World Journal of Advanced Research and Reviews

eISSN: 2581-9615 CODEN (USA): WJARAI

Cross Ref DOI: 10.30574/wjarr

Journal homepage: https://wjarr.com/

(Research Article)

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Biochemical profile of populations exposed to organophosphorus pesticides in the municipality of Bingerville versus those of Port-Bouët

Angbo Kousso Marie Angéline [1,*], Kambou Sansan Philippe [1], Bedou Kouassi Denis [2] and CISSE-CAMARA Massara [1]

[1] *Laboratory of Medical Biochemistry, Medical Sciences Training and Research Unit, Félix Hoophouse Boigny University, Abidjan, Côte d'Ivoire.*

[2] *Laboratory of Biology and Health Unit of Pharmacodynamic-Biochemistry, UFHB, Félix Houphouët Boigny University, Abidjan, Côte d'Ivoire.*

World Journal of Advanced Research and Reviews, 2025, 28(02), 1253-1262

Publication history: Received on 01 October 2025; revised on 11 November 2025; accepted on 14 November 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.28.2.3799

Abstract

Pesticides are chemical or biological agents capable of destroying parasites or controlling their growth and reproduction. Long-term exposure may have adverse effects on biochemical parameters. Several parameters were analyzed, including cholinesterase and transaminase activities, and the quantification of urea and creatinine using a Cobas c 111 analyzer and a spectrophotometer. Blood samples were collected from all participants.

The study population consisted of 30 and 34 market gardeners from the municipalities of Bingerville and Port-Bouët, respectively, and 50 control subjects of Cocody. The dominant age group (60%) was under 23 years for Port-Bouët market gardeners and the controls (46%), whereas 90% of Bingerville market gardeners were over 50 years old. Mean urea concentrations were 0.24 ± 0.05 g/L, 0.24 ± 0.02 g/L, and 0.20 ± 0.05 g/L, while creatinine levels were 8.76 ± 1.91 mg/L, 8.67 ± 1.47 mg/L, and 13.50 ± 1.80 mg/L in the controls, Bingerville, and Port-Bouët groups, respectively ($p > 0.05$). Mean blood glucose levels were 0.96 ± 0.11 g/L for the controls and 0.94 ± 0.18 g/L and 0.83 ± 0.14 g/L for the Bingerville and Port-Bouët market gardeners respectively. Mean values of AST and ALT activities for the controls, Bingerville, and Port-Bouët market gardeners were 29.12 ± 12.25 IU/L, 16.93 ± 7.9 IU/L, 30.75 ± 2.14 IU/L, and 29.30 ± 12.16 IU/L, 14.50 ± 9.36 IU/L, 26.25 ± 4.27 IU/L, respectively ($p < 0.05$). Mean cholinesterase activities were 9584.42 ± 1686.26 IU/L in controls, 7590.63 ± 1606.27 IU/L in Bingerville market gardeners, and 4249 ± 825.16 IU/L in Port-Bouët market gardeners ($p < 0.05$). The inhibition of cholinesterase activity reached 20% in Bingerville and 44% in Port-Bouët market gardeners.

The results showed that all market gardeners had received training; however, only those from Bingerville applied the safety recommendations. The biochemical parameters remained within normal limits. Nevertheless, the marked inhibition of cholinesterase activity among Port-Bouët market gardeners was concerning. Continuous monitoring of these workers could help to mitigate the harmful effects of pesticide exposure.

Keywords: Pesticides; Cholinesterase; Market Gardeners; Biochemical Parameters; Organophosphorus Compounds

1 Introduction

Cash crop agriculture has encouraged farmers to adopt new methods such as the use of machinery, fertilizers, and, most importantly, pesticides, which improve both the quality and quantity of agricultural products [1]. Pesticides are substances mainly used in agriculture to protect crops against pests, weeds, or diseases [2]. Although their use has

* Corresponding author: Angbo Kousso Marie Angéline

proven beneficial, numerous studies have revealed their harmful effects on the health of both consumers of agricultural products and the farmers who handle them [1].

