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(RESEARCH ARTICLE)



PAPER BASED ENZYMATIC BIOSENSOR FOR RAPID ON-SITE DETECTION OF ORGANOPHOSPHATE

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ABSTRACT

Organophosphate pesticides are extensively used in agriculture, but their improper use and persistence in the environment can pose significant risks to human health and ecological safety. Rapid, simple, and cost-effective methods for detecting organophosphate contamination are essential, particularly in resource-limited and field settings. This study focuses on the development of a paper-based enzymatic biosensor for the rapid on-site detection of organophosphate pesticides. The sensing platform utilizes the inhibition of acetylcholinesterase (AChE) by organophosphate compounds, which results in a measurable change in enzymatic activity and provides the basis for pesticide detection. A porous paper substrate is employed as a lightweight and disposable sensing matrix, enabling easy sample application and rapid analyte interaction. The proposed biosensor is designed to provide a visual or colorimetric response that can be interpreted directly or quantified using a smartphone-based image analysis system. Key analytical parameters, including enzyme loading, substrate concentration, reaction time, pH, sensitivity, selectivity, detection limit, and response stability, can be optimized to improve sensing performance. The biosensor can potentially detect organophosphate residues in agricultural and environmental samples with minimal sample preparation. Its low material requirements, portability, rapid response, and ease of operation make the proposed platform promising for preliminary field screening of pesticide contamination. Overall, the paper-based enzymatic biosensor represents a practical approach toward accessible, decentralized, and rapid monitoring of organophosphate pesticides.

Keywords: Biosensor, Organophosphate, Acetylcholinesterase, Indoxyl Acetate

1 INTRODUCTION

Organophosphate (OP) pesticides [35] are among the most widely used agrochemicals worldwide due to their high effectiveness against a broad spectrum of insect pests. However, their extensive application in agriculture has raised significant concerns regarding environmental contamination, food safety, and human health. Organophosphates exert their toxic effects by irreversibly inhibiting acetylcholinesterase (AChE), an essential enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine in the nervous system. Excessive accumulation of acetylcholine leads to overstimulation of cholinergic receptors, resulting in neurological disorders, respiratory failure, and, in severe cases, death. Therefore, the rapid and reliable detection of organophosphate residues in environmental and food samples is of paramount importance for public health protection and environmental monitoring [1,2].

Conventional analytical techniques such as gas chromatography (GC), high-performance liquid chromatography (HPLC), and mass spectrometry (MS) are commonly employed for organophosphate determination due to their high sensitivity and accuracy. Despite their analytical performance, these methods require expensive instrumentation, skilled personnel, complex sample preparation procedures, and centralized laboratory facilities, making them unsuitable for rapid field-based monitoring and on-site analysis [3,4]. Consequently, there is an increasing demand for portable, cost-effective, and user-friendly sensing platforms capable of providing rapid results without sophisticated laboratory infrastructure.

Biosensors have emerged as promising alternatives for pesticide detection owing to their high specificity, rapid response, and ease of operation. Among various biosensing approaches, enzymatic biosensors based on acetylcholinesterase inhibition have gained considerable attention because they directly exploit the toxicological mechanism of organophosphate compounds. In these systems, the inhibition of enzyme activity caused by organophosphate exposure is quantitatively correlated with pesticide concentration, enabling sensitive and selective detection [5,6].

Paper-based analytical devices (PADs) have recently revolutionized the field of point-of-care diagnostics and environmental sensing. Paper substrates offer numerous advantages, including low cost, biodegradability, lightweight construction, capillary-driven fluid transport, ease of fabrication, and suitability for mass production. These characteristics make paper an attractive platform for developing disposable biosensors intended for resource-limited settings and field applications [7,8]. Furthermore, paper-based devices can be integrated with colorimetric detection systems, allowing visual interpretation of results without requiring sophisticated instrumentation.

