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Research Article

Synthesis of Nanosilver Nitrate for Vector Control (*Aedes Aegypti*) in Banjarnegara, Central Java (Case of Endemic Area DHF Banjarnegara)

Mursid Raharjo[1], Agus Subagio[2], Sulistyani[3], Yulia Aryanti[4]

[1,3,4]Magister of Evironmental Health, Faculty of Public Health, Diponegoro University, Semarang - Indonesia.
[2]Faculty of Sains and Mathematics, Diponegoro University, Semarang, Indonesia.
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I N F O

Corresponding Author:
Mursid Raharjo, Magister of Environmental Health, Faculty of Public Health, Diponegoro University, Semarang, Indonesia.
E-mail Id:
mursidraharjo@gmail.com
Orcid Id:
https://orcid.org/0000-0003-4791-1242
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A B S T R A C T

Integrated vector management especially for *Aedes aegypti* mosquito is a priority to control the transmission of dengue fever. An effective and environment friendly insecticide is needed. This study aims to find the effective dose of vector control material for *Ae. aegypti*. Nanosilver nitrate used as a material for mosquitoes insecticide caused it has greater potential to enter through the pore of nervous system of *Ae. aegypti.* This field research used adult mosquitoes *Ae. Aegypti* for toxicity test. The total samples were 200. The toxicity test of organophosphate compounds, at concentration 3.5 mg/L as many as 60% mosquitoes were death. Toxicity test of synthesis two compounds on concentration 3.5 (organophospat) + 0.5 (Ag2NO3) mg/L there was 100%. Treatment synthesis of nanosilver with organophopat is more effective than only organophospat compounds. based on the *Ae. Aegypti* LC50 of organophosphate was 7.81 mg/L meanwhile LC50 for the synthesis of silver nitrate nanoparticle with organophosphate were 5.59 mg/L. Nanosilver nitrate has the potential as a synthetic material or substitute for organophosphate.

Keywords: Aedes aegypti, Nanosilver, LC50

Introduction

Global climate change is one of the potential increasing risk factors on dengue transmission dynamics.[1] Climate change affects vector reproduction, including *Aedes aegypti* as a vector of Dengue Hemoragic Fiber (DHF).[2] Climate change also caused the appearance of endangered mosquito species.[3]

The resistence of *Aedes aegypti* as a carrier of DHF is dangerous for public health.[4] Because of the resistance, it's required an increasing dose of insecticide. Increased dosage may be harmful to non target organism and vector's environment.[5] Acetylcholinesterase (AChE) is an enzyme in the nervous system, which hydrolyzes acetylcholine neurotransmitters and stops nerve impulses, and its the target for organophosphate and nanosilver.[6] IVM (Integrated Vector Management) is a decision making process to optimize vector control to improve efficacy, cost effectiveness, ecological friendly and sustainablility. Nanotechnology has a potential expanding to some utilization.[7]

Nanotechnology has developed into an alternative technology in various fields. Technology with nanoscale particles (10^{-9}) m has specific characteristics and can change materials' characteristics. In another country, insecticide materials using Nano-TiO2 and Sillica.[8] Nanosilver (Ag2NO3) can be applied in the health sector. However, the utilization of nanotechnology in the field of vector control has never been conducted since its utilization in vector control is a challenge.[9] Research for eco-nanotechnology using herbs has been assessed in India only for Culex species and has not been carried out for other species with different bio-ecological characteristics.[10]

The synthesis of nanosilver nitrate and organophosphate was expected to inhibit Acethylcholinesterase (AChe). Nanosilver particles are theoretically capable of penetrating enzyme AChe, make it more effective and can be used in lower doses.[4]

This research is in concern how to diversify Ag2NO3 nanoparticles for vector control compared with organophosphate compounds. The purpose of this research is to analyze toxicity test of synthesis nanosilver (Ag2NO3) and organophosphate to the adult mosquitoes (Aedes aegypti).

Materials and Methods

This was field research that control all of variables. The research began with the processing of nanosilver derived from Ag2NO3. Ag2NO3 materials obtained from chemical supplier. Organophosphate obtained from market product. The *Aedes aegypti* vector which used for toxicity test was adult mosquitoes.

The solutions used for test solutions are Organophosphate (Malathion 95%) and Ag2NO3 nano-particle. Toxicity test used *Ae. aegypti* species in adult stage with synthesis Organophosphate Ag2NO3 solutions.

