosphorus). They form insoluble complexes, such as calcium oxalate crystals when binds to calcium and this complexes are not readily absorbed by the gastrointestinal tract which lead to health problems such as kidney stone oxalemia [51]. Alkaloids cause infertility, gastrointestinal and neurological disorder [15].

The metal levels discovered in the soil were all within the FAO/WHO soil permissible limit. According to analysis of variance (ANOVA) conducted, the concentrations of the hazardous elements in the soil varied significantly ($p < 0.05$). In comparison to all metals analyze on soil, Pb content had the highest value in cowpea with agrochemical application. Regular consumption of Pb-contaminated foods has been shown to affect the liver, kidneys, heart, brain, nerves, and other vital organs. Pb exposure can cause heart disease, anemia, high blood pressure, and reproductive problems such osteoporosis (brittle bone disease), especially in men. Heavy metal concentrations in soil were found to be in the following order: Pb >Cr> As > Cu > Hg >Cd. The soil heavy metals were all below the WHO/FAO permissible limit, with the exception of mercury (4.48 mg/kg), which had an allowable limit of 2.0 mg/kg. Symptoms of mercury toxicity include, memory loss, headaches, hair loss, mental retardation in the fetus, fetal abnormalities, blindness, deafness, and muscle rigidity [52, 53]. Similarly, [14] discovery have shown that *Aza* and *Bt* has a lower heavy metal content in the soil than synthetic agrochemicals which is in line with this current research.

In the *Vigna u* seed and blood sample examined for heavy metals, the cowpea and blood with agro pesticide had higher heavy metals levels, and they are all above the WHO/FAO authorized limit of metal in blood and cowpea. The differences were significant ($p<0.05$) when compared to the cowpea controlled *Aza* and *Bt* solution. Heavy metals and nutrients received by the roots are often translocated to other parts of the cowpea, including the leaves and seed. On the other hand, metal availability in the soil and continual absorption by the roots could lead to higher concentrations in various areas of the cowpea. The amounts of heavy metals in *Vigna u* controlled for pest with an agricultural agropesticide decreased in the following order: Pb > Cr > As > Cu > Hg > Cd. The metal Pb showed the highest concentration (7.41 mg/kg) in the cowpea, exceeding the permissible limit (2.0 mg/kg) in agrochemical controlled cowpea. The rise in Pb and other metals could be linked to the widespread use of agricultural agropesticides. Little quantity of heavy metals are important for human health, large doses might cause metabolic disorders, according to [54]. According to the CDC, Cd causes acute and chronic poisoning, as well as damage to the immune system [55]. High dosages of Cr have been related to chronic bronchitis, and vomiting, according to studies [56]. When the brain, nervous system, and red blood cells are exposed to high levels of Pb, it causes mental deterioration, decreased reaction time, memory loss, decreased fertility, renal system damage, nausea, insomnia, anorexia, and joint weakness [57].

Heavy metals were found in varying levels in the blood of rat fed with various pesticides, which might be attributable to the presence of these trace elements in pesticides sprayed on the cowpea. In prior analyses, metals were discovered in insecticides [58-60]. Following the heavy metals concentrations mean values in *Vigna u* that neem solution biopesticide were applied Cu > Pb > As > Cr > Hg > Cd. Cu and Hg, Cu (2.78) and Hg (0.27 mg/kg) exceeded the WHO/FAO permissible limits (0.04 and 0.1 mg/kg respectively). The presence of Cu and Hg in *Vigna u* samples with neem solution may be as a result of the atmospheric conditions/air deposition [61]. Other metals were found to be below the FAO/WHO permissible level in *Vigna u* with *Aza* and *Bt* biopesticide. In this study, the *Aza* and *Bt* biopesticide demonstrated non-significant level of metals to the soil or *Vigna u* seed. Neem solution and *Bt* has a low or no harmful effect on cowpea seed, and it is biodegradable when applied to plants. Metal absorption rate depend on soil physicochemical qualities, and other factors [62] could explain the discrepancies in metal accumulation in the cowpea plant under study (Table 5).

The BAF value of Cu and Pb (0.83) was found to be more in the *vigna u* controlled with agrochemical. Plants are known to take up and accumulate trace metals from contaminated soil via absorption from the root. The BAF of other elements are less than 1 and falls within the normal range of transfer. Hyper accumulators are plants having a BF greater than one, and they could be used in bioremediation of extremely polluted soil. The element is excluded from the soil by the *Vigna u* when the BAF is less than 1. The DIM values of heavy metals were greater in the *Vigna u* that had been sprayed with an agricultural agropesticide. When *Vigna u* is consumed, the DIM values obtained show the amount of metal that will be accumulated in a day for both adults and children. In this findings the HQ values for all heavy metals were significantly higher in *Vigna u* controlled with agrochemical pesticide than that of the *Aza* and *Bt*. The findings of HQ [27] show that Pb, As, and Cd pollution pose a significant health risk to both adults and children, but that Zn (1.058) exposure poses only a little harm to children who consume onions. The dangers of consuming polluted plants are more likely to affect children than adult. *Vigna u* controlled with agroagricultural pesticides is not safe for consumption, according to the findings of the hazard index.

5. CONCLUSION

This study has concludes that exposure of cowpea plant to agrochemical products in soil could impose oxidative stress and heavy metals in the blood when consume. The values of heavy metals and antinutrient constituents in *Vigna u* seed controlled with agrochemical pesticide were significantly higher and above WHO/FAO acceptable limits in cowpea. The risk assessment indicate that consumption of these *Vigna u* controlled with synthetic pesticides can pose a health risk as a result of heavy metal intake, especially to children. Neem and *Bt* biopesticide have shown as an alternative method in the management of cowpea pest.

AVAILABILITY OF DATA AND MATERIAL

We confirm the availability of all the data included in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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