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ORIGINAL RESEARCH

Assessment of Haemato-Toxicity Induced by Chemical and Biological Pesticides in Grain-Fed Male Wistar Rats

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ABSTRACT

Haemato-toxicity, characterized by alterations in hematological profiles, poses significant health risks, mainly due to the widespread use of pesticides in agriculture. This research is driven by the need to understand the comparative toxicological effects on various haematological parameters. This study investigated the haematological effects of chemical (organophosphate) and biological pesticides in Wistar rats. Thirty-five Wistar rats were divided into seven groups and fed grains treated with either bio-pesticides or chemical pesticides for ten days. Blood samples were collected and analyzed to measure changes in key haematological parameters. The results indicate that chemical and biological pesticides did not significantly alter Packed Cell Volume (PCV) or haemoglobin levels, with all treated groups showing values comparable to the control group. However, there was a mild reduction in Red Blood Cell (RBC) count, particularly in the groups treated with fungi-based biopesticides, suggesting a potential impact on erythropoiesis or RBC integrity. Specifically, the fungi pesticide rice group showed a reduction of approximately 9.32% in RBC count compared to the control group. Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) showed slight increases in chemical pesticide-treated groups, indicating mild macrocytosis and elevated haemoglobin content. For instance, the chemical pesticide rice group exhibited a 4.76% increase in MCV, and a 4.58% increase in MCH compared to the control. In contrast, fungi-based biopesticide groups had MCV values closer to the control, suggesting less impact on RBC size. Mean Corpuscular Hemoglobin Concentration (MCHC) values remained broadly consistent across all groups, with changes of less than 0.03%, demonstrating that pesticide exposure did not significantly alter haemoglobin concentration within the red blood cells. Overall, the findings suggest that both chemical and biological pesticides have moderate haematological effects, with fungi-based biopesticides demonstrating a relatively safer profile on erythrocyte health.

Keywords: Haemato-toxicity, Bio-pesticide, Chemical pesticide, Wistar rats, Red Blood Cell

Introduction

Haematotoxicity, the harmful effect of certain drugs on the haematopoietic system and blood, is a critical concern. Assessing the impact of chemical and bio-pesticides used in grain preservation on haematological parameters in Wistar rats is essential for understanding these effects. Grains are a fundamental food source globally, providing essential nutrients and energy through cereals like rice, wheat, barley, oats, rye, millet, and maize. However, the widespread use of pesticides poses a risk due to environmental pollution and potential health hazards (Afolabi et al., 2020).

Pesticides are a concern because they are typically applied carelessly and in huge quantities, polluting the environment (Kalender et al., 2005). Severe pesticide poisoning may have several negative consequences. According to Köprücü et al. (2006), one of the harmful health impacts of pesticides is a severe and ongoing injury that results in blood problems. Diverse organ systems, including the blood, liver, kidney, and cytotoxic impact in experimental animals, have been toxicologically affected by pesticides in diverse ways. Both *in vivo* and *in vitro*, pesticides have been demonstrated to cause oxidative stress and modify the antioxidant system, which can result in hematotoxicity (Ambali et al., 2011; Elsharkawy et al., 2013).

Chemical pesticides, while effective, can have toxic effects on various organ systems, including blood, liver, and kidneys, and can induce oxidative stress (Aktar et al., 2009). However, bio-pesticides, derived from natural sources, are considered a safer alternative for pest management (Rajendra, 2013).

There is a need to understand the haematological impact of both chemical and bio-pesticides, given that grains treated with these substances are a major component of the human diet. While the toxicological effects of chemical pesticides are relatively documented, there is less clarity on the specific haematological impacts of bio-pesticides, particularly in comparison to chemical pesticides. This study uniquely addresses this gap by directly comparing the haematological effects of commonly used chemical pesticides (organophosphate) with different types of bio-pesticides (bacterial and fungal) in Wistar rats, providing a more nuanced understanding of their relative haematotoxicity.

