

ORIGINAL RESEARCH

Sex-Specific Variations in Acetylcholinesterase Activity Following Exposure to Pesticide-Treated Grains in Wistar Rats

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ABSTRACT

This study investigated the neurotoxic effects of chemical (specifically dichlorvos) and biological (bacteria - *Pseudomonas aeruginosa* and fungi - *Beauveria bassiana*) pesticides on acetylcholinesterase (AChE) activity in male and female Wistar rats fed with pesticide-treated rice and cowpea. AChE activity served as a biomarker of neurotoxicity. Seventy adult rats (35 males and 35 females) were randomly assigned to 14 treatment groups of five animals each and fed with 100 g of pesticide-treated or untreated grains for 10 days. On the eleventh day, the animals were sacrificed and the blood obtained through ocular puncture into a heparinized bottle. AChE activity was measured spectrophotometrically and analyzed using one-way ANOVA with significance at $p < 0.05$. In male rats, chemical pesticide (rice) treatment significantly reduced AChE activity by 47.74% compared to control, indicating marked neurotoxicity. Conversely, chemical pesticide (cowpea) caused a 26.91% increase, suggesting compensatory enzymatic upregulation. Bacterial and fungal pesticide treatments caused substantial AChE elevation, particularly bacterial pesticide (cowpea) with a 125.4% increase over control, suggesting low neurotoxicity or enzymatic induction. In female rats, AChE activity in the chemical pesticide (rice) group showed a 9.6% increase relative to control, while cowpea (chemical) reduced activity by 17.4%. Bacterial pesticide (rice) treatment markedly decreased AChE activity by 41.5%, suggesting potential neurotoxicity in females. Fungal pesticide treatments exhibited variable effects, with AChE activity increasing by 15.7% in the rice group and decreasing by 36.4% in the cowpea group. These findings reveal sex-specific and treatment-specific neurotoxic responses, with chemical pesticides showing stronger inhibitory effects in males. Bio-pesticides, while generally safer, displayed dose- and grain-type-dependent variability. The study underscores the need for sex-inclusive neurotoxicity assessments and supports cautious use of biopesticides in food systems.

Keywords: Neurotoxicity, Acetylcholinesterase (AChE), Chemical pesticides, Biopesticides, Wistar rats

Introduction

Grains, also known as cereals, are a vital human food group and a valuable source of fibers, proteins, carbohydrates, phytochemicals, minerals, and vitamins. Frequent consumption of grains appears to be associated with several health benefits (Zhu et al., 2017; Tieri et al., 2020). Globally, cereals and legumes (grains) are essential dietary components due to their high protein and carbohydrate content (Borneo et al., 2012). Food security is a fundamental aspect of any nation's social and economic prosperity, and agriculture gives the continent of Africa a means of subsistence and sustainability (Hollinger et al., 2015). Observational and experimental studies show that grains are abundant in polyphenols, which play a role in modulating the host immune response and regulating a variety of pathways, including oxidative and inflammatory ones (Basili et al., 2012; Akhlaghi et al., 2012).

Chemical pesticides are synthetic chemical substances produced in factories through chemical synthesis (Shah, 2021). Insects, rodents, fungus, and weeds that impede or damage the growth of crops, shrubs, trees, woodlands, and other plants utilized for human benefit are to be eradicated or their growth inhibited (Pimentel, 2005). However, all chemical pesticides are a long-term threat to human health and the environment because of their intrinsic toxicity and capacity to develop resistance in plant or human tissues. According to Damalas et al. (2011), many pesticides are non-specific and can kill both beneficial and innocuous living things. Chemical pesticides eliminate pests rapidly and efficiently. They are made from sulfur, lime, ferrous sulfate, copper sulfate, and other inorganic or artificial salts. Pests can easily absorb them because to their simple and highly soluble chemical compositions, which increases their activity and environmental longevity (Kim et al., 2017; Abubakar et al., 2020). Organophosphorus, carbamates and pyrethroids are some examples of

chemical pesticides that can also be categorized based on their chemical structure (Bortoli et al., 2018).

