Paper biosensors utilize silver nanoparticles for onsite pesticide residue detection

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Abstract. The improper use of pesticides and excessive doses in the long term contribute to climate change and even threaten human health, organisms, and the balance of the ecosystem. A pesticide detection device is needed to monitor its levels to minimize risks to human health and the environment. A paper biosensor was developed in this study to detect organophosphates by utilizing the enzyme acetylcholine esterase (AChE) and silver nanoparticles (AgNP). The Whatman filter paper was used as a visual OP detection zone. AgNP, as an indicator, is adsorbed and gives a brownish-yellow color to the paper, while AChE is immobilized into the film and layered on the paper. The addition of acetylthiocholine chloride as a substrate to the film released thiocholine products which could replace the capping AgNPs causing the AgNPs to aggregate and the paper color to pale. The presence of OP in the sample will inhibit AChE activity so that paper fading is reduced. The biosensor response is quantized as an RGB value, which is determined using an application on a smartphone. The resulting biosensor has excellent performance with a linear range of 0.05-2.00 mg/L, a detection limit of 0.04 mg/L, and a CV of 0.48%. Biosensor measurements on vegetable samples showed conformity with the GCMS results as the standard method. Therefore, this biosensor is suitable for the on-site detection of pesticides offering easy, fast, and inexpensive analysis.

1 Introduction

Pesticides have been used for quite a long time, especially in agriculture, for reducing harmful pest organisms. Using pesticides according to the rules provides benefits, increasing crops and producing a more efficient and economical impact on agriculture. Unfortunately, the improper use of excessive pesticide doses also has negative effects, especially continuous

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application over a long period. The negative effects of pesticides do not only occur in the application area. They can spread due to their mobility through evaporation, leaching, and runoff, contaminating the air, water, and soil [1]. The application of pesticides also increases greenhouse gas emissions. In contrast, increasing temperatures due to climate change encourage the use of more pesticides due to an increase in pests and the rate of pesticide degradation [2]. Pesticides reduce soil biodiversity, cause poisoning of organisms and even chronic diseases such as cancer, and damage the nervous, respiratory, and heart systems. Pesticides must be used carefully and wisely to minimize their impact. Regulations are made to ensure human health, organisms, and environmental quality. It is known that the maximum residue limit for organophosphate pesticides is 0.01 mg/kg/day [3]. Therefore, controlling pesticide levels below the maximum residue limit is very important. For this reason, developing an analytical tool to detect pesticides is needed.

High-Performance Liquid Chromatography (HPLC) or Gas Chromatography (GC) is commonly used to determine the presence of excess pesticides in vegetables [4]. However, the weaknesses of the HPLC and GC methods are the high cost of reagents and the extraction and purification treatment in the laboratory, which requires solvents and a longer analysis time, which can lead to a risk of errors. Therefore, developing a pesticide residue detector that is cheap, fast, and easy to apply is necessary. Paper biosensors are a platform capable of detecting analytes (pesticide residues) that require fewer chemicals, do not require large volumes of samples, and have short analysis times [5,6].

This biosensor works on an enzyme inhibition system by analytes in the presence of an indicator that induces a color change [7]. This system is not new, but with a smaller design, easier to operate, and less expensive, paper-based biosensors have great potential for routine analysis. Although paper-based biosensors are inherently affordable and can be delivered to end users, researchers are still researching to achieve good performance to produce analytical tools capable of rapid and accurate quantitation to enable rapid risk management decision-making. Modifying paper biosensors using nanoparticles is known to improve their performance, obtained from the unique properties of nanomaterials [8]. This research aimed to develop a biosensor as an alternative method for quantizing pesticides with the principle of inhibition of the enzyme acetylcholinesterase on paper microzone plates using Whatman filter paper. Acetylthiocholine substrate is added to produce products from the hydrolysis reaction, namely acetic acid and thiocholine, which interact with silver nanoparticles (AgNP) to indicate color change [9,10]. Hence, monitoring and controlling pesticide residue levels to stay below thresholds is easier to achieve.

2 Experimental

2.1 Chemicals

The paper biosensor in this study was fabricated on paper microzone plates using Whatman grade 41-cellulose filter paper paint no. 1441-125. The color change indicator on the paper biosensor used biogenic AgNP synthesized by Hermanto et al. [11]. It electrochemically used green tea leaf extract in a spherical form of AgNP with an average size of ~17 nm. Other materials used in this study included acetylthiocholine chloride (ATChCl) (Sigma Aldrich) substrate, acetylcholinesterase (AChE) (EC 3.1.1.7, VI-S type, 200 units/mL) (Sigma Aldrich), tris HCL buffer (Merck), and the pesticide profenofos (sigma Pestanal®). The real samples used were vegetables obtained from local markets in Mataram, Indonesia.