Materials and Methods

Formulation of Rat Feed and Incorporation of Bio-pesticides (Fungi)

The food formulation contains: 40 percent carbohydrate, 20 percent animal protein, 20 percent plant protein (cowpea), 15 percent fat and oil, 5 percent in form of salts, vitamins and minerals. The feed was milled into pellets using pellet milling machine with each pellet in the size range of 4mm. a batch of the feed was formulated using cowpea preserved with chemical pesticide, the second batch was formulated using cowpea preserved with the biopesticide while the third batch was formulated using unpreserved cowpea. 200 g of the powdered biopesticide was added to 1 kg of the pelletized feed and mixed thorougly. The formulated feeds were used in feeding the experimental animals after the first week.

Formulation of Bio-pesticides and Incorporation into Rat Feed (Bacteria)

A loopful of the bacteria were inoculated into sterilized nutrient broths and incubated in a rotary shaker at 150 rpm for 72 hours at room temperature. After 72 hours of incubation, Bacteria broth cultures were centrifuged to separate and obtain their cells from the broth. The cells were further washed by reconstituting with sterile saline and centrifuging again. The suspension was serially diluted and the concentration adjusted to 10^6 cfu/ml. The appropriate diluent was cultured to enumerate the viable bacterial cells present within. 400 ml of bacterial broth suspension, 1 kg of the purified talc powder, 15 g of calcium carbonate (to adjust the pH to neutral) and 10 g of Carboxy methyl cellulose were mixed under sterile conditions. The resulting product was shade dried to reduce the moisture content below and then packed in a polypropylene bag and sealed (Senthilraja and Vijayakumar, 2010). The dried formulation was added to the pelletized feed formulation by adding 200g of the formulation to 1 kg of the feed.

Formulation of Chemical Pesticide and Incorporation into Feed

The organophosphate chemical was used in the preservation of stored grains. They were diluted in distilled water and prepared according to manufacturer's specification. 10 ml of the chemical was sprayed on 1 kg of the feed and mixed thoroughly. The sprayed feed was allowed to air dry for 24 hours and packed.

Animals

Thirty-five (35) male Wistar rats were purchased and housed at the animal house of Federal University Lokoja, Kogi state, Nigeria. The Animals were acclimatized for 2 weeks under standard environmental conditions, with an approximately 12- hour light/dark cycle and fed a standard laboratory diet and water. After 2 weeks of acclimatization, the animals were administered the treatment feed formulated with grains preserved with chemical pesticides and bio-pesticides. The animals used in the present study were maintained by the principles and guidelines of the Canadian Council on Animal Care by Ahmadi-Noorbakhsh et al. (2021).

Experimental Design

The animals were divided into seven (7) groups of five (5) each based on their weight ranges. Treatments were carried out orally for ten (10) days in other to assess the short-term effects of the pesticides on the haematological parameters of the rats. Grains preserved with chemical and bio-pesticides were formulated into feeds and hundred (100) grams were administered to the rats daily throughout the experiment. This enables the experimental animals have access to adequate nutrition, *ad libitum*, allowing for observation of the effects of the pesticide-treated grains across all treatment groups. Also, administering a fixed amount of 100 grams daily helps prevent wastage, ensures consistency and makes comparison of the different pesticides easier across all treatment groups.

Bodyweight Changes

According to Balcombe et al. (2004), the rats were weighed daily from the day before the first day of treatment till the tenth day of treatment. They were further weighed on the eleventh day before they were sacrificed. The changes in the body weights were documented.

Blood Collection

The blood samples were obtained through ocular puncture according to the method outlined by Fizner et al. (2006) using heparinized capillary tubes were collected into labelled heparinized bottles and centrifuged at 4000 rpm for minutes to separate the whole blood into plasma and erythrocytes. The plasma was carefully removed using a syringe and stored in the deep freezer until further analysis at Eden Laboratory, along Mount Patti Road, Lokoja.

