Synthesis Nanoparticle of Silver Nitrate (Ag_2NO_3) and Carbamate (CO_2NH_3 derivatives) for Vector Control of *Anopheles*

Mursid Raharjo[1], Agus Subagyo[2], Sulistiyani[3]
{mursidraharjo@gmail.com[1], agus_fadhil@yahoo.com[2], sulisbadar@gmail.com[3]}

Magister of Environmental Health, Faculty of Public Health, Diponegoro University, Semarang - Indonesia[1,3],
Faculty of Sains and Mathematics, Diponegoro University, Semarang – Indonesia[2],

Abstract. : The climate changes affecting vector breeding. *Anopheles*' resistance is harmful to life. Synthesis of Nanosilver (Ag_2NO_3) and Carbamate (CO_2NH_3 derivatives) is a new form in control. This research aimed to toxicity test of synthesis nano-silver and carbamate on *Anopheles* larvae. This was laboratory research (true experiment) used 3-4 instar stage larvae. The 21 samples calculated using an experimental approach with a completely randomized design. The number of death larvae used carbamate toxicity test at concentration 0.001 mg/lt was 74%, 0.007 mg/lt was 83% and 0.010 mg /lt was 100%. Synthesis of nano-silver and carbamate showed the death number on concentration 0.001(carbamate)+0,05(Ag_2NO_3)mg/lt was 73%, 0.005 mg/lt (carbamate)+0,05(Ag_2NO_3) mg/lt was 100%. The larvae more active in the low concentration of Ag_2NO_3 (0.01 mg/l-0.1 mg/l). The size of Ag_2NO_3 nanoparticles is 1-100 nm, which has penetrating power on *Anopheles* larvae. The conclusion is, synthesis of nano-silver carbamate is more effective (200%) than carbamate.

Keywords: *Anopheles*, Carbamate, Synthesis Nanosilvernitrate

1 Introduction

Climate change has an impact on various lives, including vector breeding [1]. The changes in vectors, especially in bionocyclic vectors caused by micro-climate changes. Anopheles, as a transmissible malaria vector, is also affected by these changes, so it causes changes in nature and vitality [2].

Climate change also has the potential to create endangered species [3]. The changes of Anopheles behavior also triggered by the use of insecticides in controlling efforts. The occurrence of resistance to Anopheles vectors as transmitters of malaria endangers life [4]. Resistance causes the increase of insecticide dose using. The increasing of doses threaten non-target organisms and endanger the environment [2]. Carbamates, as an insecticide (CO_2NH_3 derivatives), are widely used for vector control [4]. The results of another research showed that the concentration level of 0.03 mg/lt showed 100% resistance [5]. WHO classified carbamate types carbofuran, butocarboxim dan butoxycarboxim to class Ib (Highly hazardous) [6]. The research by The International Programme on Chemical Safety (IPCS) resulted that the LD50 of carbamate on rats range from 1 mg/kg to 5000 mg/kg of body weight and on aquatic

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microorganism (fish) Bluegill *(Lepomis machrochirus)* resulted in the acute dose on concentration 0,24^{e} mg/l used the carbamate with active material carbofuran [7]

Nanotechnology, with its development, has been added to various developments [8]. In the health sector, several ingredients have been tried in other countries, with Nano-TiO_2, Silica, and herbal ingredients [9]. Nano-Silver, which is obtained from Ag_2NO_3 material, is easily obtained and can be used for the health sector. The principle of carbamate biomechanism, which inhibits Acetylcholinesterase (AChe), replaces ownership if it is synthesized with Nano Silver (Ag_2NO_3). Silver nanoparticles can only penetrate the AChe enzyme, so they are more effective and have a lower dose [4].

The problem in this research is how the diversification of Ag_2NO_3 nanoparticles for vector control and compared to carbamate compounds. The purpose of this research is to know the toxicity of nanosilver (Ag_2NO_3) and carbamate (CO_2NH_3 derivate) synthesis materials against *Anopheles* larvae.

2 Method

This was laboratory research(true experiment) by controlling all of the variables. The research began to prepare a compound of Nano-silver from Ag_2NO_3 processing. Ag_2NO_3 material is obtained from chemical suppliers. Carbamates are obtained from market products and standardized to determine concentration.

The *Anopheles* vector, which used for the toxicity test, was larvae instar 3-4. The toxicity test for larvae used the liquid-immersion method. The number of samples in this research was calculated by the experimental approach, with a completely randomized design and formulated as follows (t-1) (r-1)> 15 [24]. The number of samples was 21.

The solutions used as test solutions include carbamate (Furadan 3gr) and AgNo3 nano solution (0.2%). Steps of making the mother liquor (Furadan 3gr) to obtain two concentrations are 0,1 ppm, and 1 ppm., The making of $AgNO_3$ nano test solution was designed with a concentration of 2 ppm mother liquor.