According to World Health Organization (WHO), there are about one million cases of severe pesticide poisoning worldwide each year, resulting in approximately 220,000 deaths annually [3]. Organophosphorus compounds (OPs) are organic chemicals widely used as pesticides, plasticizers, flame retardants, and even chemical warfare agents [4]. They represent the most commonly used class of pesticides globally, accounting for about 30% of the world pesticide market. Each year, OPs are responsible for an estimated three million poisonings and 100,000 deaths, as well as for inducing disabling neurological disorders—thus representing a major public health and environmental toxicity concern.

The primary biological targets of OPs are cholinesterases, whose enzymatic activity serves as a biological marker and the principal indicator of OP intoxication and its clinical manifestations [5]. Both plasma and erythrocyte cholinesterase activities are key biomarkers used to monitor workers exposed to OPs, reflecting the cumulative effects of previous weeks' exposures or episodes of acute overexposure [6].

Prevention relies on the medical monitoring of pesticide applicators through mandatory medical examinations. This surveillance includes both a clinical assessment for possible signs of intoxication and a biochemical examination involving the measurement of serum cholinesterase activity. The WHO recommends that an investigation be initiated if plasma cholinesterase activity decreases by more than 20% from baseline, to identify the cause of exposure and apply corrective measures. Furthermore, workers should be removed from exposure if their plasma cholinesterase activity falls below 40% of the baseline value. The WHO also advises individuals exposed to pesticides to undergo semiannual biochemical screening, including assessments of cholinesterase, urea, creatinine, and transaminases. A decrease in cholinesterase activity may indicate hepatic impairment, whereas an increase is sometimes observed in nephrotic syndrome [7].

In developing countries, where pesticide use is widespread in agricultural practices, pesticide poisoning accounts for up to 99% of fatal intoxications due to the lack of regulatory monitoring systems and limited access to information and healthcare. Côte d'Ivoire, an agriculturally driven economy, uses large quantities of pesticides to enhance vegetable production. However, this practice contributes to water pollution and population exposure [8]. The growing need to supply urban centers with food drives market gardeners to use increasing amounts of pesticides [9]. Unfortunately, this intensive use is not without harmful consequences. Previous studies have demonstrated biochemical dysfunctions suggestive of hepatic [10, 11] and renal cytotoxicity [12, 13] in agricultural workers exposed to pesticides.

In Côte d'Ivoire, most studies on pesticides have focused mainly on farmers' knowledge, attitudes, and practices regarding pesticide use [14], or on pesticide residues in vegetables [15]. However, pesticide exposure can also be assessed through the measurement of the pesticide or its metabolites in biological samples (urine, blood, etc.), or through biological effect markers such as cholinesterase inhibition [16].

Despite the toxicological importance of these biochemical markers, studies exploring the impact of pesticide exposure on such biomarkers remain scarce in Côte d'Ivoire. The municipalities of Bingerville and Port-Bouët are among the main vegetable-growing areas that extensively use pesticides for crop protection. We therefore hypothesized that frequent and long-term exposure to pesticides could alter biochemical parameters among market gardeners in these areas.

The objective of this study was to establish and compare the biochemical profiles of market gardeners from Bingerville and Port-Bouët with those of unexposed controls, based on measurements of cholinesterase, transaminases, urea, creatinine, and blood glucose.

2 Materials and Methods

2.1 Materials

2.1.1 Biological Material

The biological material consisted of human blood samples.

2.1.2 Technical Material

The technical materials included Vacutainer tubes (dry), tubes containing potassium oxalate and sodium fluoride, EDTA-containing tubes, blood collection needles, a cooler, a HITACHI automated analyzer, and a spectrophotometer.

2.2 Methods

2.2.1 Study Area and Sample Collection

This was a cross-sectional analytical study conducted over a three-month period in the communes of Port-Bouët, Biglerville, and Coody. The sampling included market gardeners working in vegetable crops and non-exposed individuals. Participants of all races, genders, socio-economic statuses, and ages, whether using pesticides or not, were included. Individuals who did not provide informed consent were excluded, as were those with hepatic or renal pathologies and diabetics.

The methodology consisted of three main phases: pre-survey, survey, and biochemical assays.