Table 1: Treatment groups and Feed Administration

Groups	Treatment	Weight of feed (g)
1	Control (untreated grains)	100
2	Chemical Pesticides (Rice)	100
3	Chemical Pesticides (Cowpea)	100
4	Biopesticides Bacteria (Rice)	100
5	Biopesticides Bacteria (Cowpea)	100
6	Biopesticides Fungi (Rice)	100
7	Biopesticides Fungi (Cowpea)	100

Table 1 shows the classification of treatment groups used in the experimental evaluation of the effects of grains preserved with chemical and biological pesticides on the cardiac status of wistar rats. Each treatment group received a standardized amount of grains (100 grams) to ensure consistency across the experiment. The treatments are categorized as follows: Group 1 serves as the control, containing untreated grains (normal grains) to provide a baseline for comparison; Groups 2 and 3 are treated with chemical pesticides, applied separately to rice and cowpea respectively; Groups 4 and 5 involve treatment with bacterial-based

biopesticides, again applied separately to rice and cowpea; while Groups 6 and 7 use fungal-based biopesticides, also applied to each grain type separately.

Haematology

Haematological assessments of TLC (Th/mm3), RBC (million/mm3), HGB (gm/dl), CT. %, MCV (µmm3), MCH (pg), MCHC (gm/dl), LYM %, MO %, GRN. %, RDW %, PCT, MPV (µmm3) and PDW % were also determined using auto-analyzer through standard methods, according to Ragav et al. (2021).

Packed Cell Volume (PCV): Small quantities of the blood sample collected into the EDTA tubes were transferred into heparinized capillary tubes. The tubes were three-quarters filled, and one end was sealed immediately with plasticine. They were arranged orderly into the micro-haematocrit centrifuge machine, covered and centrifuged for 5 minutes. After this, the PCV was checked using a micro haematocrit reader in percentage (%) units (Dacie and Lewis, 1991).

Haemoglobin Estimation: Three test tubes labelled blank (B), test (T) and standard (S) were arranged in a test-tube rack. 5ml of working reagent (comprised of dihydrogen potassium phosphate, potassium ferricyanide and potassium cyanide) was pipette into all the test tubes. 20µl of the sample was aspirated into the test tube labelled (T) and 20µl of standard aspirated into test tube labelled (S) respectively. The solutions were mixed and allowed to stand for 3 minutes at 15-25^{0}C. The absorbance was then measured with a spectrophotometer against the blank. The unit is in g/dl (Cheesbrough, 2006).

Calculation

$$Haemoglobin\ conc = \frac{Abs\ of\ Test}{Abs\ of\ Standard}\ x\ Conc\ of\ standard$$

Erythrocyte (RBC) Estimation: The procedure was similar to WBC's. Using the RBC graduated pipette, the whole blood was pipette to 0.5ml mark, and the diluting fluid (comprising sodium citrate, formalin and distilled water) was added to 101 mark on the pipette. The counting chamber was covered with a cover slip

and the mixed blood in the RBC pipette was dispensed on the covered portion of the chamber and viewed at a magnification of X40 objective under the microscope. The cells were counted on the ruled squares apportioned for RBC with the aid of a hand tally counter. The portion for RBC counting in the chamber was made up of 25 bigger squares with 16 smaller squares in each of them (Dacie and Lewis, 2001).

Calculation:

$$No\ of\ cells\ of\ blood = \frac{Cells\ counted\ x\ Blood\ dilution\ x\ Chamber\ depth}{Area\ of\ Chamber\ counted}$$

$$= \frac{No\ of\ cells\ counted\ x\ 200\ x\ 10}{1/5}$$

$$= No\ of\ cell\ counted\ x\ 10000\ (x\ 10^6/\mu l)\ unit$$

Statistical Analysis

All data were collected and tabulated, and results were expressed as mean ± standard deviation (n= 4). The values were subjected to one-way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) 21st version for comparing means of all groups. Duncan Multiple Range Test (DMRT) was used to separate means with significant difference ($p<0.05$) at a 95% confidence interval (Cl).