Rearing process of mosquitoes obtained in Banjarnegara Entomology Laboratory. The number of mosquitoes needed is 5 species x 5 x 4 x 2=200 mosquitoes. Mosquitoes chosen are in 2-3 days of age. The silver nitrate nanoparticle material was obtained from Undip Integrated Laboratory in Bio-nano section, as well as making synthesis solutions and production of organophosphate solutions. The main concentration is 1,500 ppm for nanosilver and 1,000 mg/L for organophosphate. After that, form the main solution, then diluted into a smaller concentration. The dissolving process was conducted with overhead stirrer tool. The stirring process was 5 hours for silver nitrate nanoparticles, while it was 1 hour for organophosphate. The differences in duration of stirring process is due to the different shape of the raw material. Preparation for the insecticide-treated mosquito net was designed according to the rearing area of adult mosquitoes. The duration needed for the process was 2 hours in order for the concentration solution to be absorbed by the mosquito net and expected to be effective at killing mosquitoes. Observations were in the first 3 minutes and in the next 24 hours, then recorded the results. LC50 with linier method for synthesis nanosilver and organophosphate.

Result

Organophosphate ($C_{10}H_{19}O_6PS_2$) Toxicity Test

Organophosphate toxicity test was treated on 5 adult mosquitoes *Aedes aegypti* in each concentration using controls. The observations were in the first 3 minutes and after 24 hours. In the first 3 minutes, at all concentration none of tested animals were death. The Observation after 24 hours was obtained as follows. At concentration of 3.5 mg/L, 60% of tested animal were death. Its decreased at concentration of 4 mg/L, only 40% of tested animal were death. Calculation of the LC50 concentration for organophosphate followed the formula of y=0.0905x – 0.2071. Using this formula, the LC50 for organophosphate was 7.81 mg/L. The results of organophosphate toxicity test for adult mosquitoes *Ae. aegypti* are showed in the Table 1.

Table 1.Toxicity test of Organophosphate for Aedes aegypti

Concentration (mg/L)	3 min		24 h		Indoor temperature	Outdoor temperature	Humidity
	Death	%	Death	%			
Control	0	0%	0	0%	28.80C	28.80C	61%
1.0	0	0%	0	0%	28.80C	28.80C	61%
1.5	0	0%	0	0%	28.80C	28.80C	61%
2.0	0	0%	0	0%	28.80C	28.80C	61%
2.5	0	0%	0	0%	28.80C	28.80C	61%
3.0	0	0%	3	60%	27.40C	27.40C	72%
3.5	0	0%	3	60%	27.50C	27.20C	76%
4.0	0	0%	2	40%	32.40C	31.50C	58%

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Synthesis Nanosilver and Organophosphate Toxicity Test

The shynthesis of Nanosilver (Ag2NO3) with Organophosphate toxicity test was treated out on adult mosquitoes Aedes Aegypti. The total of 5 adult mosquitoes were tested with various concentration of organophosphate that shynthesized with 0.5 mg/L Ag2NO3. Observation were made in the first 3 minutes after the mosquitoes were put in the rearing cage and after 24 hours. The observations showed as follows, in the first 3 minutes, at concentration of 1 mg/L; 1.5 mg/L; 2 mg/L Organophoshate with 0.5 mg/L Ag2NO3, none of tested animals were death. Likewise, at concentration of 3 mg/L organophosphate + 0.5 mg/L Ag2NO3. At concentration of 2.5 mg/L Organophosphate + 0.5 mg/L Ag2NO3, 40% tested animals were death, while at concentration of 3.5 mg/L and 4 mg/L organophosphate + 0.5 mg/L Ag2NO3 only 20% tested animals were death.

At 24 hours observation, the result showed that at concentration of 1 mg/L; 1.5 mg/L; 2 mg/L Organophosphate with 0.5 mg/L Ag2NO3 none of tested animals died. Mortality was seen at concentration of 2.5 mg/L; 3 mg/L; 4 mg/L Organophosphate with 0.5 mg/L Ag2NO3, there were 60% of tested animals died. At concentration of 3.5 mg/L Organophosphate + 0.5 mg/L Ag2NO3, all of tested animals died (100%). In the prediction (forecast), LC50 for the synthesis of silver nitrate nanoparticle with organophosphate followed the synthesis test's plotting results. Synthesis of nanosilver nitrate (Ag2NO3) with organophosphate used the formula of y= 0.1381x – 0.2714. Using this formula, the LC50 for the synthesis results were 5.59 mg/L of organophosphate with 0.5 mg/L of nanosilver nitrate. The result of toxicity test synthesis of organophosphate with Ag2NO3 nanoparticles against adult *Aedes aegypti* are presented in Table 2.

