Nanoparticle of Silver Nitrate (Ag_2NO_3) and Organophosphate ($C_{10}H_{19}O_6PS_2$) for Vector Control of Anopheles Larvae

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***Abstract*— The climate change has an impact on various lives, including in the vector breeding. The *Anopheles* resistance as an infectious agent of Plasmodium is harmful for life, especially to non-target organisms and the environment. Nano-silver (Ag_2NO_3), is a new form of engineering in vector control. The aim of this research was the toxicity test of nano-silver (Ag_2NO_3) and Organophosfat ($C_{10}H_{19}O_6PS_2$) on *Anopheles* larvae. This was a laboratory research (true experiment), used for 3-4 instar stage larvae toxicity test . The number of samples was calculated using an experimental approach, with a completely randomized design. As simply, can formulated as follows, (t-1) (r-1)> 15. The amount of this samples were 21. The toxicity test with organophosphate compounds, at concentration 0.03 mg /lt as many as 76% larvae were death, whereas on concentrations 0.07 mg /lt as many as 95% of the instar were death. Toxicity test of synthesis two compounds on concentration 0,03 (organophospat) + 0,05 (Ag_2NO_3) mg/lt there was 83% of larvaes were death and 100% on concentration 0,07 (organophospat) + 0,05 (Ag_2NO_3) mg/lt. The conclusion are, synthesis of nano-silver with organophopat is more effective than only organophospat compounds. Silver nitrate has the potential as a synthetic material or substitute for Organophospate.**

Keywords: climate, vector, Anopheles

I. INTRODUCTION

The micro-climate of a region is affected by global climate change. These changes have an impact to various lives, including vector breeding [1]. The impact of climate change on vectors on bionomics are caused by the changes of micro-climates.

Anopheles as malaria transmissible vector which affected by these cause the changes in trait and vitality [2]. Climate change also has a potential to caused endangered species [3].

The changes of Anopheles behavior, in addition caused by climate change are also triggered by the use of insecticides in controlling efforts. The occurrence of Anopheles‟s resistance as the transmitters of malaria could endangers the life. Resistance make the increase of insecticide dose use. Increasing of doses threaten non-target organisms and endanger the environment [4]. Organophosphate ($C_{10}H_{19}O_6PS_2$) is one of insecticide materials used for vector control. The results of the organophosphate concentration showed that the concentration level of 0.03 mg / lt showed 100% resistance [5]. WHO classified carbamate types carbofuran , butocarboxim dan butoxycarboxim to class 1b (Highly hazardous) [6].

Nano technology with its development, has been added to various developments [7]. In the health sector there are several ingredients that have been tried in other countries, with Nano-TiO2, Silica and herbal ingredients [8]. Nano-Silver which is obtained from Ag_2NO_3 material, is easily obtained and can be used for the health sector. The principle of organophosphate biomechanism, which inhibits Acethylcholinesterase (AChE), had change if it synthesized with Nano Silver (Ag_2NO_3). Silver nanoparticles can penetrate the stomach thus accelerating biochemical reactions with ACHE, make it more effective and have a lower dose [4].

The problem in this research is how the diversification of Ag_2NO_3 nanoperticles for vector control is compared with the organophosphate compound and the synthesis of both materials for the reduction of Anopheles larvae. The purpose of this study was to test the toxicity of nano silver (Ag_2NO_3) and Organophosphate ($C_{10}H_{19}O_6PS_2$) synthesis materials against Anopheles larvae.

II. METHOD

This was a laboratory research (true experiment) bcontrolling all of variables. The research began with prepare a compounds of Nano-silver from Ag_2NO_3 processing. Ag_2NO_3 material is obtained from chemical suppliers. Organophosphate are obtained from market products and standardized to determine concentration. The *Anopheles* vector which used for toxicity test was larvae instar 3-4. Toxicity test for larvae used the liquid-immersion method. The number of samples in this research was calculated by the experimental approach completely randomized design. As simple, formulated as follows (t-1) (r-1)> 15 [24]. The number samples was 21. The solutions used for test solutions are Organophosphate (Malathion 95%) and $AgNO_3$ nano solution (0.2%). Steps of making the mother liquor of Organophosphate (Malathion 95%) to obtain a 50 ppm concentration, the making of Ag_2NO_3 nano test solution was designed with a concentration of 2 ppm mother liquor.

Toxicity test use the solutions were made to *Anopheles* species instar 3. The steps of this test are 1) Transparant glasses are prepared about 21 pieces used for 3 types of solutions test with 6 concentrations and 1 control in each solution. 2) Observations were did in the first 2 hours then the observations were recorded. 3) Observations carried out again in the next 24 hours then recorded the results of these observations.