Result

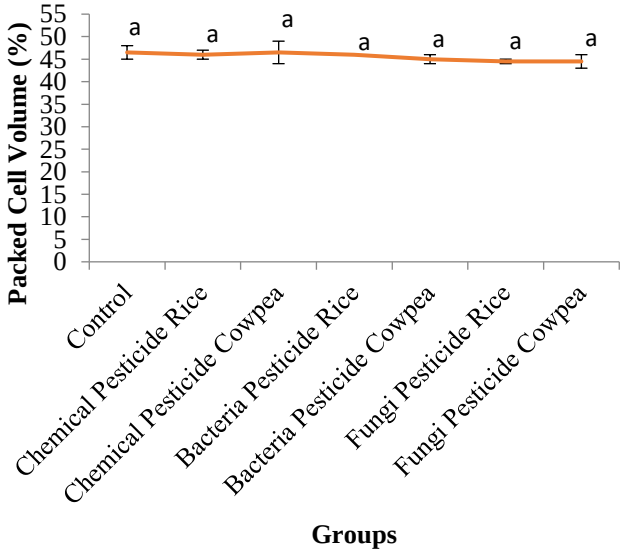

Figure 1: Effect of Chemical pesticide and Bio-pesticides on Packed Cell Volume

Values are expressed as Mean ± SD (n=4). Values with different superscripts are significantly different at P< 0.05.

From the result observed in Figure 1, there was no significant difference between the test groups compared to control.

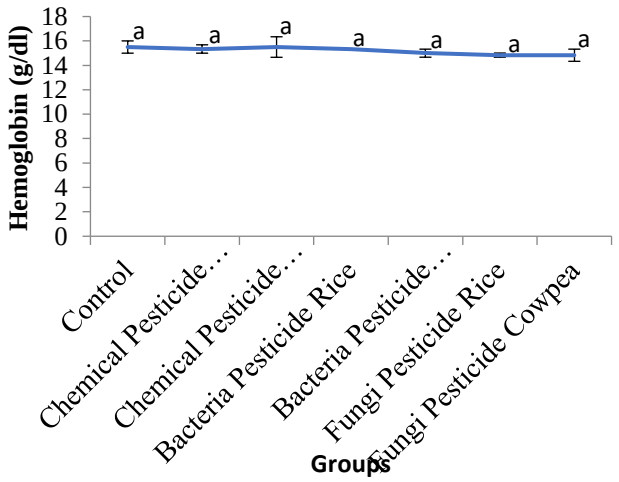

Figure 2: Effect of Chemical pesticide and Bio-pesticides on Hemoglobin level

Values are expressed as Mean ± SD (n=4). Values with different superscripts are significantly different at P< 0.05.

No significant differences were observed across any of the groups compared to the control as seen in Figure 2.

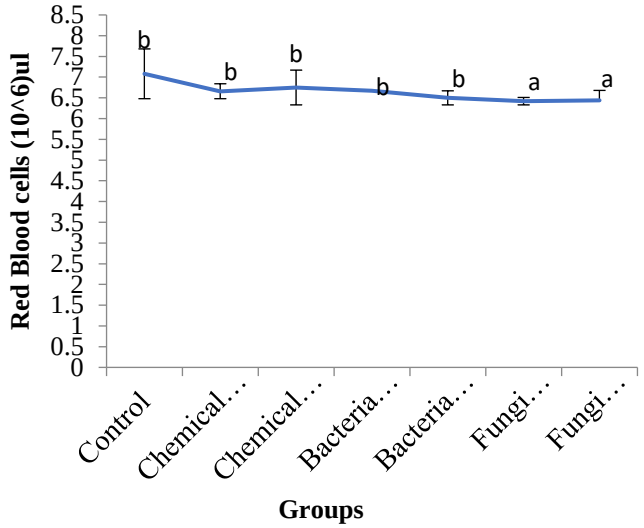

Figure 3: Effect of Chemical pesticide and Bio-pesticides on Red Blood Cell

Values are expressed as Mean ± SD (n=4). Values with different superscripts are significantly different at P< 0.05.

