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**Serum Levels of Total Antioxidant Capacity and Total Plasma Peroxide in Farm Workers Exposed to Pesticides in Southwestern Nigeria**Eliezer Oluwatimileyin Adeleke<sup>1</sup>, Surajudeen Adebayo Yaqub<sup>2</sup>, Olayinka Joshua Olufemi<sup>2</sup> Emmanuel Marvelous Oyelaran<sup>1</sup>Department of Biomedical Laboratory Science, College of Medicine, University of Ibadan, Nigeria<sup>1</sup>, Department of Immunology, College of Medicine, University of Ibadan, Nigeria<sup>2</sup>.Author for Correspondence: olayinkajoshua1@gmail.com/+234- 706-696-7702. <https://dx.doi.org/10.4314/sokjmls.v10i4.1>**Abstract**

The effect of pesticides on the levels of different serum antioxidants and oxidants have been reported in farm workers exposed to pesticides. However, there is limited research on the effect of pesticide exposure on the serum levels of Total Antioxidant Capacity (TAC) and Total Plasma Peroxide (TPP) in pesticide-exposed farm workers in South-western Nigeria. Therefore, this study was designed to investigate the impact of chronic pesticide exposure on the oxidative stress indices which are the Total Antioxidant Capacity (TAC) and Total Plasma Peroxide (TPP) with a view to adding to the growth of the body of knowledge. A total of 70 adults consisting of 40 farm workers with prolonged pesticide exposure and 30 apparently healthy non-farm workers were enrolled in this study. Serum levels of Total Antioxidant Capacity (TAC) and Total Plasma Peroxide were determined using Spectrophotometry. Data were analyzed using the Student's t test and Pearson Coefficient correlation, with a p-value < 0.05 considered statistically significant. The serum level of serum TPP was significantly higher in farm workers [ $92.35 \pm 36.67 \mu\text{M}$  vs  $61.09 \pm 42.87 \mu\text{M}$ ] compared with the controls and the serum level of TAC was slightly raised in farm workers compared with the controls. An insignificant positive correlation was also observed between serum level of TAC and TPP in the farm workers [ $1028 \pm 590 \mu\text{M}$ ]. There was a significant positive correlation observed between duration of pesticide exposure and the level of TAC and insignificant positive correlation between duration of exposure and TPP. It could be concluded from this study that

farm workers exposed to pesticides have altered serum levels of TAC and TPP and are prone to inducing oxidative stress.

**Keywords:** Pesticides, Total Plasma Peroxide, Total Antioxidant Capacity

**Introduction**

The term pesticide is generally used to describe agrochemicals such as fungicides, herbicides, insecticides, or rodenticides (Sule *et al.*, 2022). They are a group of chemicals and sometimes microorganisms (e.g., viruses) that are used for the destruction of insects, weeds, fungi, bacteria, etc (Jayaraj *et al.*, 2017). They could be grouped into different chemical families, such as organophosphates, organochlorines, organofluorines, carbamates, pyrethroids, bipyridyl herbicides, triazine herbicides, triazoles, and chloroacetanilide herbicides (García-Casillas *et al.*, 2024). Globally, approximately three billion kilograms of pesticides are used annually, yet less than 1% effectively targets insect pests on crops, while the remainder disperses into the environment (Chaudhary *et al.*, 2024). This widespread dispersal significantly increases the risk of exposure for farm workers, who are directly in contact with these chemicals during application, handling, storage, and disposal processes (Robinson *et al.*, 2024).

Although low levels of pesticide exposure may be tolerated by the immune system without causing long-term harm, sustained exposure to high concentrations over time, as often experienced by farm workers, can result in toxic

health effects (Hernández *et al.*, 2013). Exposure can also occur outside active pesticide applications, such as during entry into recently treated fields, from pesticide drift, or through contact with residues on soil or crops (Ye *et al.*, 2013; Bradman *et al.*, 2015; Damalas and Koutroubas, 2016). Dermal exposure is particularly prevalent in uncovered areas such as the hands and face (Lari *et al.*, 2022).