III. RESULTS

Organophosphate $C_{10}H_{19}O_6PS_2$ Test

Organophosphate toxicity test was carried out on 25
Anopheles instars in each bowl using controls. Observations were made in the first 2 hours and 24 hours. In the first 2 hours at concentration 0.01 mg / lt, 33% of were death. At the highest concentration was 0.07 mg / lt, as many as 67% of test animals experienced were death (Table 1).

The observation after 24 hours of Anopheles larvae as a test animal was obtained as follows. At concentration 0.01 mg/ lt 90% of larvae were death. The death decreased atconcentration 0.06 mg / lt with only 62%. Death increased to 95% at a concentration of 0.07 mg / lt. The results of the organophosphate toxicity test for 3-4 Anopheles instars are presented in Table 2.showed that the mean significance value of systolic blood pressure before and after the intervention is: p-value 0.015, p-value < 0.05, it means there is a significant difference the mean of systolic blood pressure in red watermelon juice treatment group before and after the intervention. The mean significance value of diastolic blood pressure before and after the intervention is p-value 0,053, p- value > 0,05, it means there was no significant difference the mean of diastolic blood pressure in red watermelon juice treatment group before and after the intervention

TABLE I
Toxicity Test Organophosphate for Anopheles
(2 hour)

Concentartion (mg/l)	Organofosfat (Malathion)				
	Control	Existence	%	Death	%
0,01	21	14	67%	7	33%
0,03	21	13	62%	8	38%
0,05	21	9	43%	12	57%
0,06	21	8	38%	13	62%
0,07	21	7	33%	14	67%

TABLE 2
Toxicity Test Organophosphate for Anopheles
(24 hour)

Concentartion (mg/l)	Organofosfat (Malathion)				
	Control	Existence	%	Death	%
0,01	21	2	67%	19	90%
0,03	21	5	62%	16	76%
0,05	21	8	43%	13	62%
0,06	21	8	38%	13	62%
0,07	21	1	33%	20	95%

Ag_2NO_3 (Silver Nitrate) Nanoparticle Test

Nano silver nitrate (Ag_2NO_3) toxicity test was carried out on Anopheles larvae instar 3-4. About 25 animals tested with various concentrations of nano silver nitrate particles. Observation made for the first 2 hours and after 24 hours. The observation obtained as follows. At the first 2 hour observation, at concentration 0.1 mg / lt, as many as 70% of test animals were death. Concentration 0.35 mg / lt showed that the animals were experienced death as much as 60%.

At the 24-hour observation, at concentration 0.1 mg / lt (61%) the larvaes were death. The death of all larvae was seen at a concentration 0.35 mg / lt (100%). The observations still show the instar activity on the low Ag_2NO_3 concentration of 0.1 mg / lt - 0.20 mg / lt. The results of the toxicity test for Ag_2NO_3 nanoparticles against 3-4 Anopheles instars are presented in Table 3 and Table 4

TABLE 3
Toxicity Test Nanosilver for Anopheles
(2 hour)

Concentartion (mg/l)	Nanosilver Ag_2NO_3				
	Control	Existence	%	Death	%
0,10	20	6	30%	14	70%
0,15	20	7	35%	13	65%
0,20	20	7	35%	13	65%
0,25	20	12	60%	8	40%
0,30	20	11	55%	9	45%
0,35	20	8	40%	12	60%

TABLE 4
Toxicity Test Organophosphate for Anopheles
(24 hour)

Concentartion (mg/l)	Nanosilver Ag_2NO_3				
	Control	Existenc	%	Death	%
0,10	18	7	39%	11	61%
0,15	18	5	28%	13	72%
0,20	18	7	39%	11	61%
0,25	18	5	28%	13	72%
0,30	18	1	6%	17	94%
0,35	18	0	0%	18	100%

Silver Nitrate (Ag_2NO_3) and Organophosphate ($C_{10}H_{19}O_6PS_2$) Toxicity Test

The toxicity test of Nano silver nitrate (Ag_2NO_3) and Organophosphate larvae was carried out on Anopheles larvae in instar 3-4. A total of 25 larvaein each bowl were tested with various concentrations of organophosphate and 0.05 mg / lt nano silver nitrate particle. Observations were made for the first 2 hours and after 24 hours. The observations obtained as follows. At the first 2 hours of observation, in the concentration 0.01 mg / ltd and 0.05 nano silver nitrate particles, about 89% of larvae were death.