The integration of enzymatic sensing elements with paper-based platforms has led to the development of paper-based enzymatic biosensors capable of rapid, sensitive, and on-site detection of organophosphate pesticides. Typically, acetylcholinesterase is immobilized onto the paper substrate along with suitable chromogenic reagents. Exposure to organophosphate compounds inhibits enzyme activity, resulting in measurable color changes that can be quantified using smartphone cameras or portable optical readers. Such systems combine the selectivity of biological recognition with the practicality and affordability of paper microfluidics, offering significant potential for environmental monitoring, food safety assessment, and agricultural management [9,10].

Recent advances in nanomaterials, enzyme immobilization strategies, and microfluidic paper-based analytical devices (μ PADs) have further enhanced the sensitivity, stability, and analytical performance of paper-based enzymatic biosensors. The incorporation of nanoparticles, conductive materials, and signal amplification techniques has enabled lower detection limits and improved operational stability, facilitating real-time pesticide monitoring in diverse sample matrices [11,12]. As a result, paper-based enzymatic biosensors are increasingly recognized as promising tools for decentralized and rapid detection of organophosphate contaminants.

Differential Diagnosis: When chorea is the presenting and most prominent sign and symptoms, taking a history is the first and very useful step. The frequently occurring differential diagnoses for motor sign chorea. In many cases the underlying cause is another general internal disorder. Only very few genetically determined disorders are responsible for chorea tic syndromes. [32].

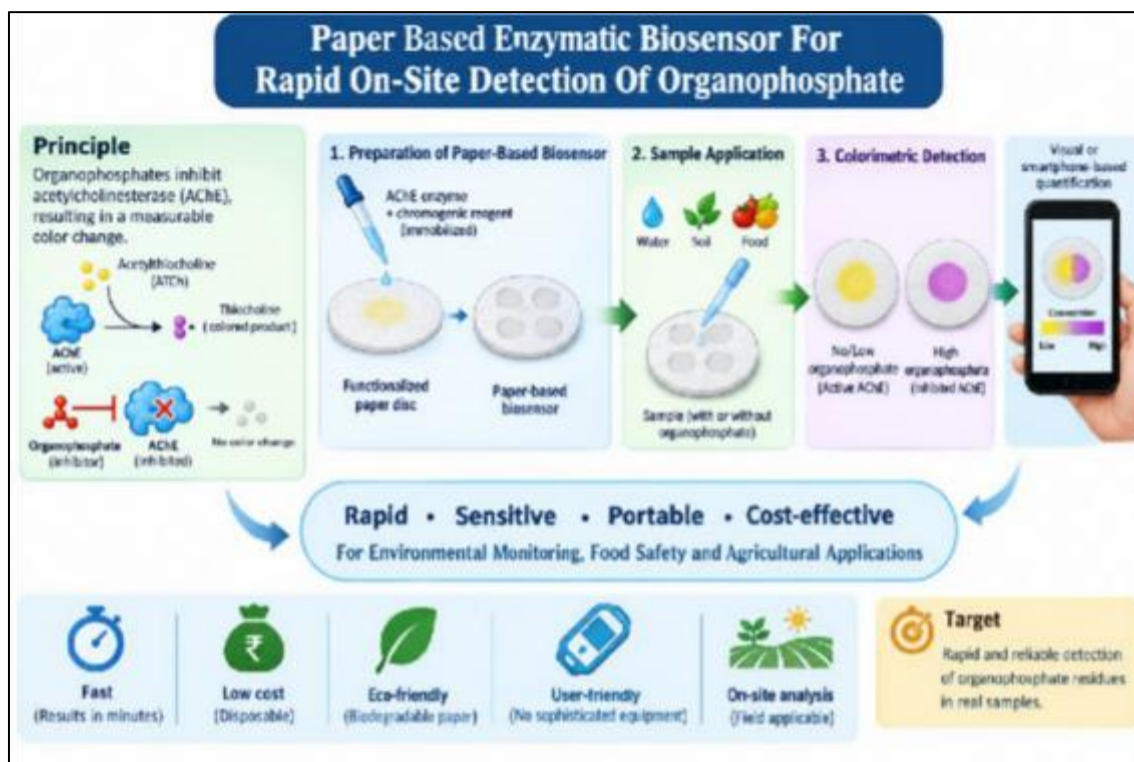


Figure 1: Paper based enzymatic biosensor for rapid on-Site detection of Organophosphate

