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Comparison of Serum Cholinesterase and Plasma Cholinesterase Levels in Oil Palm Farmers Using Pesticides

Hartini H[1]*, Adillah Kurnia Hasanah[2]
Kesehatan John Paul II Pekanbaru, Indonesia
Email: hartini.h@akjp2.ac.id

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Abstract
Pesticide exposure is a significant health concern for oil palm farmers, potentially leading to cholinesterase enzyme inhibition, which can serve as a biomarker for pesticide poisoning. However, the differences in cholinesterase levels between serum and plasma specimens in exposed farmers remain underexplored. This study aims to compare cholinesterase levels in serum and plasma samples from 15 oil palm farmers with at least five years of pesticide exposure. A cross-sectional research design was used, with blood samples collected from the farmers and analyzed for cholinesterase activity using photometric methods. The results showed no statistically significant difference between serum and plasma cholinesterase levels ($p>0.05$), indicating that both specimens are equally valid for monitoring pesticide-induced toxicity. Factors such as incomplete use of personal protective equipment (PPE) contributed to decreased cholinesterase levels, with the majority of farmers not utilizing full PPE during pesticide application. These findings suggest the importance of improving safety protocols, including proper PPE use and training, to protect farmers from the harmful effects of pesticides. Future research should explore the long-term health impacts of chronic pesticide exposure and assess the effectiveness of educational programs on PPE compliance to reduce pesticide-related health risks.

INTRODUCTION

Farmers are people who cultivate crops, one of which is oil palm farmers. Farmers use pesticides in farming to help plants develop well and protect from various plant diseases. The use of pesticides in oil palm plantations is a practical, economical and efficient way. However, exposure to pesticides also has a negative impact on farmers' health in the form of poisoning. The severity of pesticides in farmers depends on the time and concentration of exposure (Pathak et al., 2022)

Pesticides cause acute toxicity when exposure to inhaled, ingested or exposed to high doses of the skin and when prolonged causes chronic toxicity (Ahmad et al., 2024) One of the main mechanisms of toxicity of organophosphate and carbamate-based pesticides is the inhibition of the cholinesterase enzyme. The enzyme cholinesterase plays a role in breaking down acetylcholine (Ach) into acetic acid and choline (Aroniadou-Anderjaska et al., 2023; Bai & Wang, 2022; Pearson & Patel, 2016; Ranjan et al., 2018; Souza et al., 2023) Organophosphates bind acetyl cholinesterase (AChE) on the active side of the enzyme irreversibly and carbamate binds to AChE reversibly and are released for about 30 minutes. This inhibits cholinesterase activity so that there is an increase in the amount of ACh which results in cholinergic symptoms (Kaur et al., 2023) The use of oraphosphate in insects whose central nervous system uses ACh as an exitatory neurotransmitter can experience hyperstimulation of cholinergic nicotine receptors, resulting in rapid death. The use of organophosphates in humans also has life-threatening effects due to acute exposure to pesticides (Aroniadou-Anderjaska et al., 2023) At smaller levels of exposure, decreased cholinesterase activity often does not show obvious symptoms, although in the long term it negatively impacts the health of farmers.

Measurement of cholinesterase levels becomes a parameter or biomarker to evaluate pesticide exposure. Biological specimens that can be used to measure cholinesterase levels are serum and plasma (Orluwene & Ejilemele, 2006) Serum is a clear yellowish liquid obtained after blood clots and does not contain fibrinogen. Serums contain proteins, electrolytes, antibodies, antigens, hormones. Plasma is a component of blood obtained after the addition of anticoagulants and still contains fibrinogen (Institute, 2024) Some studies suggest that cholinesterase levels can vary which is influenced by technical factors in the specimen (Altilia et al., 2022) Therefore, this study aims to compare cholinesterase levels in serum and plasma to contribute to efforts to mitigate the health risks of farmers exposed to pesticides.

