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Some haematological and biochemical changes in albino rats in response to biopesticide used against *Callosobruchus maculatus* (Fab.) on *Vigna unguiculata* (L.)

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Abstract
This study investigates the hematological and biochemical impacts of formulated biopesticides from three plant species (*Ocimum gratissimum*, *Vernonia amygdalina*, and *Gongronema latifolium*) on albino rats, used in preserving *Vigna unguiculata* against *Callosobruchus maculatus*, a devastating field-to-store pest. Leaf extracts were obtained using distilled water and hexane, and then formulated into three synergistic combinations. Albino rats were divided into eight groups, including a control, and fed *Vigna unguiculata* samples treated with the biopesticide formulations and a synthetic insecticide (Deltamethrin). After 28 days, blood samples were analyzed for hematological and biochemical parameters. Results showed no significant increase in white blood cell count, but significant elevations in red blood cell count and packed cell volume in some biopesticide groups. No significant differences were observed in Liver enzymes, albumin, total protein, and globulin levels ($p>0.05$). These findings suggest the potential of these biopesticides as natural, safe, and eco-friendly alternatives to synthetic pesticides.

Keywords: *Callosobruchus maculatus*, biopesticides, synthetic pesticides, albino rats, hematological analysis, biochemical parameters

Introduction
The *Vigna unguiculata* weevil (*Callosobruchus maculatus*) is a widespread pest affecting *Vigna unguiculata* production in Africa and Asia, causing significant quantitative and qualitative losses [1, 2, 3]. In order to address these problems, farmers resort to using synthetic insecticides, which are said to be a major contributor to the increased agricultural productivity of the 21st century [4, 5]. However, long-term applications of synthetic insecticides have led to residues accumulating in different environmental components such as water, air, and soil [6]. More so, repeated application of synthetic pesticides leaves residues that may rise beyond the tolerance limits, thereby rendering Agricultural grains unsuitable for human and animal consumption [5]. Therefore, a non-toxic and ecologically tolerable control measures including the use of inert materials, plant powder, oils and extracts are sought in other to control [5].

Plants parts have considerable potential as biopesticides in controlling storage insect pests. These potentials have remained largely untapped due to continued dependence on broad-spectrum synthetic chemical insecticides by farmers [5]. Botanical insecticides have emerged as a promising alternative, with previous studies demonstrating their efficacy and safety [5, 7]. Efforts to develop and apply botanicals as alternatives to synthetic chemical insecticides have been reported by many researchers, for instance, Ogbonna *et al.* [6] reported the potential of *Zingiber officinale* in controlling *Callosobruchus maculatus*, while Oparaeke and Bunmi[8] and Oparaeke [9] showed the effectiveness of *Xylopia aethiopica* products against the common maize weevil. Idigo *et al.* [3] also demonstrated that some plant extracts could protect *Vigna unguiculata* seeds against *Callosobruchus maculatus*. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis* have been demonstrated [10].

In light of this, the present study aimed to formulate a biopesticide from the extracts of *Ocimum gratissimum* (Scent leaf), *Vernonia amygdalina* (Bitter leaf), and *Gongronema latifolium* (Utazi) and evaluate its impact on the haematological and biochemical indices of albino rats. The toxicity assessment of this biopesticide is crucial for human health, as it will provide valuable insights into its safety for use in food preservation [11]. According to Kanu *et al.* [11] chronic diseases affect blood cells adversely, therefore haematological data provides the most important information in the determination of biochemical and physiological state of animal models [12].

Damage mediated by free radicals results in a high rate of disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidative deoxyribonucleic acid (DNA), and alteration of platelet functions. The molecular damages have been linked with diabetes, cancers, inflammation, aging, and atherosclerosis [11]. From literature, many works have been done on the toxicity of *O. gratissimum, V. amygdalina,* and *G. latifolium* extracts, but to the best of our knowledge, little to no data is available on the impact of *O. gratissimum, V. amygdalina,* and *G. latifolium* (Either singly or in combination) on the haematological and biochemical indices of albino rats.

Therefore, the work evaluated the safety of biopesticides formulated with *O. gratissimum, V. amygdalina,* and *G. latifolium* extracts through levels of haematological and enzyme indices of albino rats. Data from the work is expected to enhance food security and low cost of insect pest management.