Reports of previous research on pesticides suggest that pesticides induce oxidative stress in humans (Liu, 2024). Therefore, the harmful effects of pesticides are related to the induction of oxidative stress which is usually as a result of an increase in the level of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Consequently, these overwhelm the ability of antioxidants (such as such as glutathione and the activities of antioxidant enzymes) to protect cells from oxidative damage (Ledda *et al.*, 2021). As a result, the imbalance between the oxidants and antioxidants, which are the common characteristics of oxidative stress, affect increased protein oxidation (carbonylation), lipid peroxidation and nucleic acid oxidation (Sharifi-Rad *et al.*, 2020; Ahmad *et al.*, 2024). Oxidative stress can be established by evaluating Total Antioxidant Capacity (TAC) and Total Plasma Peroxide (TPP) as biomarkers.

The Total Antioxidant Capacity, which is the moles of oxidants that is neutralized by one litre of solution, is a biomarker that measures the antioxidant potential of body fluids (Peluso and Raguzzini, 2016). It refers to the collective ability to neutralize reactive oxygen species (ROS) and free radicals, which are harmful byproducts of cellular metabolism (Silvestrini *et al.*, 2023). The assays are recognized as instrumental to establish antioxidant status of biological samples (Marques *et al.*, 2014). Antioxidants act by reacting with reactive oxygen species (ROS) and reactive nitrogen species (RNS) to neutralize or terminate the chain reaction before key molecules in the body are affected (Jomova *et al.*, 2023). The major enzymatic antioxidant defense mechanisms consist of different forms of superoxide dismutases (SODs), catalase (CAT), and glutathione peroxidases (GPXs) (Ighodaro and

Akinloye, 2017). SODs are thought to provide a first line of defense against oxygen radicals, specifically the superoxide anion ( $O_2^-$ ), which is the major ROS produced by mitochondrial respiration and various metabolic reactions (Chidambaram *et al.*, 2024). The SODs remove the potentially dangerous superoxide anions from biological systems by converting them to  $H_2O_2$ , while CAT found in peroxisomes catalyzes the conversion of two molecules of  $H_2O_2$  to two molecules of  $H_2O$  and  $O_2$  (Kiran *et al.*, 2023). The most abundant intracellular antioxidant, glutathione (GSH), is involved in the protection of cells against oxidative stress (Vázquez-Meza *et al.*, 2023).

Furthermore, exposure to pesticides has been associated with significantly increased ROS including superoxides and peroxides which consequently leads to oxidative stress induction that is beyond what the intrinsic cellular antioxidant system can reduce to normal physiological levels (Sule *et al.*, 2022). Total Plasma Peroxide as a biomarker represents the entirety of the effects all the types of peroxides present in the plasma. Plasma peroxides include hydrogen peroxides and organic peroxides which are the byproducts of many normal cellular metabolism processes that are required to generate energy for life processes.

Elevated levels of peroxides in plasma can suggest an imbalance between the production of ROS and the body's antioxidant defense mechanisms which leads to oxidative damage to lipids, proteins, and DNA (Afzal *et al.*, 2023). This study was therefore designed to evaluate serum levels of Total Antioxidant Capacity (TAC) and Total Plasma Peroxide (TPP) in farm workers exposed to pesticides in Southwestern Nigeria. It aims to determine whether pesticide exposure correlates with altered oxidative stress markers, thereby providing insight into the biological burden experienced by these individuals. In summary, oxidative stress remains a critical mechanism in pesticide-induced toxicity, and the dual evaluation of TAC and TPP provides a meaningful and measurable representation of this process.

## Materials and Methods

### Study Area

Farmers were enrolled from Oyebola Village and Alapako Village, Obafemi Owode Local Government, Ogun State and Akinlaalu Village, Ife North Local government, Osun State. Obafemi Owode Local government lies on latitude 6° - 57° North and 3° - 30° East with an area of 1410sqkm. It has a total population estimated to be three hundred and ninety-nine thousand, eight hundred (399,800) (NPC, 2022). Likewise, Ife North Local government lies on latitude Lies on latitude 7° - 31' North and 4° - 27' East with an area of 889 sqkm. It has a total population estimated to be one hundred and fifty-three thousand, six hundred and ninety-four (153,694) (NPC, 2022). The inhabitants are predominantly farmers engaging in cash crop agriculture such as cocoa and cashew.