The toxicity test used the *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 done in the first 2 hours, and then the observations were recorded. 3) Observations carried out again in the next 24 hours then recorded the results of these observations.

3 Result

3.1 Carbamate Tests (CO_2NH_3 derivatives)

Carbamate toxicity tests were carried out on 25 Anopheles instars in each bowl and using controls. Observations were made in the first 2 hours and carried out again in 24 hours. In the first 2 hours at concentration 0.001 mg / lt, there was 36% of Anopheles instar were died. At the highest concentration during the test of 0.01 mg / lt, as many as 32% of test animals died. The highest percentage occurred at concentration (0.005 mg / lt) was 40%, Table 1.

At 24 hour observation of Anopheles larvae as test animals, obtained as follows, at concentration 0.001 mg / lt, 74% of experimental animals died. This death increases up to

100% at concentration 0.01 mg / lt. The results of the carbamate toxicity test for 3-4 *Anopheles* instars are attended in Table 2.

Tabel 1. Toxicity Test Carbamat for *Anopheles* (2 hours)

Concentration (mg/l)	**Carbamate (Furadan)**				
	Control	**Existence**	**%**	**Death**	**%**
0,001	25	16	64%	9	36%
0,003	25	17	68%	8	32%
0,005	25	15	60%	10	40%
0,007	25	18	72%	7	28%
0,01	25	17	68%	8	32%

Tabel 2. Toxicity Test Carbamat for *Anopheles* (24 hours)

Concentration (mg/l)	**Carbamate (Furadan)**				
	Control	**Existence**	**%**	**Death**	**%**
0,001	23	6	26%	17	74%
0,003	23	8	35%	15	65%
0,005	23	5	22%	18	78%
0,007	23	4	17%	19	83%
0,01	23	0	0%	23	100%

3.2 Ag_2NO_3 (Silver Nitrate) Nanoparticle Test

Nanosilver nitrate (Ag_2NO_3) toxicity test was carried out on *Anopheles* larvae in instructor 3-4. There were 25 animals tested with various concentrations of nanosilver nitrate particles. Observations were made for the first 2 hours and after 24 hours. The observations obtained as follows. In the first 2 hours of observation, in the construction of 0.1 mg / lt, as many as 70% of test animals died. The 0.35 mg / lt concentration showed animals that experienced death as many as 60%.

At the 24 hour observation, it was obtained at a concentration of 0.1 mg / lt (61%) of the test animals died. The death of new test animals was seen at a concentration of 0.35 mg / lt (100%). The observations also show that an active reaction was low at concentration 0.1 mg/lt - 0.20 mg/lt. The results of the toxicity test for Ag_2NO_3 nanoparticles against instar 3-4 Anopheles participated in Table 3 and Table 4.

Tabel 3. Toxicity Test Nanosilver for *Anopheles* (2 hours)

Concentration (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%

Tabel 4. Toxicity Test Nanosilver for *Anopheles* (24 hours)

Concentration (mg/l)	Nanosilver Ag_2NO_3				
	Control	Existence	%	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%

3.3 Synthesis Test of Ag_2NO_3 Nanoparticles (Silver Nitrate) and Carbamate (CO_2NH_3 derivatives)

Toxicity test of Nanosilver nitrate (Ag_2NO_3) and Carbamate (CO_2NH_3 derivatives) performed on *Anopheles* larvae on instar 3-4. Total of 25 animals tested with various concentrations of carbamate and 0.05 mg/lt nano silver nitrate particles. Observations were made for the first 2 hours and after 24 hours. The observations obtained as follows. In the first 2 hours of observation, in the concentration of 0.001 mg / ltd and 0.05 nano silver nitrate particles, 36% of test animals died. Similarly, the 0.01 mg / lt and 0.05 silver nitrate constructions showed 50% of animals were dead.

At the 24 hour observation, at the concentration of 0.001 mg / lt with the synthesis of Ag_2NO_3 0.05 mg / lt. (73%) test animals died. 100% test animal mortality was seen at concentrations of 0.05 Carbamates with the synthesis of 0.05 mg / lt nano silver nitrate particles. The results of the toxicity test for the synthesis of carbamates and Ag_2NO_3 nanoparticles against instar 3-4 *Anopheles* are presented in Table 5 and Table 6.