Materials and Methods

Collection, Identification and Preparation of the Plant Materials

The plants that were used for the study were *Ocimum gratissimum* (Scent leaf), *Vernonia amygdalina* (Bitter leaf), and *Gongronema latifolium* (Utazi). All the plant leaves for the experiment were harvested from Chimzik farms in Aguleri, Anambra East LGA of Anambra State. The plants were identification and authentication by Prof. C. G. Ukpaka, an experienced botanist from the Department of the Biological science, Chukwuemeka Odumegwu Ojukwu University.

Preparation of Aqueous and Hexane Extracts

The fresh leaves of *O. gratissimum, V. amygdalina,* and *G. latifolium*were then washed, shade-dried, and blended into a fine powder. Two hundred grams of each properly homogenized leaves were soaked in six liters of distilled water (1:10) for aqueous extract and hexane (1:10) for hexane extract respectively for 24 hours. These were filtered using a muslin cloth and a Whatman filter paper and then concentrated using a water bath at 50-60 °C. Viscous extracts were obtained and stored separately in airtight bottles in a refrigerator maintained at 2-8 °C until when required for further work [3].

Preparation of the Synergetic Plant Extracts

A stock solution of 200 mg/ml of each extracts was prepared. This was done by dissolving 20g of each extracts in 100ml of the relevant solvent (i.e. for the aqueous extract, distilled water was used for the reconstitution while acetone and Tween80 were used for the hexane extract due to its hydrophobicity), making a total of three stock solutions i.e. aqueous, n-hexane(acetone) and n-hexane(tween80). From this stock, a 150 mg/ml concentration was also prepared by using the dilution formula: $C_1V_1 = C_2V_2$, where C_1 is the stock concentration (200 mg/ml), V_1 is the unknown volume to be taken from the stock, C_2 is the concentration of the new solution to be prepared (150 mg/ml), V_2 is the total volume (10ml). All solutions were prepared and stored in a refrigerator in airtight bottles in a refrigerator maintained at 2-8 °C until the commencement of the repellency test [3].

Test Animals

A total of Forty-eight wistar albino rats, purchased from Onyewuchi farms, Ifite, Awka, Anambra state were used to carry out these studies. Before the toxicity assessment, the test animals were allowed to acclimatize in the animal house of the Department of Applied Biochemistry, Nnamdi Azikiwe University for a period of fourteen days. They were kept in a plastic cage and given a standard feed and water throughout the study.

Experimental Design

The wistar albino rats were divided into eight groups with each group containing six animals which were approximately equal to their average body weight. 5 ml of each treatment were applied on 150 g of homogenized *Vigna unguiculata* samples used in feeding the rats. This was allowed to air dry for 48 hours before feeding. The experimental groups were designated as follows: Group 1 (Normal control); Group 2 (150 mg/ml aqueous extract); Group 3 (200 mg/ml aqueous extract); Group 4 (150 mg/ml hexane extract reconstituted in acetone); Group 5 (200mg/ml hexane extract reconstituted in acetone); Group 6 (150 mg/ml hexane extract reconstituted in Tween 80); Group 7 (200 mg/ml hexane extract reconstituted in Tween 80); Group 8 (Standard insecticide). All experimental procedures and animal use were approved by the Nnamdi Azikiwe University- Animal Research Ethics Committee (NAU/AREC/2024/0069). At the end of the 28 days feeding, the animals were starved for 12 hours prior to blood collection to ensure a fasting state in all parameters. The test animals were anesthetized using chloroform and blood was collected via cardiac puncture. Samples for hematological analysis were transferred to an EDTA bottle during blood collection while those for other biochemical parameters were transferred to a plain bottle and then spun at 4000rpm for 30 minutes to get the serum. The serum was preserved in a refrigerator at +2 to +8^{o}C prior to the analysis.

Haematological Assays

The packed cell volume (PCV) and Haemoglobin (Hb) concentration were determined with the microhematocrit and cyanmethaemoglobin procedures, respectively [13]. The erythrocyte count was determined using the haematocytometry method [13]. Total white blood cells (WBC) counts in a haemocytometer with the WBC diluting fluid and differential leucocytes counts was done by counting the different types of WBC from Giemsa stained slides viewed from each of the 30 fields of an oil immersion objective of a microscope [14].