At the 24-hour observation, obtained at 0.01 mg / lt organophosphate concentration with 0.05 mg / lt of Ag_2NO_3 synthesis (91%) larvae were death. As much as 100% test animal mortality was seen at concentrations of 0.07 Organophosphate with synthesis of 0.05 mg / lt of nano silver nitrate particles. The results of the toxicity test of the synthesis of Organophosphate and Ag_2NO_3 nanoparticles against instar 3-4 Anopheles are presented in Table 5 and Table 6.

TABLE 5
Toxicity Test Synthesis of
Organophosphate and Nanosilver for Anopheles
(2 hour)

Concentartion (mg/l)	Organophosphate (Malathion) + 0,05 Ag_2NO_3				
	Control	Existence	%	Death	%
0,01 + 0,05	18	2	11%	16	89%
0,03 + 0,05	18	4	22%	14	78%
0,05 + 0,05	18	2	11%	16	89%
0,06 + 0,05	18	7	39%	11	61%
0,07 + 0,05	18	6	33%	12	67%

TABLE 6
Toxicity Test Synthesis of
Organophosphate and Nanosilver for Anopheles
(24 hour)

Concentartion (mg/l)	Organophosphate (Malathion) + 0,05 Ag2NO3				
	Control	Existence	%	Death	%
0,01 + 0,05	23	2	9%	21	91%
0,03 + 0,05	23	4	17%	19	83%
0,05 + 0,05	23	2	9%	21	91%
0,06 + 0,05	23	6	26%	17	74%
0,07 + 0,05	23	0	0%	23	100%

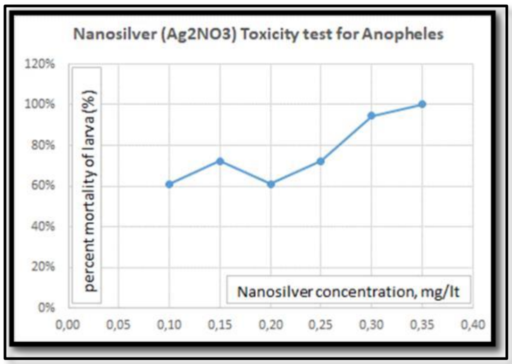

FIGURE 1
Graphic of Organophosphate Toxicity Test

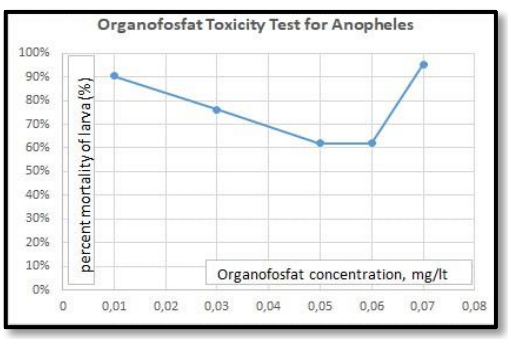

FIGURE 2
Graphic of Nanosilver Toxicity Test

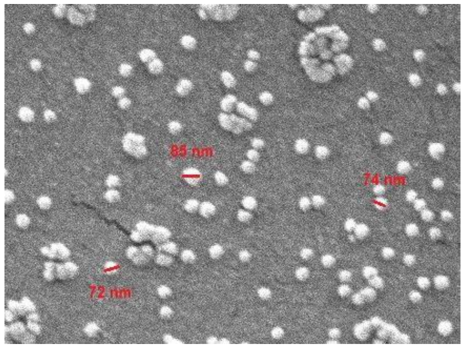

FIGURE 3
Garaphic Synthesis of Nano Silvernitrate
(Ag2NO3) and Organophosphate Toxicity Test