The socio-economic development in Indonesia's rural areas is influenced heavily by government expenditure and taxation. These two factors play a crucial role in addressing poverty rates, particularly in provinces where poverty is rampant. The impact of government spending on sectors like education, health, and infrastructure has long been debated. While such expenditures contribute to the overall development of the nation, their effectiveness in directly reducing poverty remains unclear. Particularly in remote regions, the allocation of resources often doesn't reach the people who need it most, leading to stagnant poverty levels despite governmental efforts to alleviate them.

Government expenditure on public goods like infrastructure and social services is crucial, but without proper targeting, these investments may not lead to significant poverty reduction. This study reveals the need for more focused government spending in poverty-stricken areas, where targeted subsidies or direct interventions could make a larger impact. Moreover, it's vital that expenditures align with specific community needs, ensuring that marginalized groups benefit from the improvements in public services. Understanding how government spending impacts poverty reduction requires not only the measurement of total expenditures but also the analysis of how effectively these funds are distributed among different sectors and regions.

Taxation is another important tool in the fight against poverty. Tax policies that promote equity can help redistribute wealth, which in turn can alleviate income inequality. However, the regressive nature of some taxes in Indonesia, particularly indirect taxes, has exacerbated the wealth gap, leaving low-income populations disproportionately burdened. By focusing on progressive taxation policies and ensuring that tax revenue is used efficiently, Indonesia could make significant strides in reducing inequality and improving living standards in rural areas.

This research also highlights the need for improving the effectiveness of both taxation and government expenditure to address poverty. The study suggests that while government spending on education, health, and infrastructure has increased, poverty rates have remained high in specific regions, especially in rural areas. This indicates a potential mismatch between public spending and the actual needs of the population. Therefore, future policies should focus on the targeted allocation of resources, with clear indicators of performance and outcomes in poverty reduction.

For future research, it is recommended to expand the scope to assess the long-term impacts of specific public expenditure programs on poverty alleviation. Additionally, quantitative analyses focusing on the efficiency and targeting of government spending, as well as its correlation with poverty rates, would provide a more comprehensive understanding of how public expenditure influences economic well-being. Moreover, studying the direct impact of taxation reforms on poverty and inequality, and comparing Indonesia's experience with other developing nations, could yield valuable insights for refining future policy interventions aimed at eradicating poverty.

RESEARCH METHODS

This study used a cross-sectional design involving 20 oil palm farmers in the Kualu Nenas Village area, Kampar. The inclusion criteria are farmers who have actively used pesticides in the past year, while the exclusion criteria include farmers who have a history of liver disease. Samples were taken by venipuncture and processed to obtain serum and plasma. Cholinesterase levels were measured using photometric methods based on enzyme activity against *butryrilthiocholine iodide* substrates. Data were analyzed using a paired t-test to compare serum and plasma chlolinesterase levels.

The tools used in this study were ICHEM-535 photometer, *coolbox* and micropipette. The materials used in this study are serum, plasma, red vacutainer tube, purple vacutainer tube (EDTA), syringe, 70% alcohol cotton, sterile gauze, tourniquet, centrifuge, test tube, test tube rack, micropipette, yellow tip and blue tip.

Blood sampling follows the (Rosales-Rimache et al., 2023) Farmers are asked to straighten and clench their arms. The position of the farmer's arm is adjusted so that it bends slightly and a tourniquet is installed about 10 cm above the vein where the blood will be taken (median cubitis). Palpation or palpation to confirm the position of the vein. and Serum making by taking blood from venous veins. Blood

The manufacture of Serum is carried out following the procedure(Lestari et al., 2019) The vacuum tube contains centrifugal blood at a speed of 3000 rpm for 10 minutes. Serum and red blood cells are separated using a Pasteur pipette. The serum is inserted into the test tube.

EDTA plasma manufacturing. Blood samples are collected in EDTA tubes and centrifuged to obtain a plasma sample.