2.2.2 Pre-survey

This phase involved preparation for the main survey at the selected sites. It allowed contact with administrative authorities at the vegetable production sites and helped collect information on worker participation and their willingness to provide biological samples.

2.2.3 Survey

To characterize the market gardeners, interviews were conducted on-site using a structured questionnaire covering sociodemographic data (age, sex, professional experience, training).

Blood samples were collected via superficial venous puncture in the morning after a 12-hour overnight fast. Samples were collected in dry tubes with red caps and immediately transported in a cooler with ice packs to the laboratory. Samples were centrifuged at 3000 rpm for 3 minutes, and plasma was stored at +4 °C until analysis (ISO 15189, 2022) for determination of hepatic enzymes (ALT and AST), cholinesterase activity, and urea, creatinine, and glucose levels.

2.2.4 Biochemical Assays

Glucose assay

Blood glucose was measured using the enzymatic method described by Kaplan and Trinder [17]. This method is based on the oxidation of glucose-by-glucose oxidase to form gluconic acid and hydrogen peroxide (H_2O_2). In the presence of peroxidase (POD), H_2O_2 oxidizes a chromogenic substrate (amino-4-phenazone + phenol) to produce a colored compound, the intensity of which is proportional to the glucose concentration. Reagents were prepared according to the manufacturer's instructions. For the assay, 10 µL of plasma was mixed with 1 mL of working reagent (WR) and incubated at 37 °C for 10 minutes. Optical density (OD) was read at 505 nm against the reagent blank.

Urea assay

Urea was measured enzymatically as described by Tietz [18]. Urease hydrolyzes urea into ammonia (NH_3) and carbon dioxide (CO_2). Ammonia reacts with α-ketoglutarate in the presence of glutamate dehydrogenase (GLDH), with simultaneous oxidation of NADH to NAD^+. The rate of NADH decrease is directly proportional to the urea concentration and measured photometrically. Reagents were prepared according to the manufacturer's instructions. Ten microliters of serum were mixed with 1 mL of working reagent, and the reaction followed photometrically.

Creatinine Assay

Creatinine concentration was determined using the kinetic Jaffe reaction [19]. Creatinine reacts with alkaline sodium picrate at 37 °C, producing an orange color measured at 510 nm. The color intensity is proportional to creatinine concentration. The working reagent (RT) was prepared by mixing equal volumes of picrate reagent (R1) and sodium hydroxide solution (R2). Ten microliters of plasma were mixed with 1 ml of WR and incubated for 10 minutes at 37 °C before measuring OD.

ALT (Alanine Aminotransferase) Assay

ALT activity was determined following the International Federation of Clinical Chemistry (IFCC) method [20]. A 100 µL sample was added to 1 ml of working reagent (R1) and mixed. Optical density was measured at 340 nm, and enzyme activity was calculated using the formula: [\text {ALT Activity (U/L)} = \text {OD (sample)} \times 1745]

AST (Aspartate Aminotransferase) Assay

AST activity was measured according to Karmen (1955) and IFCC recommendations. A 100 µL sample was added to 1 mL of R1, mixed, and OD measured at 505 nm. AST activity was calculated as: [\text {AST Activity (U/L)} = \text {OD (sample)} \times 1745]

Serum Cholinesterase Activity

Cholinesterase activity was determined kinetically [21]. Butyryl thiocholine is hydrolyzed to butyrate and thiocholine, which reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to form a colored compound proportional to enzyme activity. Working reagents were prepared as described [22]. In the assay, 1.5 ml of R1, 50 µL of R2, and 10 µL of 1: 2 diluted sera were mixed, and OD measured at 405 nm..

2.3 Statistical Analysis

All analytical data were initially entered into Microsoft Excel. Statistical analyses were then performed using GraphPad Prism (version 10). Mean comparisons were conducted using Tukey's test to determine the presence of statistically significant differences between calculated means. Differences were considered statistically significant at a p-value < 0.05.

3 Results

3.1 Sociodemographic characteristics

The results revealed that market gardening is an activity carried out by men, with a proportion of 96% and 94% respectively for market gardeners in Bingerville and Port Bouet; in contrast, 82% of the control group were female.