### Study Design

This is a case control study.

### Study Setting

The study site included Osun State and Ogun State, Nigeria. They are part of the major farming states in southwestern part of Nigeria. Participants for study were those that were exposed to pesticide and were from major farmlands. The study involved both field and laboratory work.

### Study Population

A total of 70 participants were enrolled in this study. A total of 40 farmworkers who were exposed to prolonged use of pesticides and 30 apparently healthy non farmworkers were enrolled into this study.

### Determination of Sample Size

According to a previous study in pesticide-exposed farm worker by Arunsi *et al.* (2022), the sample size was calculated using the formula:

$$n = \frac{(Z_{\alpha/2} + Z_{1-\beta})^2 (\sigma_1 + \sigma_2)^2}{(\mu_1 - \mu_2)^2}$$

Where: n = Sample size  $Z_{1-\beta}$  = One-sided percentage point of the normal distribution corresponding to 100% minus power, 1.28 for 80% power ( $1 - \beta = \text{Power of study} = 80\%$ )

$Z_{\alpha/2}$  = Percentage point of the normal distribution corresponding to the (two-sided) significance level = 1.96 (95% confidence level).

$$\mu_1 = 201.2$$

$$\mu_2 = 387$$

$$\sigma_1 = 25.6$$

$$\sigma_2 = 15.5$$

Substituting the values into the equation:

$$n = 20.37$$

However, to increase the power of study, 40 farm workers were enrolled.

### Inclusion criteria

#### Exposed

Individuals who met the following inclusion criteria were enrolled into the study:

1. At least 25 years of age
2. Must be a farmer who have been using pesticides for at least ten years within southwest Nigeria.
3. No history of chronic medical conditions such as hypertension, diabetes mellitus, stroke, chronic kidney disease (CKD), and chronic liver disease (CLD)
4. Provision of written informed consent

#### Controls

1. At least 25 years of age
2. Non farmworker and have no history of using pesticides
3. No history of chronic medical conditions such as hypertension, diabetes mellitus, stroke, chronic kidney disease (CKD), and chronic liver disease (CLD)
4. Provision of written informed consent

### Exclusion Criteria for Cases and Controls

Participants who satisfied any of the following criteria were excluded from the study:

1. Failure to meet any of the inclusion criteria as appropriate
2. Pregnant women
3. Refusal to give informed consent

### Ethical Consideration

Ethical approval UI/EC/23/0545 was obtained from the University of Ibadan/University College Hospital Joint Ethics Committee before the commencement of the study. Also, informed consent was obtained from each study

participants before enrolment into the study.

### **Pesticide Exposure Assessment and Data Collection**

Structured interviews and questionnaires were used to gather data on types, frequency, and duration of pesticide use. Information on clinical history were also obtained using a structured questionnaire.

### **Sample Collection**

Using aseptic technique, 10ml of venipuncture blood sample was collected into a clean dry plain sample container and kept away from sunlight. The blood sample was allowed to clot, then dislodged and spurned at 3000 rpm for 10 minutes to obtain serum. The serum was collected and dispensed into a dry, chemically clean serum container after which the samples were stored at - 20°C until analyzed.

### **Laboratory Analysis**

#### **Determination of Total Antioxidant Capacity (TAC)**

Serum TAC was assessed using the Ferric Reducing Antioxidant Power (FRAP) assay, based on the method of Benzie and Strain (1999). The assay measures the reduction of the Fe<sup>3</sup> - TPTZ complex to Fe<sup>2</sup>, producing a blue-colored product measured spectrophotometrically at 594 nm. Results were expressed in μM equivalents of ascorbic acid.

#### **Determination Of Total Plasma Peroxide (TPP)**

TPP levels were determined using the FOX-2 assay, a modified method described by Miyazawa (1989). This method detects the oxidation of ferrous ions to ferric ions by plasma peroxides, forming a ferric-xyleneol orange complex measured at 560 nm. The peroxide content was quantified using hydrogen peroxide standards and expressed in μM.