Biochemical Analysis of Serum

Kidney Function

The levels of urea and serum creatinine were determined using commercial kits from Randox Laboratories by a

method described by Fawcett and Scott [15] and Bartels and Bohmer [1] respectively.

Liver Function

Serum samples were assayed for alanine amino transferase (ALT) and aspartate amino transferase (AST) using standard diagnostic kits with a clinical spectrophotometer. Serum total protein (TP) was ascertained using the method of Reitman and Frankel [16] while albumin (ALB) was ascertained using the method of Dumas [17].

Data Analysis

The data was analysed using descriptive statistics such as mean and standard deviation (SD). The analysis of variance (ANOVA) at 95% confidence interval was used to compare the means across the different groups. The Tukey's post hoc test was used for pairwise comparison of the means in groups that had statistically significant difference. Statistical significance was determined at 5% probability level (p<0.05).

Results

Hematological Indices of Albino Rats Fed on Bio Preserved *Vigna unguiculata*: There was a non-significant (p>0.05) reduction in White Blood Cell Count (WBC) of rats in the insecticide diet group when compared with the other extract groups. However, a higher WBC count was recorded in the 150 mg/ml Hexane in Tween 80 group. There was a significant increase (p<0.05) in Red blood cell count (RBC) and Packed cell volume (PCV) count in the 200mg/ml acetone reconstituted hexane extract group when compared to other groups. The Hemoglobin (Hb) values for rat fed on the various diet groups do not differ significantly when compared (p>0.05). There was also no significant difference in platelet count when all the groups were compared (p>0.05) as presented in Table 1.

Table 1: Mean haematological indices as compared to controls

Groups	White blood cell count (10^9/L)	Red blood cell count (10^{12}/L)	Hemoglobin count (g/dl)	Packed cell volume (%)	Platelet count (10^9/L)
Normal control	9.45±0.15^a	7.22±0.45^{abc}	12.85±0.55^a	43.90±0.40^a	478.00±94.00^a
150 mg/ml Aqueous	13.75±0.05^a	6.92±0.41^a	6.92±0.41^a	45.10±2.00^b	619.00±61.00^a
200 mg/ml Aqueous	15.90±3.92^a	6.79±0.25^a	6.79±0.25^a	44.60±0.69^a	401.67±149.92^a
150 mg/ml Hexane in Acetone	11.93±2.87^a	6.50±0.10^a	6.50±0.10^a	41.53±0.98^a	366.33±100.71^a
200 mg/ml Hexane in Acetone	11.40±1.61^a	7.88±0.32^c	7.88±0.32^a	47.67±1.04^b	535.33±59.69^a
150 mg/ml Hexane in Tween 80	17.97±3.07^a	6.67±0.11^a	6.67±0.11^a	42.60±1.21^a	586.00±45.46^a
200 mg/ml Hexane in Tween 80	16.40±0.72^a	6.95±0.15^a	6.95±0.15^a	43.27±1.46^a	425.67±57.84^a
Standard insecticide	7.40±1.91^a	7.10±0.10^{ab}	7.10±0.10^a	44.27±0.46^a	531.33±47.98^a

The results are (mean ± SD) for six rats. Values followed by the same letter(s) in the same column were not significantly different at (p<0.05) using Tukey's post hoc test.

Biochemical Indices of Albino Rats Fed on Bio Preserved *Vigna unguiculata*

Kidney Function Assessment

The kidney function assessment shows the determination of two parameters *viz*; Blood Urea and Creatinine levels. The results show that there were significant elevations in creatinine levels (p<0.05) in aqueous and acetone reconstituted groups compared to the controls. Similarly, the urea levels were also significantly different when compared with the standard (p<0.05) as presented in Table 2.