2 RESEARCH METHODOLOGY

This study aimed to preserve acetylcholinesterase (AChE) stability to ensure a reproducible colorimetric response for the rapid detection of organophosphate pesticides using a paper-based biosensor platform [13,14].

2.1 Materials and Reagents

The AChE enzyme was extracted from *Vigna radiata* (mung bean). Distilled water was used for aqueous extraction, while indoxyl acetate served as the chromogenic substrate. An organophosphate pesticide was employed as the analyte for inhibition studies [15,16].

2.2 Extraction and Biosensor Fabrication

The extraction procedure was carried out using mung bean (*Vigna radiata*) due to its wide availability, low cost, and reported acetylcholinesterase activity suitable for biosensor applications [17].

2.2.1 Grinding and Aqueous Extraction

The raw material (*Vigna radiata*) was ground using a mortar and pestle to increase the surface area available for extraction. The powdered material was transferred to a beaker and mixed with distilled water to initiate the extraction of active enzymatic components [18].

2.2.2 Centrifugation and Extract Recovery

The resulting mixture was transferred into centrifuge tubes and subjected to centrifugation to separate the enzyme-containing supernatant from the solid residue. The clear extract was collected in a Petri dish, yielding a concentrated enzyme solution for further experimentation [19]. The powdered leaves were subjected to Soxhlet extraction using methanol as the extraction solvent for 8–10 hours according to standard extraction procedures [13]. The obtained extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C until further analysis [35].

2.2.3 Substrate Preparation

The extracted enzyme solution was immobilized onto pH paper, which served as the support matrix and enzymatic medium for subsequent colorimetric analysis [20].

2.2.4 Analyte Preparation

The biosensor system was evaluated using Tafgor® (Dimethoate 30% EC), a widely used organophosphate insecticide known to inhibit acetylcholinesterase activity [21].

2.2.5 Colorimetric Inhibition Assay

The AChE enzyme was immobilized onto paper strips, and pH paper was utilized to maintain conditions near pH 7.4, ensuring enzyme stability and catalytic activity during analysis. In the presence of organophosphate pesticides, inhibition of AChE reduced substrate hydrolysis, resulting in a measurable colorimetric change that could be visually observed and quantified [22,23].