Biopesticides, also known as biological pesticides, are defined by the Environmental Protection Agency (EPA) in the United States as insecticides made from natural components, such as bacteria, plants, animals, and specific minerals (Shah, 2021). Biopesticides can be defined as natural biological agents that kill pests by causing specific biological effects instead of chemical poisoning, or as a type of pesticide based on naturally occurring microorganisms or natural products that are considered active substances but do not harm people or the environment (Leahy et al., 2014). According to Rajput et al. (2020), biopesticides—which can include entomopathogenic viruses, bacteria, fungi, nematodes, or secondary plant metabolites—have complicated definitions and are becoming more and more significant in agricultural practice as substitutes for synthetic chemical pesticides. They are also a crucial part of many pest management programs. Third-generation pesticides include biopesticides, which are ecologically friendly products that are either extremely similar to or identical to natural chemicals (Chandler et al., 2011). In order to protect themselves from illnesses and insects, plants and some microorganisms produce a variety of natural chemicals. In a similar vein, natural pesticides are substances derived from natural sources. Biopesticides include commonly used natural pesticides such as nicotine, rotenone, neem, pyrethrins, sabadilla, fluoroacetate, ryania, *Bacillus thuringiensis* (Bt), and spinosad (Regnault et al., 2008).

When chemical, biological, or physical agents negatively impact the structure and function of the nervous system, especially the brain, this is referred to as neurotoxicity (Tilson 2008). The brain is an incredibly intricate biological system that controls perception, behavior, and reaction to outside stimuli. It is in charge of coordinating the actions of every other organ system and mediating communication with the outside world (Bilge, 2022). Both observational and experimental studies

show that grains are abundant in polyphenols, which play a role in modulating the host immune response and regulating a variety of pathways, including oxidative and inflammatory ones (Akhlaghi et al., 2012). Dietary polyphenols have been widely studied for their neuroprotective effects, particularly against pesticide-induced neurotoxicity. Pesticides such as organophosphates, carbamates, and pyrethroids exert neurotoxic effects through oxidative stress, neuroinflammation, mitochondrial dysfunction, and acetylcholinesterase (AChE) inhibition. Polyphenols counteract these effects via antioxidant (Ahmed et al., 2021), anti-inflammatory (Chhillar and Dhingra, 2018), and enzyme-modulating mechanisms (Kaur et al., 2019).

The widespread use of chemical pesticides in grain preservation poses significant public health concerns due to their known neurotoxic effects. In regions where food safety regulations may be less stringent, especially in developing nations, there is a pressing need to evaluate the potential health risks associated with pesticide residues in staple foods. Rice and cowpea were chosen for this study because they are part of the most vulnerable grains/crop to post harvest spoilage and pest attack. They are also the most common grains together with maize that are famously preserved. Understanding how different types of pesticides affect biological systems, particularly the nervous system, is critical in advocating for safer agricultural practices and protecting consumer health. Despite the known hazards of chemical pesticides, there is a paucity of comparative data on the neurotoxic effects of chemical versus biological (biopesticide) treatments, particularly in staple grains like rice and cowpea. This study aims to address this gap by directly assessing the neurotoxic impact of both categories of pesticides using acetylcholinesterase (AChE) activity as a biomarker in a controlled experimental setting.

This research uniquely investigates the sex-specific neurotoxic responses to both chemical and biopesticides in Wistar rats fed with pesticide-treated rice and cowpea. By evaluating AChE activity,

a sensitive indicator of neurotoxicity, this study provides novel insights into the relative safety of biopesticides, as well as the potential variability in susceptibility based on sex, which is often overlooked in toxicological studies. The primary aim of this study is to evaluate and compare the neurotoxic effects of chemical and biological pesticides on acetylcholinesterase activity in male and female Wistar rats fed with treated grains. The findings aim to inform safer pest management strategies and contribute to evidence-based recommendations for pesticide regulation and food safety.