Table 2: Mean kidney function parameters as compared to controls

Groups	Average Urea levels (mg/dl)	Average Creatinine levels (mg/dl)
Normal control	47.01±6.17^{cde}	3.96±0.89^b
150 mg/ml Aqueous	48.50±3.48^e	5.26±0.16^b
200 mg/ml Aqueous	23.29±3.23^a	4.23±1.33^b
150 mg/ml Hexane in Acetone	23.64±4.38^{ab}	4.58±1.38^b
200 mg/ml Hexane in Acetone	46.62±7.81^{cd}	5.65±1.82^b
150 mg/ml Hexane in Tween 80	18.76±4.38^a	1.11±0.29^a
200 mg/ml Hexane in Tween 80	30.29±5.27^{abc}	1.19±0.25^a
Standard insecticide	34.23±3.65^c	0.96±0.22^a

The results are (mean ±SD) for six rats. Values followed by the same letter(s) in the same column were not significantly different at (p0.05) using Tukey's post hoc test.

Liver Function Assessment

The Liver function assessment showed the determination of five parameters *viz*; alanine amino transferase (ALT), aspartate amino transferase (AST), albumin, total protein and globulin levels. From the results, there were slight non-significant (p>0.05) elevations in ALT and AST activity in the extract groups (ALT in the hexane extract group and AST in the 200 mg/ml aqueous group) but not in the insecticide group. There was a slight case of high albumin levels in the insecticide groups, though this wasn't significant (p>0.05) when compared to the extract groups and normal control. The total protein level showed no significant difference (p>0.05) in rats that consumed *Vigna unguiculata* from any of the extract groups when compared with the insecticide group. However, a lower protein level of 94.71 g/dl and 89.85 g/dl was observed in rats on 200mg/ml (HA) and 200 mg/ml (HT80) bio-preserved *Vigna unguiculata* diets respectively. The serum globulin content of rats across the groups was not significantly different (p>0.05) but is lower with values of 89.35 g/dl and 83.83g/dl in200mg/ml (HA) and 200mg/ml (HT80) groups respectively. However, it was more in the standard insecticide group (135.02 g/dl) as presented in Table 3

Table 3: Mean liver function parameters as compared to controls

Groups	ALT (U/L)	AST (U/L)	Albumin (g/dl)	Total protein (g/dl)	Globulin (g/dl)
Normal control	37.13±7.96[a]	119.60±12.69[a]	4.44±0.65[a]	99.48±14.63[a]	95.07±14.06[a]
150 mg/ml Aqueous	27.00±5.20[a]	109.50±11.50[a]	4.85±1.13[a]	117.02±33.43[a]	112.17±84.57[a]
200 mg/ml Aqueous	30.45±2.21[a]	129.67±24.68[a]	4.83±1.07[a]	127.32±16.46[a]	122.48±15.42[a]
150 mg/ml Hexane in Acetone	27.33±7.94[a]	124.60±21.91[a]	5.86±0.78[a]	106.55±7.22[a]	100.69±7.53[a]
200 mg/ml Hexane in Acetone	25.04±4.57[a]	87.00±10.98[a]	5.36±0.62[a]	94.71±11.82[a]	89.35±11.65[a]
150 mg/ml Hexane in Tween 80	23.20±3.73[a]	113.20±9.38[a]	4.43±0.56[a]	98.70±7.63[a]	94.00±7.63[a]
200 mg/ml Hexane in Tween 80	45.10±3.54[a]	111.13±11.52[a]	6.02±0.96[a]	89.85±16.41[a]	83.83±16.09[a]
Standard insecticide	35.00±2.29 [a]	102.90±13.46[a]	8.03±1.60[a]	128.58±17.14[a]	135.02±14.25[a]

The results are (mean ±SD) for six rats. Values followed by the same letter(s) in the same column were not significantly different at (p<0.05) using Tukey's post hoc test.