Result from Figure 3 showed no significant difference in the Chemical Pesticide Rice, Chemical Pesticide Cowpea, Bacteria Pesticide Rice, Bacteria Pesticide Cowpea groups when compared to the control group while a significant decrease of 9.32% and 9.04% was observed in Fungi Pesticide Rice and Fungi Pesticide Cowpea groups respectively when compared to the control group

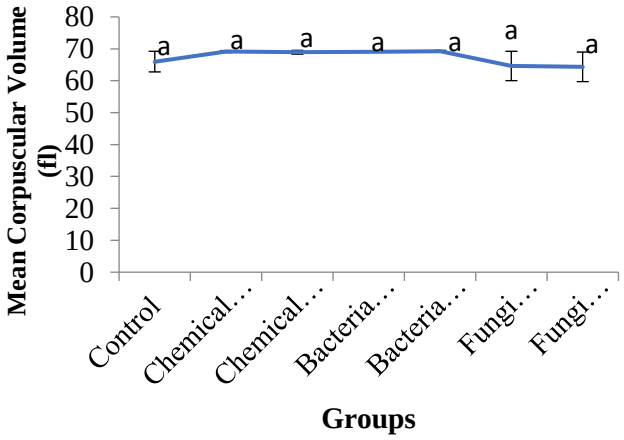

Figure 4: Effect of Chemical Pesticide and Bio-pesticide on Mean Corpuscular Volume

Values are expressed as Mean ± SD (n=4). Values with different superscripts are significantly different at P< 0.05.

No significant differences were observed in the Mean Corpuscular Volume (MCV) across any of the groups compared to the control (Figure 4).

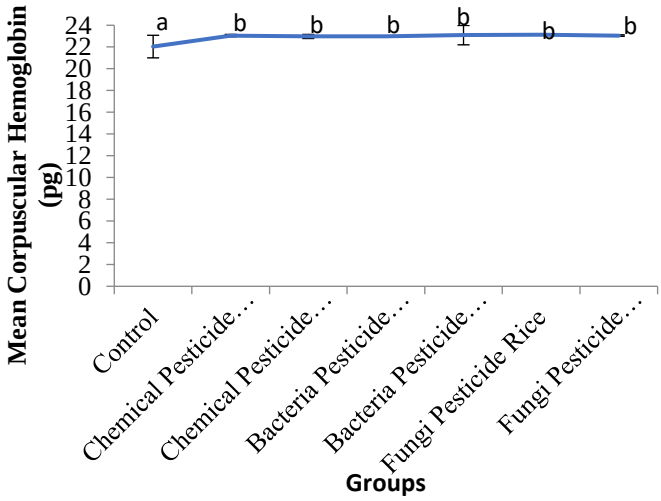

Figure 5: Effect of Chemical Pesticide and Bio-pesticide on Mean Corpuscular Hemoglobin

Values are expressed as Mean ± SD (n=4). Values with different superscripts are significantly different at P< 0.05.

Figure 5 showed a significant increase in MCH (Mean Corpuscular Hemoglobin) in all pesticide-treated groups (Chemical Pesticide Rice, 4.58%; Chemical Pesticide Cowpea, 4.22%; Bacteria Pesticide Rice, 4.26%; Bacteria Pesticide Cowpea, 4.76%; Fungi Pesticide Rice, 4.95% and Fungi Pesticide Cowpea, 4.58%) when compared to the control group.

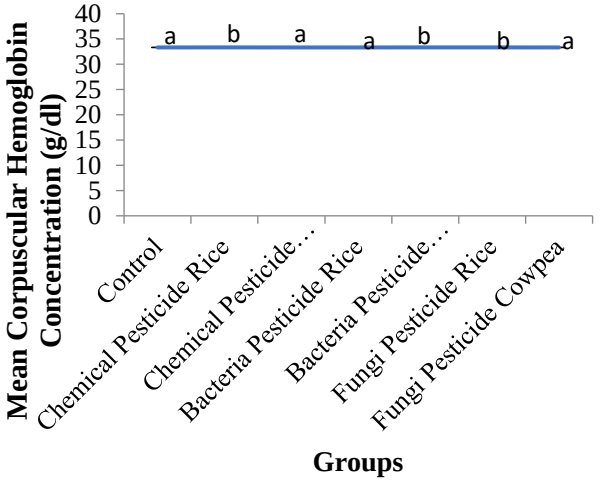

Figure 6: Effect of Chemical Pesticide and Bio-pesticide on Mean Corpuscular Hemoglobin Concentration

Values are expressed as Mean ± SD (n=4). Values with different superscripts are significantly different at P< 0.05.