2.2 Fabrication process and operation paper biosensor

Paper biosensors are designed using modified filter paper as analytical tools. Whatman 41 filter paper was chosen as the biosensor medium and biogenic AgNPs for chemical color reactions, as seen in the biosensor design (Figure 1). First, with a piece of filter paper cut to the size of 1 x 1 cm², 10 µL of 10 µg/mL colloidal biogenic AgNP in 1% PVA solution is
absorbed into the filter paper medium, where PVA serves to bind biogenic AgNPs to the filter paper fibers. Absorption was carried out by soaking overnight. The color-adsorbed filter paper from biogenic AgNPs was allowed to dry at a chiller temperature of around 10°C for 24 h. A 10% PVA solution was prepared with Tris HCl buffer solution pH 7.0 and 10 µL of AChE solution (prepared with 200.0 units/mL AChE in 40 µL of Tris-HCl buffer solution pH 7.0). The dried filter paper coating was then coated with a PVA solution containing AChE by immersion for 10 s, and the results were dried at 10°C for 1 h. This coating process was repeated 3 times. This paper-based biosensor uses filter paper as a medium for chemical color reactions and coats it with a particular material, as previously described. Then, the filter paper was further modified to aid the occurrence of color changes due to chemical color reactions on the paper due to the catalytic activity of enzymes and redox in AgNPs for further testing and detection of pesticides.

![Biosensor scheme.](https://example.com/biosensor.png)

**Fig. 1.** Biosensor scheme.

### 2.3 Establishment of profenofos standard curve with paper biosensor

The fabricated paper biosensor is used for the detection approach based on chemical color reaction and integrated with the inspection machine [12]. Chemical color changes are observed as three base colors, R (red), G (green), and B (blue), which are conferred in the appropriate values. Digital cameras (smartphone cameras) and ImageJ image processing software can quantify the resulting image's color and identify differences in image resolution. The inspection machine is used to quantify the RGB values of chemically colored sectors on a paper biosensor under constant temperature and maintained light conditions. The standard curve is made from the change in RGB value (color intensity with increasing concentration) series of standard solutions. Then, the sample is determined, and the RGB value obtained from the sample is calculated using the standard curve linear regression equation to obtain the concentration of the test substance in the sample.

### 2.4 Real sample analysis

Method validation for determining the performance of paper biosensors in pesticide detection, the GCMS method (QP210 Ultra, Shimadzu) was used as a comparison [8]. The RTX®-5MS column and helium gas were stationary and mobile phases. The injection temperature is 250°C. The qualitative is based on the retention time of the chromatogram peaks. At the same time, for the quantitative one, the calibration curve is used by plotting the concentration vs. chromatogram peak area. The sample concentration was determined from the chromatogram area and calculated based on the linear regression equation. Vegetable samples obtained from a local market in Mataram, Indonesia, including cayenne pepper (fruit), long beans (pod), and chinese cabbage (leaves and stem), were used as real samples. 100 g of the fresh sample was mashed, and 50 mL of Tris-HCl buffer pH 7.0 was stirred. The mixture was separated by centrifugation at 8000 rpm for 5 minutes, the supernatant was
3 Results and discussion

3.1 Biosensor scheme

This work uses a paper biosensor for pesticide residue detection in vegetables. It is based on AChE inhibition and utilizes AgNP as a color-changing agent (Figure 1). The AgNP that has been adsorbed onto the filter paper evenly has a brownish-yellow color, and then it is coated with a PVA film that already contains AChE. The paper biosensor surface containing AChE makes it easier for enzymatic reactions to occur first. The ATCh substrate interacts with AChE, and a hydrolysis reaction occurs to produce acetic acid and TCh [13]. TCh has a thiol group, -SH, which further interacts with AgNPs on the paper side. The -SH group tends to interact more easily with Ag to form Ag-SH. Loss of Ag in AgNP to Ag-SH causes a change in the color of the biosensor paper from brownish yellow to pale yellow [14]. Pesticides inhibit the AChE enzymatic reaction, which correlates to the color change of the paper biosensor. Paper biosensors utilize silver nanoparticles for pesticide residue detection, making them easy and simple analytical devices, but they still have high sensitivity in detecting pesticides. To date, there have been very few reports on paper biosensors for pesticide detection in real samples, so testing the performance of paper biosensors has always been an interesting challenge. For this reason, testing on real samples is needed to validate the performance of this paper biosensor.