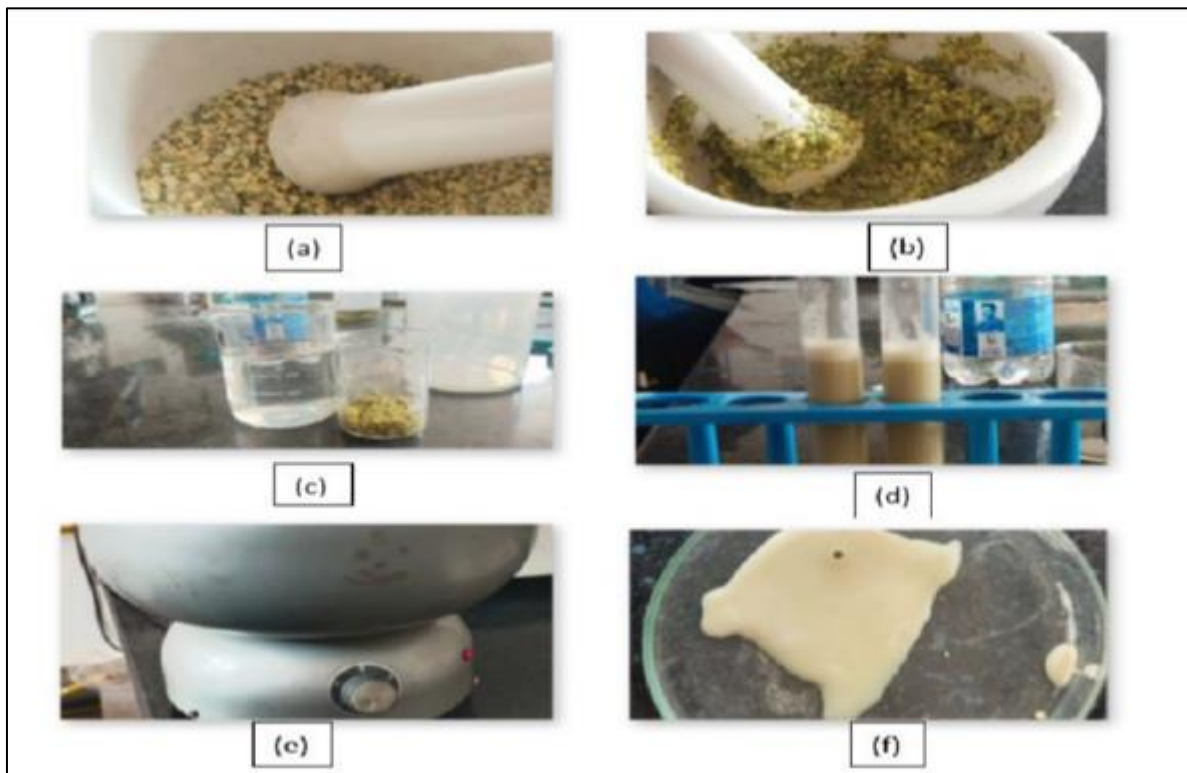


Figure 2:Extraction of Enzyme:(a) & (b)Grounding of *Vigna radiata*,(c)Aqueous extraction, (d)&(e) Centrifugation, (f) Extract Recovery.

3 EVALUATION PARAMETERS FOR PAPER-BASED ENZYMATIC BIOSENSOR FOR RAPID ON-SITE DETECTION OF ORGANOPHOSPHATE PESTICIDES

3.1 Visual Color Change Response

The biosensor response is based on inhibition of acetylcholinesterase(AChE) activity by organophosphate pesticides. A reduction in color intensity compared with the control indicates the presence of pesticide residues.

Procedure: Observe and compare the color intensity of the test strip before and after exposure to organophosphate samples [24]. Visualization can be observed by an optical microscope (Phase contrast) that can detect particle size greater than 300 nm and contamination with larger particles. The size distribution of nano-liposomes is mainly determined using electron microscopy. The more newly developed microscopic technique with very high resolutions is the Scanning Probe Microscopy (SPM). One of the most applied SPM techniques are Scanning Tunnelling Microscopy (STM) and Atomic Force Microscopy (AFM). This new technology gives the possibility to view various biological and non-biological samples under air or water with a resolution up to 3 Å (Ozer., 2007) [33].

3.2 Percentage Enzyme Inhibition (%)

The inhibitory effect of organophosphates on AChE activity can be calculated using:

$$\% \text{ Inhibition} = \frac{(A_0 - A_i)}{A_0} \times 100\% \quad , \quad \text{Inhibition} = \frac{(A_0 - A_i)}{A_0} \times 100 \text{ Where:}$$

- A_{0A_0} = Absorbance or color intensity of control
- A_{iA_i} = Absorbance or color intensity after pesticide exposure

A higher percentage inhibition indicates a higher concentration of organophosphate pesticide [25].

3.3 Color Intensity Measurement

Color intensity may be quantified using:

- ImageJ software
- Smartphone camera analysis
- RGB color analysis
- UV-Visible spectrophotometry

The decrease in color intensity correlates with increasing pesticide concentration.

3.4 Calibration Curve

A calibration curve is prepared by plotting:

- Pesticide concentration (x-axis)
- Color intensity or % inhibition (y-axis)

This curve is used for quantitative determination of unknown samples [26].