Materials and Methods

Equipment and Reagents

Separation of the whole blood was performed using table top centrifuge (ET-80-3). The chemical reactions were monitored spectrophotometrically with visible spectrophotometer (V721). The samples and chemicals were preserved in the refrigerator (HTF-100H). Heparinized tubes were used for blood collection which was carried out through ocular puncture under anesthesia. Other apparatus used were beakers, eppendorf tubes, syringes and cuvettes. The micropipette tips were dried using the thermostat oven (ET-9025A). All chemicals used in this study were of analytical grade and were used without further purification. Acetylthiocholine iodide, sodium hydrogen carbonate, DTNB and sodium dihydrogen phosphate were obtained from Bridge Biotech Limited, Ilorin, Kwara state.

Formulation of Rat Feed and Incorporation of Biopesticides (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 (200 g/kg) and mixed thoroughly. The formulated feeds were used in feeding the experimental animals after the first week.

Formulation of Biopesticides 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 (dichlorvos) 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

Seventy (70) adult wistar rats (35 male and 35 female) of 120 grams to 150 grams were purchased from the Department of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria. They were housed in standard laboratory cages under controlled environmental conditions (temperature: $25 \pm 2^{\circ}\text{C}$; relative humidity: 50–60%; 12-hour light/dark cycle) at the animal house of the Department of Zoology, Federal University Lokoja, Kogi State, Nigeria. The animals were acclimatized for a period of fourteen days prior to the commencement of the experiment and had free access to standard pelleted feed and clean drinking water *ad libitum*. Bedding material was changed twice weekly, and all animals were monitored daily for signs of stress or illness. After 14 days of acclimatization, the animals were divided into 14 groups (7 groups per sex) of five animals each. These groups were subjected to various treatments for a period of 10 days and were euthanized on the eleventh day after of the experiment following anaesthesia with diethylether. The animals used in the present study were maintained in accordance with the principles and guidelines of the Canadian Council on Animal Care as outlined in “Guide for the Care and Use of Laboratory Animals” (1993). Every effort was made to minimize animal suffering and reduce the number of animals used.

Table 1: Treatment Groups and Feed Administration for Both Male and Female Groups

Groups	Feed	Weight (g)
1	Control	100
2	Chemical pesticide rice	100
3	Chemical pesticide cowpea	100
4	Bacterial biopesticide rice	100
5	Bacterial biopesticide cowpea	100
6	Fungi biopesticide rice	100
7	Fungi biopesticide cowpea	100

Table 1 shows the classification of treatment groups per sex used in the experimental evaluation of the effects of grains preserved with chemical and biological pesticides on the cardiac status of wistar rats using AChE as biomarker. 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. This classification allows the study to assess and compare the impact of grains preserved with chemical pesticides and the two forms of biopesticides (bacterial and fungal) on AChE in Wistar rats.

Experimental Design

Each sex of animals was divided into seven (7) groups of five (5) animals each based on their weight ranges. The weight of the animals ranges from 120 grams to 150 grams with those of similar weights pooled together. The administration of the feed (100g daily) and water was done *ad libitum*.

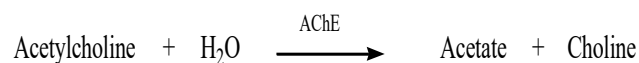
Bodyweight changes

The rats were weighed daily from the period of acclimatization to the eleventh day of the treatment period when they were sacrificed. The changes in the body weights were documented.

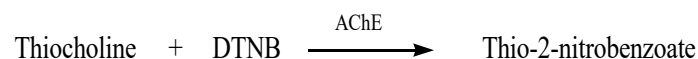
Determination of Acetylcholinesterase (AChE) Activity

Determination of acetylcholinesterase activity was done according to the method of Whittaker et al. (1983).