With regard to age, among the market gardeners in Port-Bouet, the predominant age group (60%) consisted of individuals under 23 years old, as was also the case for the control group (46%). In contrast, in Bingerville, the majority of respondents (90%) were over 50 years old.

In terms of professional experience, 68% of market gardeners in Port-Bouet had less than 5 years' experience, while those in Bingerville (90%) had more than 50 years' experience.

In terms of professional training, both groups of market gardeners reported having benefited from the support of the National Agency for Rural Development (ANADER).

3.2. Biological parameters

3.1.1 Average values of biochemical parameters

The mean values of the various biochemical parameters analyzed are summarized in Table 1 below.

Table 1 Mean values of biochemical parameters

Biochemical Parameters	**Market Gardeners (Port-Bouet)**	**Market Gardeners (Biglerville)**	**Controls**
Cholinesterase (U/L)	4249 ± 825.16	7590.63±1606.27	9584.42±1686.26
Urea (g/L)	0.20±0.05	0.24±0,02	0.24±0.05
Blood glucose (g/L)	0.83 ± 0.14	0.94±0.17	0.96±0.11
Creatinine (mg/L)	13.50±1.81	8.67±1.47	8.76±1.91
ALT	30.75±2,14	16.93±7,90	29.12±12,25
AST	26.25 ± 4,27	14.50±9,36	29.30±12,16

3.1.2 Cholinesterase

The results (Table 2) show highly significant differences between all pairs of groups.

Table 2 Comparison of cholinesterase levels in the study population

Tukey's comparison test	Mean Difference (IU/L	95% Confidence Interval for the Difference	Summary	Adjusted P-value
Control vs Biglerville	1994	1193; 2795	****	<0.0001
Control vs Port-Bout	5335	4564; 6106	****	<0.0001
Biglerville vs Port-Bout	3342	2473; 4210	****	<0.0001

Table 3 Variation in the inhibition of enzymatic activity among market gardeners

Study population	Mean values of cholinesterase (U/L)	Cholinesterase activity (%)	Cholinesterase inhibition (%)
Biglerville market gardeners	7590.63 ±1606,27	79.20	20.80
Port-bouet market gardeners	4249 ± 825.16	55.67	44.33

3.1.3 Renal Markers

Urea

The mean differences in blood urea levels between the control group and the market gardeners from Bingerville and Port-Bouët are summarized in the table below

Table 4 Comparison of urea concentrations among the different study populations

Tukey's comparison test	Mean Difference (IU/L	95% Confidence Interval for the Difference	Summary	Adjusted P-value
Control vs Biglerville	0.0017	-0.0256; 0.0290	Ns	0.9880
Control vs Port-Bout	0.0420	0.01568; 0.0683	***	0.0007
Biglerville vs Port-Bout	0.04030	0.01064; 0.0699	**	0.0046

Creatinine

The mean differences in blood creatinine levels between the control group and the market gardeners from Biglerville and Port-Bout are summarized in the table below:

Table 5 Comparison of creatinine concentrations among the different study populations

Tukey's comparison test	Mean Difference (IU/L	95% Confidence Interval for the Difference	Summary	Adjusted P-value
Control vs Bingerville	0.09000	-0.884; 1,064	Ns	0.9738
Control vs Port-Bouët	-4.740	-5.678 ;3.802	****	<0.0001
Bingerville vs Port-Bouët	-4.830	-5.887; -3.773	****	<0.0001

3.2 Hepatic Markers

3.2.1 AST

AST (aspartate aminotransferase) is an enzymatic marker used to assess liver function. The mean AST levels were measured in the control group as well as in market gardeners from Biglerville and Port-Bouet. The results are presented in Table 6.

Table 6 Comparison of AST concentrations among the study populations

Tukey's comparison test	Mean Difference (IU/L	95% Confidence Interval for the Difference	Summary	Adjusted P-value
Control vs Biglerville	12.19	7.164; 17,22	****	<0,0001
Control vs Port-Bouët	-1.630	-6.468; 3.208	Ns	0.7036
Bingerville vs Port-Bouët	-13.82	-19.27; -8,368	****	<0.0001

3.2.2 ALT

ALT (alanine aminotransferase) is an enzymatic marker used to assess liver function. The mean ALT levels were measured in the control group as well as in market gardeners from Biglerville and Port-Bouet. The results are presented in Table 7.