Discussion

Following ingestion, toxicants are transported by the blood to various organs including the liver and kidney where they may eventually cause harmful effects [11]. Blood can act as a pathological and physiological indicator of animal health [12]. In this study, there was a non-significant reduction in WBC count in the insecticide group. This finding is in disagreement with that of Kanu *et al*. [11] who noted an increase in WBC (p<0.05) at day 14, 21 and 28 indicative of leukocytosis. The reduction entails an onset of leucopenia which is a cause for concern since WBCs play a huge role in the body's immune system, defending against infection and foreign invaders. This occurrence opens the doors for the risk of increased infections. The mechanism through which this occurs remains unknown but might be attributed to increased inhibition of absorption of nutrients from the *Vigna unguiculata* consumed or depleted nutrient composition of the *Vigna unguiculata* since depletion in nutrients like: Vitamin B_{12}, folic acid and copper can inhibit WBC production. The non-significant WBC in the extract groups are not signs of a diseased state but normal upsurges in WBC production, though the solvents used in reconstitution could have played a role in that (especially Tween 80). There was a significant increase in RBC and PCV count in the 200mg/ml acetone reconstituted hexane extract group when compared to other groups. This might be described as an onset of polycythemia. Since the insecticide group showed no significant increase in RBC and PCV, this is therefore in agreement with the earlier findings [11, 18] after the administration of dichlorvos-based insecticide. The underlying cause of the adnormal rise in the RBC and PCV associated with the 200 mg/ml HA group might be the solvent of reconstitution (acetone) inhibiting the production of erythropoietin, a hormone that regulates red blood cell formation, thus up regulation of red blood cells. There was no significant difference in platelet count when all the groups were compared (p>0.05).

Storage of food products is very necessary to ensure food safety and prevent food scarcity but most of these storage agents (Insecticides) might have detrimental effects on organ function. These effects could be on homeostatic organs (Like the liver and kidney). The liver is the organ most commonly involved in the metabolism of endogenous and foreign compounds. Blood is transported to the liver through the portal vein which carries blood containing digested nutrients from the gastrointestinal tract and the hepatic artery which carries oxygenated blood from the lungs.[19] Liver enzymes AST and ALT are frequently used as biomarkers of liver injury because they are released by hepatocytes into the extracellular space [20]. This study showed a slight non-significant elevations in ALT and AST activity in the extract groups (ALT in the hexane extract groups and AST in the 200mg/ml aqueous group) but not in the insecticide group. These elevations being non-significant (p>0.05) showed no onset of liver dysfunction. There was a slight case of high albumin levels in the insecticide groups, though this wasn't significant, it shows onset of systemic dehydration associated with using this synthetic insecticides. The study also shows a slight non-significant increase in the total protein in the aqueous extract (AQW) group and also in the insecticide group compared to those of other groups. There was no significant difference in globulin when all the groups were compared (p>0.05). However, there was a slight non-significant increase in globulin level in the AQW group and also in the insecticide group compared to those of other groups. The slight increases in the total protein in the AQW group and the insecticide group; seem to be attributed to the slight increase in the albumin and globulin level.

The study also showed significant elevations in creatinine levels (p<0.05) in some of the herbal extract groups when compared with the controls especially in the hexane extract reconstituted with acetone (HA) groups. This increase in creatinine levels might be attributed to the solvent used in the reconstitution of the extracts, especially acetone. The urea levels were also significantly different in the 200 mg/ml aqueous, 150 mg/ml Hexane in Acetone and 150 mg/l Hexane in Tween 80 groups from that of the control. The lower creatinine level in the insecticide group might be attributed to its lower growth rate (Just a 20% increase over a month) since lower body mass can be attributed to lower creatinine levels and thus malfunction in the ultra-filtration mechanism of the kidneys. Previous studies have however observed an increase in creatinine [21, 22] and blood urea [22] as the duration of the exposure increased.

Conclusion

The non-significant increases and reductions in majority of the haematological and biochemical indices of the albino rat tested on an acute scale showed a healthy state in the rats that consumed the *Vigna unguiculata* preserved with the formulated biopesticides. There was a significant increase observed in the RBC count and PCV in 200mg/ml hexane extract reconstituted with acetone (HA) group when compared to other groups. This significant increase in PCV in 200 mg/ml HA group might have serious health implication in the long run. Also, the significant reduction in creatinine level in the standard insecticide and hexane extract reconstituted with tween 80 (HT80) group, may have chronic effect if the research is left for a longer period. The significant reduction in urea level might have occurred by chance since it involved aqueous, acetone and tween 80 reconstituted groups at different concentrations. Therefore,

the use of these herbal insecticides doesn't have any detrimental effect on acute scale and can as well make a good substitute to synthetic insecticides owing to their high insecticidal potential against *Vigna unguiculata* weevils and thus help to reduce the development of resistance. More work is recommended on the topic at a longer period of exposure.

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