### **Data Analysis**

All data were carefully entered on an excel spreadsheet and reassessed for accuracy and completeness before statistical analysis. The data analysis was done using SPSS version 23.0. Before analysis, data was assessed for Gaussian distribution and thereafter Student's t-test was used to determine differences in means of variables between the groups. Pearson's correlation coefficient was used to examine the strength of linear relationships between quantitative variables. P-values less than 0.05 (2-tailed) was considered as statistically significant. The results were expressed as Mean ± Standard Deviation (S.D).

### **Results**

The characteristics of the study participants are shown in table 1. The mean age for the farmers is 37.6±8.2 and that of the control is 32.2±6.8. The farm workers had low lower education attainment compared with controls, only 10% had tertiary education while 2.5% had no formal education at all. 37.5% of them occasionally use pesticide, especially organophosphate and glyphosate. The majority of them (82.5%) had no pesticide usage training and 52.5% of them did not use Personal Protective Equipment (PPE). As shown in table 2, the serum level of TPP was significantly higher in farm workers compared with controls and the serum level of TAC was slightly elevated in farm workers compared with the controls. Correlation between serum TAC and TPP levels in farm workers and controls are shown in table 3. There was no significant correlation between serum TAC and TPP in farm workers in both groups. Table 4 shows a correlation between levels of the serum TAC, TPP and the duration of pesticide exposure in farm workers. However, there was an insignificant correlation between them.

**Table 1: Characteristic of Study Participants**

| Variables                       | Farm Workers (n=40) | Controls (n=30) |
|---------------------------------|---------------------|-----------------|
| <b>Age</b>                      |                     |                 |
| Mean age±SD (years)             | 37.62±8.25          | 32.2±6.8        |
| <b>Educational status</b>       |                     |                 |
| No formal education             | 1(2.5%)             | 0               |
| Primary six                     | 23(57.3%)           | 0               |
| SSCE                            | 12(30%)             | 3(10%)          |
| Tertiary                        | 4(10%)              | 27(90%)         |
| <b>Type of Pesticide Used</b>   |                     |                 |
| Paraquat                        | 5(12.5%)            | N/A             |
| Organophosphate and glyphosate  | 35(87.5%)           | N/A             |
| <b>Pesticide usage training</b> |                     |                 |
| Yes                             | 7(17.5%)            | N/A             |
| No                              | 33(82.5%)           | N/A             |
| <b>Use of PPE</b>               |                     |                 |
| Yes                             | 19(47.5%)           | N/A             |
| No                              | 21(52.5%)           | N/A             |

*Values are in number (percentage)*

**Table 2: Total Antioxidant Capacity (TAC) and Total Plasma Peroxide (TPP) in study participants**

| Parameters | Farm Workers (n=40) | Controls n=30 | p-value |
|------------|---------------------|---------------|---------|
| TAC(μM)    | 1028±590            | 865.90±355.48 | 0.158   |
| TPP(μM)    | 92.35±36.67         | 61.09±36.     | 0.002*  |

*\*Significant at P<0.05, TAC= Total Antioxidant Capacity, TPP= Total Plasma Peroxide*

**Table 3: Correlation between Serum Levels of TAC and TPP among study participant**

| Parameter    | r-value | p-value |
|--------------|---------|---------|
| Control      | 0.061   | 0.744   |
| Farm Workers | 0.058   | 0.719   |

**Table 4: Correlation between levels of serum TAC, TPP and duration of Pesticide Exposure in farm workers**

| Parameter | Farm Workers(n=40) | p-value |
|-----------|--------------------|---------|
| TAC       | 0.349              | 0.027*  |
| TPP       | 0.083              | 0.608   |

\*Significant at  $P < 0.05$ , TAC= Total Antioxidant Capacity, TPP= Total Plasma Peroxide

### Discussion

The modern agricultural practices rely heavily on the usage of pesticides to control pest and then to improve both farm yield quality and quantity (Sharma et al., 2019). While the benefits of the usage of pesticides are central in ensuring food security for the ever-growing population around the globe, close attention must be paid to health consequences of prolonged exposure to pesticides. These health consequences have been linked to the induction of oxidative stress in farm workers by pesticides (Sule *et al.*, 2022). Therefore, studying the effect of pesticides on total antioxidant capacity and total plasma peroxide which are both oxidative stress indices is important.