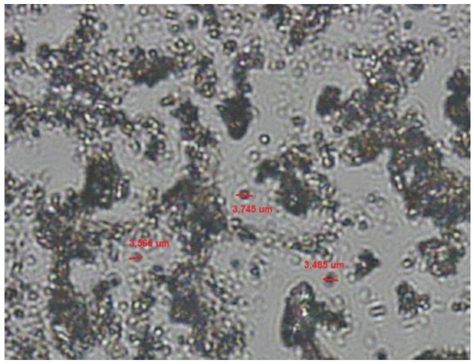

FIGURE 4
Size of Nanoparticle Silver

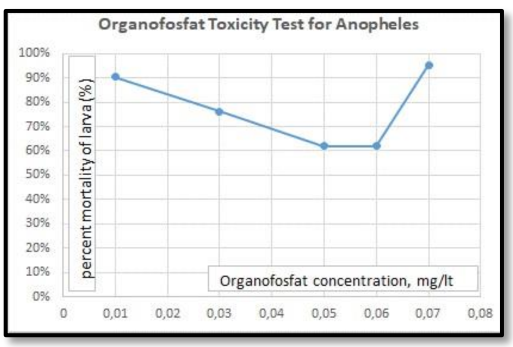

FIGURE 5
Size of Organophosphate particle

IV. DISCUSSION

Chemical control by utilizing various of synthetic materials, preparations and derivatives of organophosphate and carbamate compounds is still widely used. Control is carried out on vectors in the larva-pupa phase until adulthood. Instar phase 3-4 of Anopheles in the water utilizes oxygen and minerals in the water for its life. Oxygen absorption through the surface of the skin and minerals through the mouth is a biochemical mechanism in the larvae life cycle. Biochemical mechanisms in the body of the larvae are used to choose the form of intervention so that the larvae are easily destroyed and can‟t grow up to adult mosquitoes.

The results of the toxicity test of nano silver particles (Ag_2NO_3), showed that at low concentrations (0.10 - 0.2 mg / lt) the activity of Anopheles larvae showed the suitability of the mineral Nitrate as an essential material for growth and development by encouraging the activeness of the larvae. The Acetylcholinesterase (AChE) enzyme can grow optimally. This is consistent with Leibig's theory, that the growth of organisms is controlled by limiting factors [9].

At concentration 0.35 mg / lt, all Anopheles larvae (100%) were died. This concentration is greater than organophosphate. Organophosphate concentration ($C_{10}H_{19}O_6PS_2$) to kill 95% of test animals is needed at 0.07 mg / lt. So to kill all of animals tested requires a concentration greater than 0.07 mg / lt. Extrapolation simulation results need concentration 0.1 mg / lt to kill all of larvae. In the test by synthesizing organophosphate and nano silver material, the following results were obtained. At 0.01 and 0.05 nanosilver and organophosphate synthesis doses, 91% of the larvae were death. Organophosphate concentration and nanosilver synthesis needed to kill 100% of test animals, as much as 0.07 mg / lt was synthesized with 0.05 mg / lt nanosilver. The concentration is smaller than when used organophosphate alone, which requires a concentration of 0.1 mg / lt (1.43 times greater). Nanosilver synthesis of 0.05 mg / lt can increase organophosphate effectiveness up to 143%.

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 mosquito larvae‟s abdominal is 0.5 mm [André Pereira 2018]. Estimated pore size of the abdomen from the larvae is 0.5 mm x 1000000/1000 = 500 nm. The size is bigger than the size of silver nitrate particles. The potential for the occurrence of intrusion of nanomaterial through the abdominal pores of the larvae is very large. This intrusion encourages more effective reactions between nanoparticles and organiphosphate to react with Acethyl Chloniesterase (AChE) enzyme.

Nanoparticles size is between 1-100 nm, have high absorption properties to all media around them. Anopheles larvae breathe through a funnel on their tail. Food sources enter through the gastrointestinal tract and through the pores of the abdomen [10]. Nanosilver size is 72-86 nanomater has a smaller size compared to other materials. For comparison, the wavelength of visible light rays is between 400 nm and 700 nm. Blood cells (leukocytes) size is 10,000 nm, a bacterium has size between 1000-10000, a virus 75-100 nm, protein 5-50 nm, deoxyribonucleic acid (DNA) ~ 2 nm (width), and an atom of ~ 0.1 nm. On this scale, the physical, biological and chemical characteristics of each material have fundamentally different from each other and often unexpected actions are seen from them [11].

Nano Ag_2NO_3 particles with its size have high absorption power in Anopheles larvae. Ag_2NO_3 nanoparticles are dispersions of solid particles or colloidal structures ranging in diameter from 1 - 1000 nm. The nanosilver material consists of synthetic polymers, semi-synthetic and natural active therapeutic molecules having the ability to be trapped, encapsulated, dissolved, absorbed, or chemically attached [12]. The characteristics which are owned so that biodegradability, biocompatibility, and flexibility in utilization [13] [14].

The size of nano silver nitrate has greater potential to enter through the pore and food channel (gastrointestinal). The speed of particles to interact with the enzyme system in the larval body increases, so that in low concentrations it has accumulated in the larval body that is able to inhibit the working system of the enzyme Acetylcholinesterase (AChE).