3.2 Biosensor response analysis

Profenofos is used to control insects (especially Lepidoptera) and mites. The largest agricultural market regarding the total weight of active ingredients uses profenofos pesticides in vegetable crops. Profenofos is a non-systematic insecticide and acaricide that works as a skin contact poison, inhalation poison if it enters the respiratory system, and stomach poison if ingested. Profenofos can biochemically inhibit the action of the acetylcholinesterase enzyme. Profenofos inhibits AChE irreversibly through covalent binding of the serine hydroxyl group of the AChE active site by the phosphoryl group of profenofos, rendering it unable to hydrolyze ACh [15]. Therefore, profenofos was used as a test material for detecting paper biosensors in this study.

Digital image information has been used in analytical applications, showing precision and accuracy in quantitative measurement results data. This procedure determines color information through the chromaticity of the standard red, green, and blue (RGB) color components. These intensities can be collected and provide a basis for quantitative measurements via the ImageJ tool, which is a colorimetric measurement method with a portable format [5]. The standard curve in the quantitative analysis determines the relationship between the concentration of the standard solution and the color intensity (mean RGB). It is used to quantitatively ensure the feasibility of the colorimetric test (Figure 2).
The paper biosensor shows the sensor response to catalytic activity by changing the color of the paper from brownish yellow to pale yellow. Pesticides inhibit the catalytic activity, which can be seen quantitatively in the standard curve in Figure 2a. Meanwhile, changes in the color of the paper biosensor can be seen in Figure 2b. The corresponding change in the color of the paper biosensor, which can be detected by the naked eye in quantizing several concentrations of pesticides (Fig. 2b), implies the simplicity of paper biosensors for this purpose due to the elimination of time-consuming sampling, sample preparation, and procedures in traditional analytical laboratories [5]. As a result, this feature is appropriate for point-of-care (POC) testing. A more accurate inference about the response of the paper biosensor can be obtained by plotting the standard curve between observed color intensity (mean RGB) vs. pesticide concentration, as shown in Figure 2a. The curve shows a linear response in the region of the desired concentration from 0.05 to 2.5 mg/L of profenofos with a correlation coefficient ($r$) of 0.994. The paper biosensor’s detection limit was 0.04 mg/L of profenofos. Repeating measurements of the profenofos standard solution using a paper biosensor 3 times gives a coefficient of variance (CV) of 0.48%. The lower the CV means the lower level of dispersion around the average value, indicating the more precise biosensor measurement results.

3.3 Biosensor performance

The next stage in the analysis of biosensor performance is method validation, which is carried out by determining pesticide residues in real samples and comparing the results obtained using certified reference methods. In this case, the method used is GCMS, which is a method commonly used in the quantitative analysis of pesticide residues in the laboratory. Here, the samples were spiked with profenofos at a 0.5 mg/L concentration. The results of the quantitation of profenofos in several vegetables summarized in Table 1. Table 1 shows the measured pesticide content values corrected by adding a standard profenofos solution. The results of measuring the pesticide content using the paper biosensor and the GCMS method showed that several samples were detected to contain pesticides. However, they were still below the threshold allowed by the Indonesian authorities, so they were still at a safe level for consumption [16]. Additionally, low levels of pesticides might be caused by several natural factors including degradation by temperature, leaching by water, etc. The paper biosensor developed for this purpose agreed well with the GCMS method. It was not significantly different using the t-test. Therefore, paper biosensors utilizing AgNP can be applied on-site in detecting pesticide residues, offering easy, fast, and inexpensive analysis.
Table 1. Measurement of pesticide concentration in real samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Various vegetable samples</th>
<th>Biosensor, mg/L</th>
<th>GCMS, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cayenne pepper</td>
<td>0.020±0.003</td>
<td>0.023±0.001</td>
</tr>
<tr>
<td>2</td>
<td>Long beans</td>
<td>0.013±0.002</td>
<td>0.010±0.001</td>
</tr>
<tr>
<td>3</td>
<td>Chinese cabbage</td>
<td>0.055±0.004</td>
<td>0.048±0.001</td>
</tr>
</tbody>
</table>

4 Conclusions
A paper-based colorimetric biosensor for detecting profenofos has been successfully designed using AgNPs as an indicator. Profenofos in the sample inhibits AChE activity and reduces color fading of the paper (which contains the AgNP), a colorimetric method with a portable format via the ImageJ tool. The sensor response was measured as the average RGB value of the paper and showed a linear relationship with the profenofos concentration in the range of 0.05-2.50 mg/L. The detection limit of the paper biosensor is 0.04 mg/L. The results of biosensor measurements on vegetable samples showed conformity with GC-MS method. Therefore, this paper biosensor can be applied to determine profenofos easily and quickly.

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