In this study, the majority of the farm workers were observed to be less educated and also the majority had no pesticide-usage training. These findings are in accordance with multiple studies that have reported limited education and training on pesticide usage as major factors that contribute to the risk of pesticide exposure (Ahmadipour and Nakhei, 2024; Damalas *et al.*, 2024). Furthermore, the irregular usage of PPE as observed from the studies further consolidated the reports from Okoffo *et al.* (2016) and Lari *et al.* (2023) that associated the risk of exposure and toxicities of pesticides with inadequate usage of PPE.

Findings of this study showed that farm workers exposed to pesticides have significantly higher TPP than the controls. The significant elevation serum TPP level might be due to excessive production of free radicals, which is as a result of effect of pesticide on NADPH oxidases (NOXs) and superoxide ( $O_2^-$ ) levels, leading to an increase in ROS. This is consistent with many previous studies that reported increase in ROS due to exposure to pesticides (Cortés-Iza and Rodríguez, 2018; Sule *et al.*, 2022; Sajad *et al.*,

2024). Considering the harmful effects of excessive ROS on molecules, elevation could predispose the exposed farmers to infection and susceptibility to cancers and other diseases.

In addition to the above, pesticides, especially organophosphate and carbamates have been reported in many articles to be inhibitors of the enzyme acetyl cholinesterases (Vale and Lotti, 2015). The inhibition could lead to production of calcium ( $Ca^{2+}$ ) influx into neurons via muscarinic receptors and the elevated intracellular  $Ca^{2+}$  levels can trigger mitochondrial dysfunction, leading to increased production of reactive oxygen species (ROS) such as superoxide radicals ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) (Pearson and Patel, 2016).

However, a slightly elevated serum level of TAC in farm workers was observed compared with the controls in this study. This observation contradicts the report of Arunsi *et al.*, (2022) in pesticide exposed farm workers. This contradiction might be due to the differences in the duration of exposure to pesticides and their diets. The elevated antioxidant capacity could be as a result of compensatory mechanism to mitigate the effects of the rising ROS in farm workers (Savu *et al.*, 2012). This could be done by mobilization of different antioxidants.

Correlating the serum levels of TPP and TAC in farm workers together, an insignificant positive correlation was observed. The positive correlation further buttresses compensatory response where the body increases the production of antioxidants to oxidative stress. However, the insignificance could suggest that the response might not be adequate, and the farm workers are prone to developing oxidative stress.

Finally, there was a significant positive correlation observed between duration of pesticide exposure and the level of TAC and insignificant positive correlation between duration of exposure and TPP. This may suggest that the longer the duration of exposure the higher the level of TPP. The positive significant correlation between TAC and duration of exposure could further reveal the compensatory mechanism which is protective measures exhibited by the human body to counteract the potential of ROS. This observation is supported by previous studies where exposure to pesticides have been associated with high plasma peroxides and oxidative stress (Anderjaska *et al.*, 2020; Aroniadou *et al.*, 2023; Garud *et al.* 2024).

### Conclusion

It could be concluded from this study that chronic exposure to pesticide decreases the serum level of Total Antioxidant Capacity and increases serum level of Total Plasma Peroxide compared with controls. The alteration in the levels of both Total Antioxidant Capacity and Total Plasma Peroxide predisposes the farm workers to oxidative damage of molecules especially when there is no medical intervention. Therefore, safety measures including the use of PPE and pesticide-use training are necessary for the farm workers.

### Limitations

Small sample size and inability to conduct thorough medical tests to ascertain the absence of other free-radical generating conditions were limitations in this study. Exposure to environmental toxins and as well as dietary factors such as nutrition and food quality may also influence the levels of TAC and TPP. These were not accounted for in this study and could have impacted on the accuracy of the results.

### Recommendations

Based on the observations from this study, the following could be recommended:

- The farm workers should be well trained in the safe and effective method of pesticide usage
  - As government at all levels empower farmers with farm tools, Personal Protective Equipment should equally be seen as vital equipment for effective farming.
- Farm workers should be well taught on the correct and importance of usage of Personal Protective Equipment.
  - Regular medical checkups and personal hygiene should be encouraged among the farm work.

### Conflict of Interest Declaration

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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