Measurement of cholinesterase levels. Pipette 2700 μL reagent 1 then insert it into the test tube and add 300 μL reagent 3 and homogenize it as a control reagent. Negative control reagents are made by pumping 1.5 mL of control reagent then put it into the test tube, add 50 μL of reagent 2 and homogenize. Pipette 10 μL of control serum then put it in a test tube and homogenized. Test the sample by inflate 1.5 mL of reagent 1, then put it in the test tube, add 50 μL of reagent 2 and homogenize. Pipette 10 μL of farmer's serum then put it in a test tube, homogenized and analyzed.

Data analysis. The Shapiro-Wilk test was used for the normality analysis of the data and the paired t-test was used to compare the average of the two groups.

RESULTS AND DISSCUSSION

Characteristics of respondents

This study involved 15 farmers who worked using pesticides in oil palm plantations. Farmers are 80% male (12 people) and 20% female (3 people). The age range of farmers is 30-50 years old and all of them have worked for more than 5 years. At work, 53.3% (8 people) of farmers sprayed pesticides in the direction of the wind and 46.7% (7 people) sprayed in the opposite direction of the wind. The personal protective equipment (PPE) used included 0% head coverings, 0% special glasses, 13.3% masks, gloves 33.3% and boots 53.3% (table 1).

Table 1. Characteristics of respondents

Variabel		**f (person)**	**%**
Gender	Man	12	80,0
	Woman	3	20,0
Long working time	< 5 years	0	0,0
	≥ 5 years	15	100,0
Direction of pesticide spraying	Following the direction of the wind	8	53,3
	Opposite Wind	7	46,7
Using personal protective equipment		Ya	No
	Head covering (hat)	0	15
	Special glasses	0	15
	Mask	2	13
	Glove	5	10
	Boots	8	7

The data presented in table 2 are presented with serum and plasma cholinesterase enzyme levels. The average cholinesterase level in the >3,500 U/L (normal) category in serum was 5106.15 U/L (13.3%) and plasma 3883.78 U/L (13.3%). The average cholinesterase level with an amount of >1,401-3,500 U/L (mild poisoning) in serum was 2470.19 U/L (40%) and plasma 2676.64 U/L (53.3%). Meanwhile, the average cholinesterase level with a total of 701-1, 400 U/L (moderate poisoning) in serum was 1182.19 U/L (20%) and plasma 1041.01 U/L (13.3%). The average cholinesterase level <700 U/L (severe toxicity) in serum was 310.75 U/L (26.7%) and plasma 437.40 (20%). The average cholinesterase level in serum was 1988.20 U/L and plasma 2171.66 U/L.

Table 2. Serum and plasma cholinesterase levels

No	Category (Banday et al., 2015)	Specimen types					
		Serum			Plasma		
		Installment-installment (U/L)	n	%	Installment-installment (U/L)	n	%
1.	>3,500 U/L (Normal)	5106,15	2	13,3	3883,78	2	13,3
2.	>1,401-3,500 U/L (Mild poisoning)	2470,19	6	40,0	2676,64	8	53,3
3.	701-1, 400 U/L (Moderate poisoning)	1182,19	3	20,0	1041,01	2	13,3
4.	<700 U/L (Severe poisoning)	310,75	4	26,7	437,40	3	20,0
	Total		15	100,0		15	100,0

The results of the analysis of the paired t-test (Table 3) obtained a significant value (p) of 0.0681 (p>0.05). The difference in cholinesterase levels was 183.46 U/L (IK95% - 1119.29251 - 752.36984). Based on this, it is known that statistically there is no statistically significant difference in the average serum and plasma cholinesterase in oil palm farmers.