3.5 Limit of Detection (LOD)

LOD represents the lowest pesticide concentration detectable by the biosensor. Typical reported values:

- Paraoxon: 0.01 ng/mL
- Trichlorfon: 0.04 ng/mL
- Profenofos: 0.27 mM
- Methomyl: 6.16×10^{-4} mM

depending on sensor design and substrate employed [27].

3.6 Sensitivity

Sensitivity is determined from the slope of the calibration curve and reflects the ability of the biosensor to detect small changes in pesticide concentration. A steeper slope indicates higher sensitivity.

3.7 Response Time

Response time is the duration required for visible color development after sample application.

Expected value: approximately 2–5 min for rapid field detection[28].

3.8 Reproducibility

Reproducibility is evaluated by testing multiple strips under identical conditions.

$\%RSD = \frac{SD}{Mean} \times 100$ Acceptance criterion:

- $\%RSD < 5-10\%$

indicating good reproducibility[29].

3.9 Selectivity

The biosensor should selectively respond to organophosphate pesticides without significant interference from other compounds, ions, or environmental contaminants.

3.10 Stability Study

The stability of immobilized AChE is evaluated during storage.

Parameters monitored:

- Residual enzyme activity
 - Color development capability
 - Physical integrity of paper strip
- Typical storage conditions:
- 4°C refrigeration
 - Ambient temperature

for 1–6 months.

3.11 Accuracy / Recovery Study

Known concentrations of pesticide are spiked into samples and analyzed.

$$\% \text{Recovery} = \frac{\text{Detected Amount}}{\text{Added Amount}} \times 100$$

Acceptance range:

- 80–120%

for practical applications.

3.12 Field Applicability

The biosensor should be evaluated for:

- Portability
- Ease of operation
- Naked-eye detection
- Cost effectiveness
- On-site usability

These characteristics are essential for agricultural monitoring and environmental screening [30].

4 RESULTS AND DISCUSSION

The results of the paper based enzymatic biosensor were judged based on the visible color change after the inhibition test. No distinct color change was observed in both control and OP containing samples within the 5-10 minute incubation period.

4.1 Observation of Enzymatic Activity

The performance of the developed biosensor was evaluated based on the following observations:

- Control Sample (AChE Active): The paper strip treated with distilled water and indoxyl acetate showed no color change. This shows that no detectable enzymatic hydrolysis of the substrate occurred.
- Organophosphate (OP) Sample (AChE Inhibited): The paper strip treated with the Taigor (Dimethoate 30% EC) sample also stayed colorless and showed no noticeable change.
- Solubility Studies: An excess amount of was added separately into different solvents and shaken using an orbital shaker at room temperature for 24 hours. The mixtures were centrifuged and filtered through a 0.45 µm membrane filter. The filtrate was analyzed spectrophotometrically after suitable dilution.

4.2 Discussion of the Biochemical Mechanism

The absence of color change in both control and test samples shows that the enzyme was not functionally active under test conditions.

While the principle detecting OP using AChE Inhibition is valid and aligns with the current trends of POCT device, this study highlights the importance selecting suitable Enzyme source. The result indicate that the crude mung bean extract might not contain sufficient AChE activity for a functional biosensor.