Table 7 Comparison of ALT concentrations among the study populations

Tukey's comparison test	Mean Difference (IU/L	95% Confidence Interval for the Difference	Summary	Adjusted P- value
Control vs Biglerville	14.80	9.491; 20.11	****	<0.0001
Control vs Port- Port-Bouët	3.050	-2.060; 8.160	Ns	0.3352
Biglerville vs Port- Port-Bouët	-11.75	-17.51; 5.99	****	<0.0001

3.2.3 Blood Glucose

The mean blood glucose levels were measured in the control group as well as in market gardeners from Bingerville and Port-Bouët. The results are summarized in the table 8.

Table 8 Comparison of blood glucose levels among the study populations

Tukey's comparison test	Mean Difference (IU/L	95% Confidence Interval for the Difference	Summary	Adjusted P-value
Control vs Biglerville	0.022	-0.054: 0.099	ns	0.762
Control vs Port-Bout	0.136	0.062; 0.210	****	<0.0001
Biglerville vs Port-Bout	0.113	0.030; 0.196	**	0.0044

4 Discussion

The mean values of urea, blood glucose, creatinine, and transaminases among market gardeners from Port- Port-Bouët, Bingerville, and the control group were within normal limits. These results are consistent with those reported by Angbo et al. (23; 24). However, they differ from findings by Araoud and Hu R. [11, 13] and others, who reported biochemical dysfunctions potentially reflecting hepatic and renal cytotoxicity [12, 13] among agricultural workers exposed to cholinesterase-inhibiting pesticides.

The mean cholinesterase activity of the control group (9584 U/L) was significantly higher than that of the Bingerville group (7591 U/L), with a mean difference of 1994 U/L (95% CI = [1193; 2795]; p < 0.0001). Similarly, the comparison between the control group and the Port-Bouët group showed a mean difference of 5335 U/L (95% CI = [4564; 6106]; p < 0.0001), also highly significant.

Finally, comparing the two groups of market gardeners revealed that the mean activity observed in Bingerville (7591 U/L) was significantly higher than that recorded in Port-Bouët (4249 U/L), with a mean difference of 3342 U/L (95% CI = [2473; 4210]; p < 0.0001). This significant difference is confirmed by the degree of cholinesterase inhibition. Indeed, cholinesterase activity was inhibited in market gardeners from Bingerville and Port-Bouët by 20.80% and 44.33%, respectively (Table 3).

Overall, comparisons performed using Tukey's test highlighted highly significant differences (p < 0.0001) among all populations considered for serum cholinesterase activity. Our results are in agreement with those of Chanese et al. [25] in Thailand, who reported a significant decrease in cholinesterase activity among agricultural workers compared to controls, as well as with Cestonaro et al. [26] and Muller et al. [27]. Cholinesterase activity among Port-Bouët gardeners is significantly lower than that of Bingerville gardeners. Both groups are supervised by ANADER (National Agency for Rural Development Support). According to interviews, Bingerville gardeners strictly followed the recommendations provided by their supervisors, whereas Port-Bouët gardeners did not. This hypothesis is supported by professional experience. Indeed, the market gardeners from Port-Bouet, who have mostly been exposed for less than five years, showed a marked decrease in cholinesterase activity compared to those from Bingerville, most of whom have been exposed for more than fifty years. The predominant age of under 23 years among Port-Bouët gardeners may explain the non-compliance with recommendations due to immaturity, compared to the more responsible Bingerville gardeners who are mostly over 50 years old.

The cholinesterase inhibition observed (44.33% for Port-Bouët gardeners, Table 3) is particularly concerning, as the WHO recommends removing workers from exposure if plasma cholinesterase activity drops below 40% [28]. A decrease below 60% for BuChE or the presence of clinical signs (regardless of activity level) requires suspension from work until activity returns to 80% of baseline [29].