Figure 6 showed no significant difference in groups administered with Chemical Pesticide Cowpea, Bacteria Pesticide Rice, and Fungi Pesticide Cowpea compared to the control while a significant increase of 0.03% was observed in groups administered with Chemical Pesticide Rice, Bacteria Pesticide Cowpea, and Fungi Pesticide Rice compared to the control group.

Discussion

The haematological parameters, including Packed Cell Volume (PCV), haemoglobin concentration, and Red Blood Cell (RBC) count, provide crucial insights into the physiological effects of pesticide exposure on blood health. In this study, the PCV and haemoglobin levels were not significantly affected across all the groups. The control group exhibited a PCV of 46.50% and a haemoglobin level of 15.50 g/dL, consistent with the values observed in pesticide-treated groups. The absence of major changes in these parameters suggests that pesticide exposure did not induce anaemia or disrupt the oxygen-carrying capacity of the blood. Similar

findings were reported by Gokcimen et al. (2007), who observed no significant changes in PCV or haemoglobin levels in rats exposed to sub-lethal doses of pesticides.

However, the RBC count was slightly reduced, particularly in the groups treated with fungi-based pesticides. The fungi pesticide rice group had a significantly lower RBC count (6.42 × 10^6/µL) of 9.32% compared to the control group (7.08 × 10^6/µL), indicating a potential negative impact on erythropoiesis or RBC survival. This reduction in RBCs could be linked to oxidative stress or direct toxic effects on the bone marrow, as reported in previous studies on pesticide toxicity (Aly et al., 2010). Aly et al. (2010) demonstrated that pesticide exposure can disrupt haematopoiesis by inducing oxidative damage and reducing RBC count. In the present study, the slight decrease in RBCs in the fungi pesticide groups aligns with the findings of Aly et al., (2010) supporting the notion that certain pesticides, even biological ones, can mildly affect blood cell production. Chemical pesticides, particularly in the rice and cowpea groups, did not cause significant changes in RBC count compared to the control. The chemical pesticide cowpea group had an RBC count of 6.75 × 10^6/µL, slightly lower than the control but not statistically significant (4.66%), indicating that chemical pesticides may have a less severe impact on RBC production at the doses used in this study. These results are consistent with the findings of Ahmed et al. (2016), who reported minimal haematological alterations in rats treated with moderate doses of chemical pesticides. Bacterial pesticides also had a limited effect on RBC count. Both the bacteria pesticide rice (6.67 × 10^6/µL) and cowpea (6.50 × 10^6/µL) groups showed slight reductions (5.80% and 8.19% respectively) in RBCs. However, the changes were statistically insignificant, suggesting that bacterial pesticides, often considered environmentally safer, may still pose mild haematological risks, particularly in long-term exposure scenarios. This observation is supported by literature showing that some biopesticides can induce mild haemato-toxic effects, likely due to byproducts or metabolic disturbances caused by microbial agents (Singh et al., 2018).