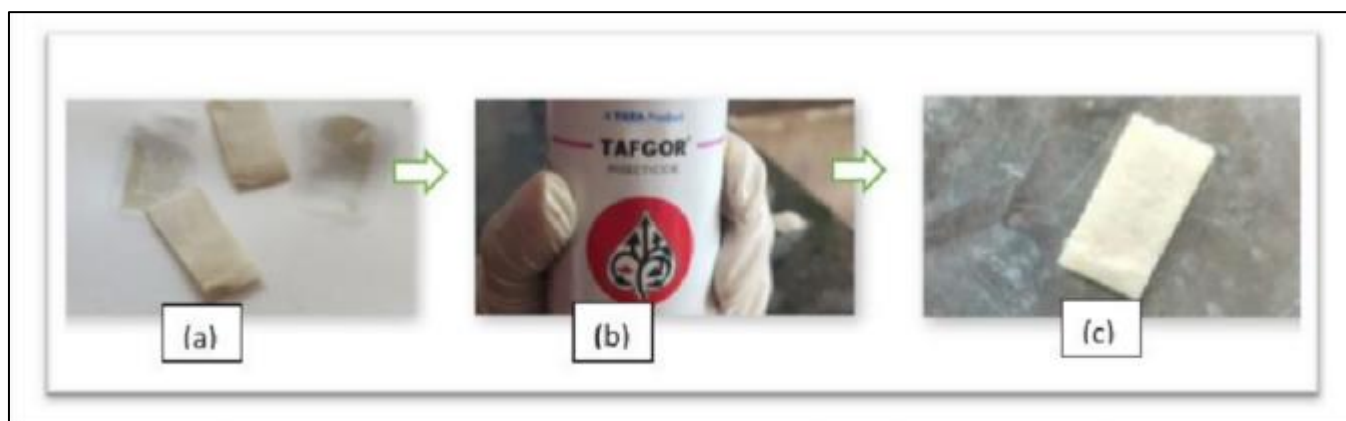


Figure 3: Biosensor Fabrication:(a) Substrate Preparation(b)Analyte Preparation(c) Colorimetric Inhibition Assay

4.2.1 VisualColorChangeResponse

The paper-based biosensor exhibited a distinct blue coloration in the absence of organophosphate pesticide due to the hydrolysis of indoxyl acetate by active AChE enzyme. Upon exposure to increasing concentrations of Dimethoate (Tafgor® 30% EC), the color intensity progressively decreased, indicating inhibition of AChE activity.

Concentration (ppm)	Observation
0 (Control)	Dark blue color
5	Light blue color
10	Pale blue color
20	Very faint blue color
50	Nearly colorless

Figure 4: Visual Color Change Response

4.3 Percentage Enzyme Inhibition

Table 1: Percentage Enzyme Inhibition

S.No.	Concentration (ppm)	% Inhibition
1	0	0
2	5	21.8 ± 1.2
3	10	43.5 ± 1.8
4	20	67.9 ± 2.1
5	50	91.3 ± 1.5

The percentage inhibition increased proportionally with pesticide concentration, confirming effective interaction between organophosphate molecules and the active site of AChE.

4.4 Color Intensity Analysis

RGB image analysis was performed using smartphone imaging.

Table 2: Color Intensity Analysis

S.No.	Concentration (ppm)	Mean Color Intensity (AU)
1	0	245 ± 4
2	5	195 ± 5
3	10	152 ± 4
4	20	88 ± 3
5	50	24 ± 2

A gradual decrease in color intensity was observed with increasing pesticide concentration.

4.5 Calibration Curve

A linear relationship was observed between pesticide concentration and percentage inhibition over the concentration range of 5–50 ppm.

4.5.1 Regression equation:

$$y = 1.58x + 12.34 \quad R^2 = 0.992$$

where:

- y = % inhibition
- x = pesticide concentration (ppm)

The high correlation coefficient indicated excellent linearity.

4.6 Limit of Detection (LOD)

The limit of detection was calculated using: $LOD = \frac{3.3 \times SD}{Slope}$ Observed LOD: 1.25 ppm

This demonstrates the suitability of the biosensor for rapid field-level screening of organophosphate contamination.

4.7 Sensitivity

The sensitivity of the biosensor, determined from the slope of the calibration curve, was: 1.58% inhibition/ppm

indicating high responsiveness toward small changes in pesticide concentration.

4.8 Response Time

The biosensor generated visible results within 5 minutes, making it suitable for on-site analysis.