Regarding renal parameters, urea and creatinine concentrations were within normal limits. Previous studies have reported a significant increase in uremia in exposed groups [30]. Our analysis (Table 4) showed no significant difference in mean blood urea between the control group and the Bingerville group (mean difference = 0.0017 g/l; 95% CI = [-0.02564; 0.02904]; p = 0.9880). However, a statistically significant difference was observed between the control group and Port-Bouët group (mean difference = 0.0420 g/l; 95% CI = [0.01568; 0.06832]; p = 0.0007), as well as between Bingerville and Port-Bouët groups (mean difference = 0.0403 g/l; 95% CI = [0.01064; 0.06996]; p = 0.0046). This significant difference may be explained by the high cholinesterase inhibition.

Mean serum creatinine in the control group did not differ significantly from that of the Bingerville group (mean difference = 0.09 g/l; 95% CI = [-0.8841; 1.064]; p = 0.9638). However, a statistically significant difference was observed between the control group and Port-Bouët (mean difference = -4.740 g/l; 95% CI = [-5.678; -3.802]; p < 0.0001), as well as between Bingerville and Port-Bouët groups (mean difference = -4.830 g/l; 95% CI = [-5.887; -3.773]; p < 0.0001).

Hepatic enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are used as biological markers of liver disorders. In this study, the analyzed serum enzymes reflecting hepatic damage (AST, ALT) were within normal limits. Our results differ from those of Abdul [13] in Egypt and Kori [31] in India, who reported a significant increase in AST and ALT levels.

The test results (Table 6) revealed that the mean AST value in the control group differed significantly from that of the Bingerville group (mean difference = 12.19 g/l; 95% CI = [7.164; 17.22]; p < 0.0001), as well as between Bingerville and Port-Bouët (mean difference = -13.82 g/l; 95% CI = [-19.27; -8.368]; p < 0.0001). In contrast, no statistically significant difference was observed between the control group and Port-Bouët (mean difference = -1.630 g/l; 95% CI = [-6.468; -3.802]; p = 0.7036).

Regarding mean ALT values (Table 7), significant differences were observed between the control group and Bingerville (mean difference = 14.80 U/L; 95% CI = [9.491; 20.11 p < 0.0001), as well as between Bingerville and Port-Bouët (mean difference = -11.75 g/l; 95% CI = [-17.51; -5.991]; p < 0.0001). No statistically significant difference was observed between the control group and Port-Bouët (mean difference = -3.050 g/L; 95% CI = [-2.060; 8.160]; p = 0.3352).

The results for blood glucose (Table 8) showed no significant difference between the control group and Bingerville (mean difference = 0.02 g/l; 95% CI = [-0.05401; 0.09941]; p = 0.7622). However, significant differences were observed between the control group and Port-Bouët (mean difference = 0.1364 g/l; 95% CI = [0.06257; 0.2102]; $p < 0.0001$), as well as between Bingerville and Port-Bouët (mean difference = 0.1137 g/l; 95% CI = [0.03050; 0.1969]; $p = 0.0001$).

5 Conclusion

This study aimed to determine the biochemical profile of market gardeners in the communes of Bingerville and Port-Bouet compared to non-exposed controls in the Cocody commune. The study included 30 and 34 gardeners from Bingerville and Port-Bouet, respectively, and 50 controls. The male population predominated among both groups of gardeners, while females predominated among controls (82%). All gardeners had received training, but those in Port-Bouet did not strictly follow recommendations.

Most biochemical parameters were within normal limits, as was cholinesterase activity in Bingerville gardeners. However, cholinesterase activity among Port-Bouet gardeners showed significant inhibition of 44%. Pesticide exposure had a negative impact on Port-Bouet gardeners, as WHO guidelines indicate that inhibition above 40% requires removal from exposure until activity returns to 20% below baseline. These results highlight the need for regular monitoring of gardeners to reduce their exposure to the harmful effects of pesticides.

Compliance with ethical standards

Acknowledgments

The authors would like to thank the laboratories of medical biochemistry, authorities administrative of the communes of Bingerville, Port-Bouet and Cocody.

Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of informed consent

Consent was obtained from all individual participants included in the study. All the participants of this study were informed of its benefits. In addition, informed consent was obtained from each individual before the study began.

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