The MCV values in this study were generally consistent across groups, with some variation. The control group had an MCV of 65.99 fl, while the chemical pesticide rice and cowpea groups exhibited slightly elevated MCV values of 69.13 fl and 68.93 fl, respectively. These increases suggest a mild macrocytic effect, which could indicate altered RBC production or response to stress caused by chemical pesticide exposure. Previous studies have reported that pesticide exposure can cause RBC enlargement, often as a compensatory mechanism in response to oxidative stress or reduced erythropoiesis (Ahmed et al., 2018). The bacteria pesticide groups (rice: 69.02 fl; cowpea: 69.24 fl) showed similar MCV levels to the chemical pesticide groups, indicating that biopesticides may induce macrocytosis to some extent. However, the fungi pesticide groups (rice: 64.63 fl; cowpea: 64.36 fl) displayed lower MCV values, which are closer to the control, suggesting a lesser impact on RBC size. This finding aligns with research indicating that fungi-based biopesticides might cause less severe alterations in RBC morphology than chemical pesticides (Ortiz-Hernández et al., 2019).

The MCH values, which reflect the amount of haemoglobin per RBC, were relatively uniform across all groups. The control group had an MCH of 22.04 pg, and other groups exhibited slightly higher values, ranging from 22.97 to 23.13 pg. As observed in other studies, an increase in MCH could suggest a mild increase in haemoglobin synthesis or a compensatory response to oxidative stress from pesticide exposure (Dhouib et al., 2016). However, the differences in MCH are small, and the overall values remain within normal ranges, suggesting that pesticide exposure did not drastically impair haemoglobin synthesis. This result is consistent with research indicating that short-term pesticide exposure may not always cause significant reductions in haemoglobin content, particularly moderate exposure (Ahmed et al., 2018).

The MCHC values, representing hemoglobin concentration in RBCs, were consistent across most groups, with slight variations. The control group had an MCHC of 33.33 g/dl, and all other groups, except the chemical pesticide rice and bacteria pesticide cowpea groups, maintained similar MCHC values. The slight increase

observed in the chemical pesticide rice (33.34 g/dl) and bacteria pesticide cowpea (33.34 g/dl) groups indicates a minor alteration in RBC haemoglobin concentration, though the overall consistency suggests that pesticide exposure had limited effects on haemoglobin concentration within RBCs. This finding aligns with studies showing that MCHC is often less sensitive to moderate pesticide exposure than other haematological parameters (Figueiredo et al., 2021).

Conclusion

In conclusion, this study reveals that both chemical and bio-pesticides have effects on various haematological parameters in Wistar rats. Chemical pesticides can have toxic effects on organs and induce oxidative stress. Bio-pesticides, although considered safer, also exhibit haematological impacts. The findings indicate that fungi-based bio-pesticides have a more noticeable effect on RBC count compared to other pesticides. Overall, the research emphasizes the need for careful evaluation of both chemical and bio-pesticides to understand their haematological consequences.

Recommendations

Based on the findings of this study, minimizing the use of chemical pesticides is recommended due to their potential, albeit mild, haematological effects, particularly on RBC morphology. Integrated Pest Management (IPM) strategies, which combine biological control methods and reduced pesticide application, should be encouraged to limit the negative impacts on blood health. Fungi-based biopesticides, which showed fewer alterations in haematological parameters, can be considered a safer alternative for pest control. Additionally, further research is needed to explore the long-term effects of both chemical and biological pesticides on haematological parameters, especially under different exposure conditions. Monitoring the blood health of individuals frequently exposed to these pesticides, such as agricultural workers, could help to detect and prevent potential adverse effects early.

Authors' Contributions

Faokunla Opeyemi designed the research, carried out the laboratory experiment, and compiled the manuscript; Mohammed Sani Sade designed the research and corrected the manuscript; Owoeye Fisayo Deborah designed the research, carried out the laboratory experiment and the statistical analysis; Kelly Babatunde Abiodun designed the research, formulated the chemical and biological pesticides preserved feed; Amoo Olumayowa Temiotan designed the research. Ajayi Boluwatife Mercy and Jimoh Tijani carried out the laboratory experiment and statistical analysis, and compiled the first draft; Abel Ikoojo Grace, Adejoh Vivian Uyo, Adeoye Theresa Meka, Ojo Dorcas Bisola, Luke David Peculiar, Ajayi Anuoluwapo Mary, Oladimeji Fatimah Bolanle and Alfred Temitope Oladimeji carried out the laboratory experiments.

Conflict of Interest

None

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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