Table 2.Synthesis of Organophosphate and Nanosilver toxicity test for Ae. aegypti

Concentration (mg/L)	Oragnofosfat + Ag2NO3				Indoor temperature	Outdoor temperature	Humidity
	3 min		24 h				
	Death	%	Death	%			
Control	0	0%	0	0%	290C	290C	56%
1.0 + 0.5	0	0%	0	0%	29.20C	29.20C	57%
1.5 + 0.5	0	0%	0	0%	29.20C	29.20C	57%
2.0 + 0.5	0	0%	0	0%	29.20C	29.20C	57%
2.5 + 0.5	2	40%	3	60%	27.40C	28.10C	72%
3.0 + 0.5	0	0%	3	60%	27.40C	28.10C	72%
3.5 + 0.5	1	20%	5	100%	27.30C	27.20C	72%
4.0 + 0.5	1	20%	3	60%	27.50C	27.20C	72%

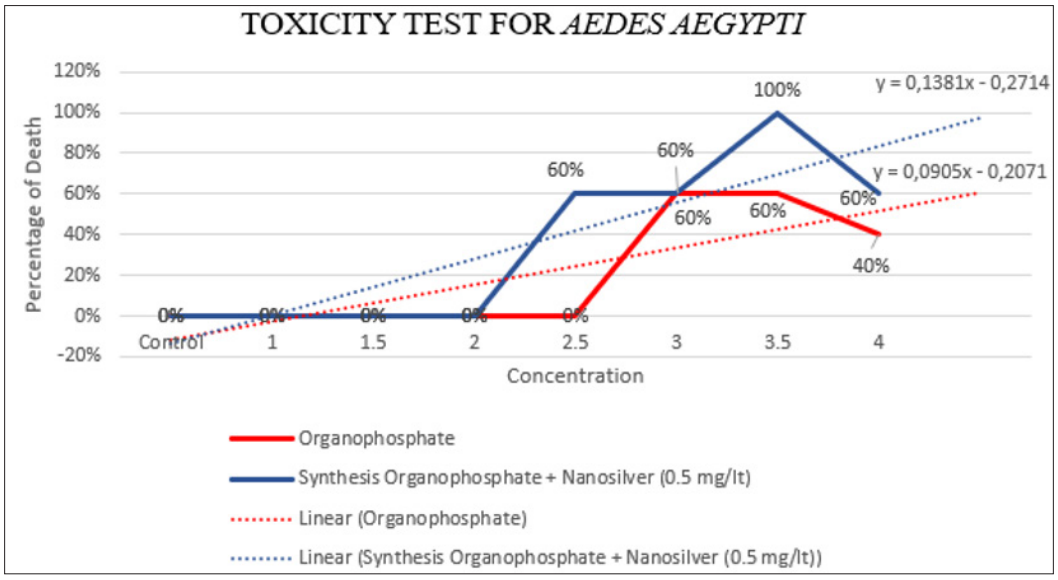

Figure 1.Prediction (forecast) on Lethal Concentration of synthesis of nanosilver nitrate (Ag2NO3) and organophosphate (C10H19O6PS2)

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Discussion

The majority of chemicals used to control the mosquitoes were carbamate and organophosphate materials. Control is carried out on vectors in the larva-pupa phase until adult hood. The use of insecticides to control the mosquitoes was carried out by Indoor Residual Sprayer (IRS) method, or with Insecticide-Treated Nets (ITNs).

The result showed that shyntesis of nanosilver with organophosphate can kill 100% of tested animals at concentration of 3.5 mg/L Organophosphate + 0.5 mg/L Ag2NO3. This concentration is greater than organophosphate. Organophosphate only kill 60% of tested animals at concentration of 3.5 mg/L and 40% at concentration of 4 mg/L. The LC50 for organophosphate was 7.81 mg/L and for the synthesis was 5.59 mg/L. The concentration of synthesis organophosphate with Nanosilver (Ag2NO3) is smaller than when used organophosphate alone. Nanosilver synthesis of 0.5 mg/L can increase organophosphate effectiveness and has a higher chance of killing insects.