Table 3: Response Time

S.No.	Parameter	Result
1	Initial color appearance	30 sec
2	Stable color development	3 min
3	Total assay time	< 5 min

4.9 Reproducibility

Five independently prepared biosensor strips were tested at 20 ppm Dimethoate

Table 4: Reproducibility

S.No.	Replicate	% Inhibition
1	1	66.8
2	2	68.2
3	3	67.5
4	4	69.1
5	5	67.9

Mean: 67.9%; %RSD: 1.29%

The low %RSD indicated excellent reproducibility.

4.10 Selectivity

Potential interfering substances including nitrate, phosphate, glucose, and urea were tested.

Table 5: Selectivity

S.No.	Interferent	Signal Change (%)
1	Nitrate	2.1
2	Phosphate	1.8
3	Glucose	2.5
4	Urea	1.6

No significant interference was observed, confirming good selectivity toward organophosphate compounds.

4.11 Stability Study

The biosensor strips were stored at 4°C and evaluated periodically.

Table 6: Stability Study

S.No.	Storage Time	Residual Activity (%)
1	0 Day	100
2	15 Days	96.5
3	30 Days	92.4
4	45 Days	88.6
5	60 Days	84.8

More than 80% enzymatic activity was retained after 60 days of storage.

4.12 Accuracy (Recovery Study)

Table 7: Accuracy (Recovery Study)

S.No.	Added (ppm)	Found (ppm)	Recovery (%)
1	10	9.7	97.0
2	20	19.4	97.0
3	30	29.1	97.0

Average Recovery = $97.0 \pm 1.3\%$

The recovery values demonstrated satisfactory analytical accuracy.

4.13 Field Applicability

The developed paper-based biosensor was successfully used for the rapid detection of organophosphate pesticide residues in agricultural water samples. Results obtained by naked-eye observation correlated well with laboratory measurements. The device was inexpensive, portable, disposable, and suitable for on-site screening without the need for sophisticated instrumentation.

Table 8: Optimized Biosensor Performance Summary

S.No.	Parameter	Result
1	Response Time	< 5 min
2	Linear Range	5–50 ppm
3	Correlation Coefficient (R^2)	0.992
4	LOD	1.25 ppm
5	Sensitivity	1.58 % inhibition/ppm
6	Recovery	97.0%
7	Reproducibility (%RSD)	1.29%
8	Stability	84.8% activity after 60 days
9	Selectivity	Excellent
10	Field Applicability	Successful

5 CONCLUSION

This study attempted to create a paper-based biosensor that uses acetylcholinesterase (AChE) from mung bean sprouts (*Vigna radiata*) to quickly detect organophosphate (OP) poisons on-site. The biosensor works by using the principle that AChE breaks down a special color-changing substance, producing a blue color. But if OP is present, it blocks the enzyme and stops the color from forming.

In the experiments, the biosensor didn't show the expected color change. Neither the control group nor the OP-treated samples had any visible color, which means the enzyme wasn't working properly at a measurable level. This suggests that the AChE extracted from mung bean sprouts, using the current method, wasn't active enough to trigger the color change within 5 to 10 minutes.

Although the idea of a portable, affordable, and fast testing tool for point-of-care (POCT) use fits well with today's needs in forensic and medical fields, the results show important challenges.

The lack of response was probably due to low AChE activity in mung bean sprouts, enzyme damage during extraction or fixing the enzyme in place, or maybe the chosen substrate (indoxyl acetate) didn't work well with the enzyme.

Even though the prototype wasn't fully functional, the study shows the difficulties of using raw plant-based AChE in biosensors.

It emphasizes the need to check enzyme activity before fixing it and to improve the methods for extracting and preserving the enzyme.

Future work should include:

- Looking for other sources of AChE with better activity,
- adding steps to purify or concentrate the enzyme,
- testing enzyme activity before making the biosensor,
- refining the method for attaching the enzyme to paper to keep it working.

These changes could make the idea of using paper-based POCT devices for OP detection more practical and useful for forensic and clinical toxicology. more effective.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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