Synthesis of Nanosilver and Organophosphate

Every material that was mixed will experience mixing and react with each other to form new compounds. Ag_2NO_3 compound is a salt compound and has clear color. Silver nitrate still very toxic and corrosive. Brief exposure will not produce immediate side effects other than purple, brown or black spots on the skin, but at constant exposure to high concentrations, side effects will be seen, including burns. Long-term exposure can cause eye damage. Silver nitrate is known as a substance that irritates the skin and eyes [15]. Organophosphate ($C_{10}H_{19}O_6PS_2$) is a compound which leaves little residue [16]. Organophosphate is a carbon compound containing carbamic acid. Synthesis of both materials will occur as follows.

Based on HKSA equation model, new carbamate derivatives obtained which have better predictive insecticide activity than existing carbamate insecticide compounds, namely 3-ethyl-2- isopropoxyphenyl methylcarbamate [17]. Organophosphate inhibits the action of pseudokholinesterase in plasma chlolinesterase in red blood cells and in synopsis. The enzyme normally hydrolyzes acethylcolin to acetate and chlorine. When enzym is inhibited, the amount of acethylcholine increases and associated with muscarinic and nicotinic receptors in the central nervous system [18]

The results showed that synthesis of organophosphate and silvernitrate was able to increase the effectiveness of organophosphate use from a concentration of 0.1 mg / lt to 0.07 mg / lt with the addition of nanosilvernitrate of 0.05 mg /lt. This efficiency increases 1.43 times (143%), when only using a single organophosphate compound. This research showed the results of synthesis produced a new compound. In a previous study conducted by Agus using silica ions, it produced 3-ethyl-2-isopropoxyphenyl methylphosphate. Substituting silica with silvernitrate is strongly suspected to also produce compounds with the same properties.

This research reinforces the previous research. There are better benefits, especially Ag_2NO_3 nano pertikel to another organisms, compared to the use of Nano Ag_2NO_3 insecticides which are more environmentally friendly. Silver nitrate nanoparticles have been known to have no effect on non-target aquatic organisms [19]. There is already information about the occurrence of acute toxicity to non-target aquatic species. Silver synthesized with Plumeria rubra and Pergularia daemia nanoparticles did not show a toxicity effect on P. reticulata fish after 48 hours of LC50 exposure [20]. Silver nanoparticles were synthesized using a non-toxic Solanum nigrum berry extract against two mosquito predators, Toxorhynchites larvae and Diplonychus annulatum [21]. Nano Silver can be applied to a variety of health care products [8]. An interesting is the potential use of silver nanoparticles (AgNP) in mosquito control. Many groups have reported synthesizing AgNP which can limit the growth of larvae and / or pupa forms from Anopheles [22] [23].

These properties are controlled by the metabolism intensity of the enzymes Monooxygenases, Esterases, Acetylcholinesterase (AChE) and Glutathione – S - transferase (GST)) in the insecticide detoxification process [4].

The performance of the enzyme as a determinant of the absorption of insecticides on target mosquitoes. Specific site targets are usually in the form of enzymes or proteins that act as effectors of insecticides [10]. Other studies have shown that the high metabolic activity of the

Esterases enzymes in Anopheles, in Sri Lanka, causes resistance potential to malathion [24] [25] [26].

Isopropyl phosphate which is a compound of synthesis, works to inhibit the enzyme acetylcholinesterase (AChE) which results in the accumulation of acetylcholine (ACh) Acetylcholine that is deposited in the Central Nervous System (CNS) will induce tremors, incoordination, convulsions and others [5]. Acetylcholinesterase (AChE) is a proven target for controlling Anopheles mosquitoes. A single amino acid mutation (G119S) in Anopheles forms AChE-1 (AgAChE), which provides resistance to AChE inhibitors. G119S resistance, AgAChE mutants, and high toxicity to An. gambiae has the effect of changing nature. Inhibitors can be generated from carbamates in the form of 'small nuclei' such as aldicarb and pyrazole-4-yl methylcarbamate 4a - e. Although none of these compounds, shows selectivity that is useful for the inhibition of AgAChE to AchE [27].

High concentrations of organophosphate synthesis to kill Anopheles larvae, due to the process of entry of the material only through the digestive tract (gastrointestinal). High concentrations are needed to achieve the enzyme system disruption in Anopheles larvae. The enzyme Acetylcholinesterase (AChE), as a controlling metabolism of Anopheles larvae, is incomplete with an organophosphate concentration of 0.01 mg / lt

V. CONCLUSION

Nano synthesis of Ag_2NO_3 particles at concentration 0.05 mg/lt was able to reduce the concentration of organophosphate used ($C_{10}H_{19}O_6PS_2$) by 143% (from 0.1 ml/lt to 0.07 mg/lt) to kill 100% of Anopheles larvae.

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