Nanosilver size is 72-86 nanomater has a smaller size compared to other materials. Such as the wavelength of visible light is between 400 nm and 700 nm. Blood cells (leukocytes) size is 10,000 nm, a bacterium has size between 1,000-10,000 nm, a virus 75-100 nm, protein 5-50 nm, deoxyribonucleic acid (DNA) ~ 2 nm (width), and an atom ~ 0.1 nm. At this scale, the physical, biological and chemical characteristics of individual substances differ substantially from one another and often unexpected reaction occur.[11]

Test results using the UV-VIS Spectrophotometer Test and Particle Size Analyzer (PSA) show the size of the Nanosilver was 72-86 nanomater, while the size of organophosphate was 3,568 - 3,745 µm. The size of nervous cell *Aedes aegypti* is bigger than Nanosilver nitrate particles. The potential for the occurrence of intrusion of nanomaterial through nervous system of mosquito is very large. This intrusion encourages more effective reactions between nanoparticles and organophosphate to react with Acethyl Chloniesterase (AChE) enzyme.[12]

Synthesis of organophosphate and nanosilver nitrate cause damage mosquitoes nervous system and its nerve membranes depolarize, resulting death. Acetylcholinesterase (AChE) is an enzyme in the nervous system, which hydrolyzes acetylcholine neurotransmitters and stops nerve impulses, and its the target for organophosphate and nanosilver.

Ag2NO3 nanoparticles were dispersions of solid particles or colloidal structures. Nanosilver material consists of synthetic polymers, semi synthetic and natural active molecules that have ability to be encapsulated, trapped, absorbed, dissolved or chemically attached.[13] The characteristics which are owned so that biocompatibility, biodegradability, and flexibility in utilization.[14,15] Every material that was mixed will experience mixing and react with each other to form new compounds. Ag2NO3 compound is a salt compound and has clear color. Organophosphate (C10H19O6PS2) is a compound which leaves little residue.[16] Organophosphate is a carbon compound containing carbamic acid. Organophosphates (OPs) are pesticides of low persistence in the environment. The resistance to OPs is connected with acetylcholinesterase insensitivity. Organophosphates (especially malathion) have become selected insecticide during dengue fever outbreaks, so a new strategy are urgently needed to replace fogging and ULV to quickly kill infected Aedes mosquitoes.[17]

This research showed the results of synthesis produced a new compound. In a previous study conducted by Agus using silica ions, it produced 3-ethyl2-isopropoxyphenyl methylphosphate. Substituting silica with silvernitrate is strongly suspected to also produce compounds with the same propertie.[7] This research reinforces the previous research. There are better benefits, especially Ag2NO3 nanoparticle to another organisms, which are more environmentally friendly. Ag2NO3 nanoparticles have been known to have no effect on non-target organisms. There are previous research about the occurrence of acute toxicity to non-target aquatic species. Synthesis nanosilver with Plumeria rubra and Pergularia daemia didn't show a toxicity effect on P. reticulata fish after 48 hours (LC50).[18] Synthesis nanosilver with a non-toxic Solanum nigrum (berry extract) against two mosquito predators, Toxorhynchites larvae and Diplonychus annulatum.[19] Nanosilver can be applied to a various health care product. The interesting thing is the potential use of nanosilver (AgNP) in mosquito control.[20]

Aedes aegypti has become resistant to insecticides that often used, such as organophosphate. So, it requires a higher concentration to control dengue vector. Meanwhile, organophosphate are dangerous insecticides when used in high doses. Therefore, the synthesis of nanosilver with organophosphate will reduce dose of insecticides and not harm non-target organisms.

The result of LC50 calculations, there was a decrease from 7.81 mg/L to 5.59 mg/L. Synthesis with nanosilver gives hope in reducing the resistance of using organophosphate. The result of the synthesis of Organophosphate (C10H19O6PS2) and nanosilver material, produces a new compound (C10H19O6PS2-Ag). This compound is more active than the original compound. This release of Ag+ coincides with the organophosphate ion, and interacts with ACHe. Research is in line with other research. New areas of great promise in nanotechnology, such as voltage-driven nano-spintronics. In Which nanosilver toxicity is eliminated by releasing Ag+ ions in aqueous solution.

Conclusion

Synthesis of nanosilver nitrate with organophosphate (LC50

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5.59 mg/L) has a better killing power than organophosphate (LC50 7.81 mg/L) alone at the same concentration, which is effective for controlling Aedes aegypti. Nanosilver provides a compound to be an alternative material for *Aedes aegypti* vector control.

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Conflict of Interest: None

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