Principle: Acetylcholinesterase catalyses the hydrolysis of acetylcholine to acetate and choline.



The catalytic activity was measured following the increase of 5-thio-2-nitrobenzoate produced from thiocholine when it reacts with 5,5-dithio-bisnitrobenzoic acid (DTNB).



Calculation:

$$\text{Enzyme Activity} = \frac{\Delta \text{Abs} \times T.V \times 1000 \times \text{dilution factor}}{\epsilon \times l \times t \times S.V}$$

where:

T.V = Total assay volume in litres

ϵ = Molar extinction coefficient ($1.361 \text{ M}^{-1} \text{ cm}^{-1}$)

l = Path length (1cm)

t = Assay time (3 minutes)

S.V = Sample volume in litres

Statistical analysis

All values are expressed as means \pm standard deviations. Data were analyzed by one-way ANOVA and significant differences between groups were determined by Duncan's multiple range test. Statistical analyses were done using SPSS 20, the statistical package. The acceptable level of significance was $p < 0.05$. The superscripts were used to depict significantly different ($p < 0.05$) mean values at 95% confidence interval (CI).

Results

All data were collected and tabulated, and results were expressed as mean \pm standard deviation of at least four representatives per group.

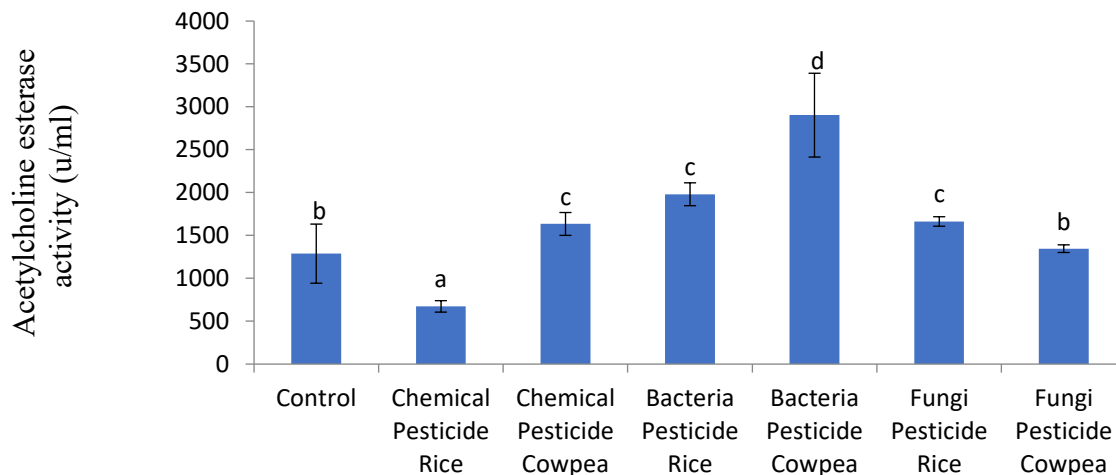


Figure 1: Acetylcholinesterase (AChE) activity in male Wistar rats fed with grains treated with chemical (Groups 2 and 3) and biological (Groups 4–7) pesticides.

Data are presented as mean \pm SD (n=4). Different superscripts indicate statistically significant differences ($p < 0.05$).

The results in Table 1 show that AChE activity in male Wistar rats varied markedly following exposure to pesticide-treated grains. Rats fed with chemically treated rice grains showed a 47.76% decrease in AChE activity compared with the control, indicating a significant ($p < 0.05$) inhibition of the enzyme. In contrast, the chemical pesticide cowpea group demonstrated a 26.87% increase in enzyme activity relative to control, while the bacteria pesticide rice group recorded a 53.73% increase. The bacteria pesticide cowpea group showed the highest and most significant increase, with enzyme activity rising by 125.37%. Similarly, rats in the fungi pesticide rice group exhibited a

29.10% increase compared to control values. However, the fungi pesticide cowpea group recorded only a 4.48% increase which was statistically non-significant ($p > 0.05$).