Table 3. ***Results of the analysis of the paired t-test***

	Rerata (U/L)	Selisih (U/L)	IK96%	Nilai p
cholinesterase serum	1988,20	183,46	-1119.29251 - 752.36984	0,681
cholinesterase plasma	2171,66			

Remarks: *p=0.000 (p>0.05), IK is a 95% confidence interval

Discussion

In this study, the oil palm farmers who were the research sample were farmers who actively sprayed pests with pesticides. The farmers in the study sample were 15 people with 80% men and 20% women and all of them had worked for more than 5 years. Working as an oil palm farmer for a long time has the potential to increase the accumulation of pesticide exposure in the farmer's body. This is in line with research conducted by (Mayaserli et al., 2022) which stated that farmers who work in plantations ≥ 5 years are at risk of experiencing a decrease in cholinesterase levels in the blood (pesticide poisoning). Pesticide poisoning in a person can occur from the time they are first exposed to two weeks after contact with pesticides. Researchers used serum and plasma samples to determine cholinesterase levels by looking at their activity against *butryrilthiocholine iodide substrates.* Serum and plasma are specimens measured as butyrylcholinesterase (BuChE).

One of the factors that results in pesticide poisoning is the direction of the wind. In this study, 8 farmers (53.3%) sprayed according to the wind direction and 7 farmers (46.7%) sprayed in the opposite direction. Spraying pesticides against the wind can increase the risk of exposure to pesticides through inhalation and skin. This is because the spray pellets are carried back towards the farmers (Desmarteau et al., 2020)

In this study, it was also found that farmers did not use Personal Protective Equipment (PPE) completely and correctly. PPE such as masks, gloves, protective glasses, long-sleeved clothing, and closed shoes. The oil palm farmers in this study did not all use special hats and glasses. Farmers who do not use PPE completely result in direct exposure to pesticides through skin, inhalation and eye contact. Examination of the cholinesterase enzyme in the blood serves to determine the exposure or level of pesticide poisoning (Apriyuni et al., 2024).

Based on the serum cholinesterase levels contained in Table 2, it was found that 86.7% of farmers experienced poisoning consisting of 40% mild poisoning, 20% moderate and 26.7% severe poisoning. In the plasma sample, the farmers' cholinesterase levels also experienced 86.6% poisoning, consisting of 53.3% mild poisoning, 13.3% moderate poisoning, and 20% severe poisoning. Pesticide poisoning in a person can occur from the time they are first exposed to two weeks after contact with pesticides. Decreased activity of the cholinesterase enzyme can result in nervous system disruption, poisoning, and death. Enzyme activity is the amount of active cholinesterase enzymes in blood plasma and red blood cells that play a role in maintaining the balance of the nervous system (Aulia et al., 2022) (Sari et al., 2018).

There was no significant difference in serum and plasma cholinesterase levels (p=0.734). It can be caused by biological composition, stability of the cholinesterase enzyme, examination methods and clinical function. Serum is a blood fluid without fibrinogen, while plasma contains fibrinogen. However, serum and plasma contain almost equal amounts of cholinesterase because they are not affected by fibrinogen. The cholinesterase enzyme produced by the liver circulates in the blood so that serum and plasma can show the same levels of cholinesterase to determine the level of pesticide exposure (Rohayati et al., 2024).

CONCLUSION

This study found that 80% of oil palm farmers experienced toxicity, as indicated by a decrease in cholinesterase levels in serum and plasma specimens. The decline in cholinesterase enzyme activity is closely related to the improper or incomplete use of personal protective equipment (PPE) by the farmers. Although no significant difference was observed in the cholinesterase levels between serum and plasma, the findings highlight the health risks associated with pesticide exposure due to inadequate safety measures. It is essential to provide proper education regarding the use of PPE and ensure that these protective measures are easily accessible to minimize the risk of exposure and safeguard the health of oil palm farmers.

For future research, it is recommended to conduct a more extensive study involving a larger sample of oil palm farmers across different regions to evaluate the effectiveness of various PPE interventions in reducing pesticide exposure. Additionally, further studies could explore the long-term effects of chronic exposure to pesticides on cholinesterase levels and overall health. Furthermore, research should investigate the impact of educational programs on improving farmers' awareness and adherence to PPE usage, as well as the feasibility of integrating sustainable farming practices that reduce pesticide reliance.

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