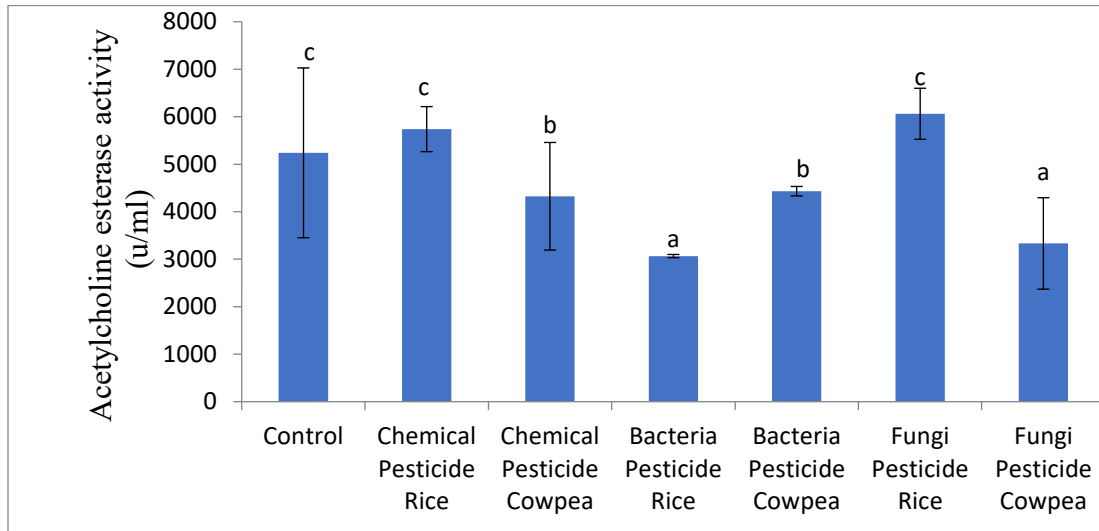


Figure 2: Acetylcholinesterase (AChE) activity in female Wistar rats fed with grains treated with chemical (Groups 2 and 3) and biological (Groups 4–7) pesticides.

Data are presented as mean \pm SD (n=4). Different superscripts indicate statistically significant differences ($p < 0.05$).

As shown in Table 2, the pattern of AChE activity in female rats differed notably from that of the males. Rats fed with chemical pesticide-treated rice grains showed a 9.54% increase, which was statistically non-significant ($p > 0.05$). Likewise, fungi pesticide rice-treated rats displayed a 15.72% increase compared to control, also without statistical significance. Conversely, chemical pesticide cowpea treatment led to a 17.43% decrease in AChE activity. The most substantial reductions were observed in the bacteria pesticide rice (41.47% decrease) and fungi pesticide cowpea (36.39% decrease) groups, both of which were highly significant ($p < 0.05$). The bacteria pesticide cowpea group also showed a 15.41% decrease in enzyme activity compared to the control.

Discussion

This study revealed sex-specific and pesticide-type-dependent alterations in acetylcholinesterase (AChE) activity in Wistar rats following dietary exposure to grains preserved with either chemical or biological pesticides. AChE serves as a critical biomarker for neurotoxicity, and its inhibition is closely associated with excessive synaptic acetylcholine accumulation, leading to overstimulation of cholinergic pathways, neuronal damage, and in severe cases, mortality (Costa, 2018).

Significant reductions in AChE activity were observed in rats fed with chemically preserved rice and cowpea (Groups 2 and 3), consistent with the known neurotoxicity of organophosphates such as dichlorvos, the chemical used in this study. These compounds exert toxicity by irreversibly phosphorylating the serine residue in the active site of AChE, inhibiting enzymatic breakdown of acetylcholine at synapses (Eddleston et al., 2008; Kim et al., 2021). The AChE inhibition was more pronounced in male rats, highlighting possible sex-linked susceptibility to pesticide-induced neurotoxicity.

Several mechanisms may underlie this difference. Female rodents are reported to express higher levels of detoxifying enzymes such as cytochrome P450 isozymes, glutathione-S-transferases, and paraoxonase-1 (PON1), which contribute to faster metabolism and clearance of organophosphate metabolites (Rettberg et al., 2014; Engin et al., 2021). Moreover, estrogen and other ovarian hormones are known to exert neuroprotective effects, possibly by modulating oxidative stress responses and maintaining cholinergic neurotransmission (Simpkins et al., 2020). These factors likely contributed to the higher AChE activity observed in female rats within comparable treatment groups.

In contrast, rats administered grains treated with bacterial or fungal biopesticides (Groups 4–7) showed no significant reduction in AChE activity, with values remaining comparable to control animals. This observation supports the safety of biopesticides, whose active compounds typically function through mechanisms unrelated to cholinesterase inhibition, such as disruption of insect midgut membranes (e.g., *Bacillus thuringiensis*) or interference with insect hormonal systems (e.g., neem extracts) (Mandal et al., 2023; EFSA, 2022). Biopesticides are widely recognized for their selective toxicity to pests with minimal adverse effects on mammals and non-target organisms (Rajput et al., 2020).

Interestingly, AChE activity in female rats exposed to biopesticide-treated grains was slightly higher than in males, further suggesting sex-based differences in neurological resilience even in low-risk exposure scenarios. This sex variation underscores the importance of incorporating gender as a biological variable in neurotoxicological risk assessments.

The observed suppression of AChE activity in chemically exposed rats aligns with classical cholinergic neurotoxicity and validates AChE as a robust early biomarker of neural dysfunction. Several recent studies confirm the utility of AChE activity in identifying subclinical neurotoxic exposure to organophosphates and other xenobiotics, both in laboratory animals and human epidemiological contexts (Abdel-Rahman et al., 2019; Anifandis et al., 2022).

The findings have meaningful implications for food safety and public health, particularly in regions with limited regulation of post-harvest pesticide application. The preservation of AChE activity in biopesticide-exposed rats strongly supports their adoption as safer alternatives in grain storage. This aligns with growing international emphasis on sustainable agriculture and reduction of synthetic pesticide dependence (FAO and WHO, 2023).

Given the sex-specific sensitivity observed, regulatory toxicology protocols should consider sex as a critical factor in pesticide risk assessment. Furthermore, the study advocates for routine biomonitoring of neurotoxicity biomarkers, including AChE, in populations with potential dietary pesticide exposure.

Conclusion

This study highlights the neurotoxic risks of chemical pesticide-treated grains, particularly in male rats, as evidenced by significant AChE inhibition. In contrast, biopesticide treatments showed minimal or no neurotoxic effect, reaffirming their suitability for food preservation. The study also underscores the importance of sex-based considerations in toxicology and calls for regulatory and research shifts toward safer pest control strategies and comprehensive health risk evaluations.

Author's 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 both the chemical-preserved grains and biological pesticides preserved grains; Amoo Olumayowa Temiotan designed the research. Ojo Dorcas Bisola carried out the laboratory experiment and statistical analysis, compiled the first draft; Abel Ikoojo Grace carried out the laboratory experiment; Adeoye Theresa Meka carried out the laboratory experiment; Ajayi Boluwatife Mercy carried out the laboratory experiment; Jimoh Tijani carried out the laboratory experiment and statistical analysis, compiled the first draft; Luke David Peculiar carried out the laboratory experiment; Ajayi Anuoluwapo Mary

carried out the laboratory experiment; Oladimeji Fatimah Bolanle carried out the laboratory experiment; Alfred Temitope Olademeji carried out the laboratory experiment.

Conflict of Interest

The authors declare no conflict of interest.

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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