Tabel 5. Toxicity Test Synthesis Nanosilver and Carbamate for *Anopheles* (2 hour)

Consentration (mg/l)	Carbamate + 0,05 Ag_2NO_3				
	Control	Existence	%	Death	%
0,001 + 0,05	22	14	64%	8	36%
0,003 + 0,05	22	15	68%	7	32%
0,005 + 0,05	22	13	59%	9	41%
0,007 + 0,05	22	12	55%	10	45%
0,01 + 0,05	22	11	50%	11	50%

Table 5 shows the results of observations used nanosilver and carbamate toxicity tests within 2 hours. The death of test animals has not reached 100% at 2 hours observation at the highest concentration. The lowest mortality was at concentration 0.003 + 0.05 mg / l (32%) and mortality on the highest at a concentration 0.01 + 0.05 mg / l was 50%.

Tabel 6. Toxicity Test Synthesis Nanosilver and Carbamate for *Anopheles* (24 hour)

Consentration (mg/l)	Carbamate + 0,05 Ag_2NO_3				
	Control	Existence	%	Death	%
0,001 + 0,05	22	6	27%	16	73%
0,003 + 0,05	22	5	23%	17	77%
0,005 + 0,05	22	0	0%	22	100%
0,007 + 0,05	22	0	0%	22	100%
0,01 + 0,05	22	0	0%	22	100%

Table 6 shows the results of observations used nanosilver and carbamate toxicity tests within 24 hours. At concentrations 0.005 + 0.05 mg / l, carbamate and nanosilver synthesis showed there was 100% death animals and at lower concentrations 0.001 + 0.05 mg / l there was 73% death animals and at 0.003 + 0.05 mg / l there was 77%.

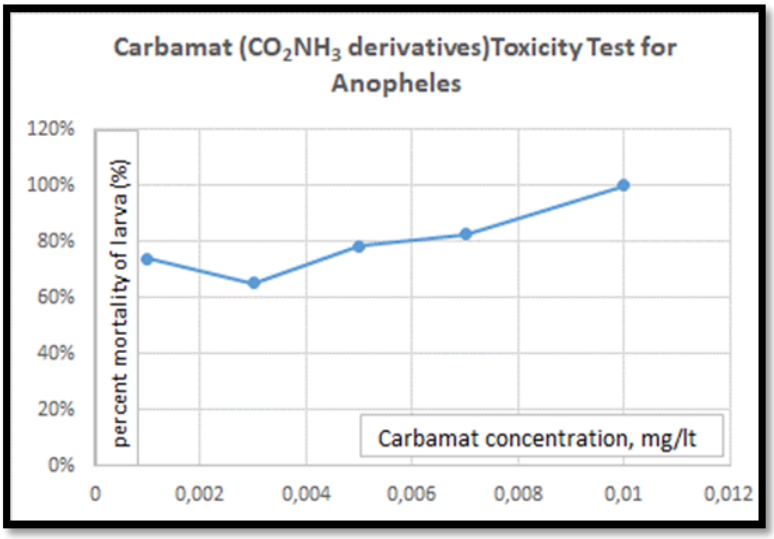

Fig. 1. Graphic of Carbamate Toxicity Test

In the graph above (figure 1) illustrates the death of test animals continues to increase along with increasing concentrations of a solution except at concentrations of 0.003. Test animal mortality of 100% was seen at concentrations of 0.01 mg / l carbamate.

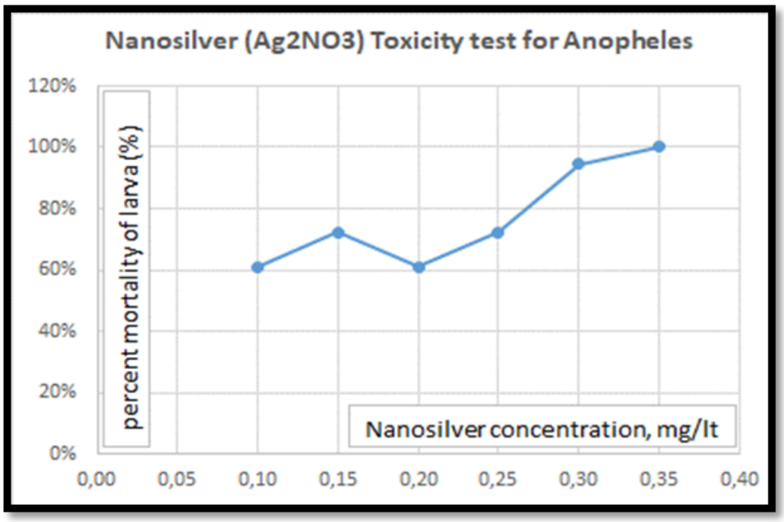

Fig. 2. Graphic of Nanosilver Toxicity Test

The death of test animals using a single nanosilver solution can be seen in figure 2. The death of test animals looks fluctuating showed by figure 2, and the lowest mortality occurs at a concentration of 0.30 mg / l was 61%. The death of 100% of test animals occurred at a concentration of 0.35 mg / l.

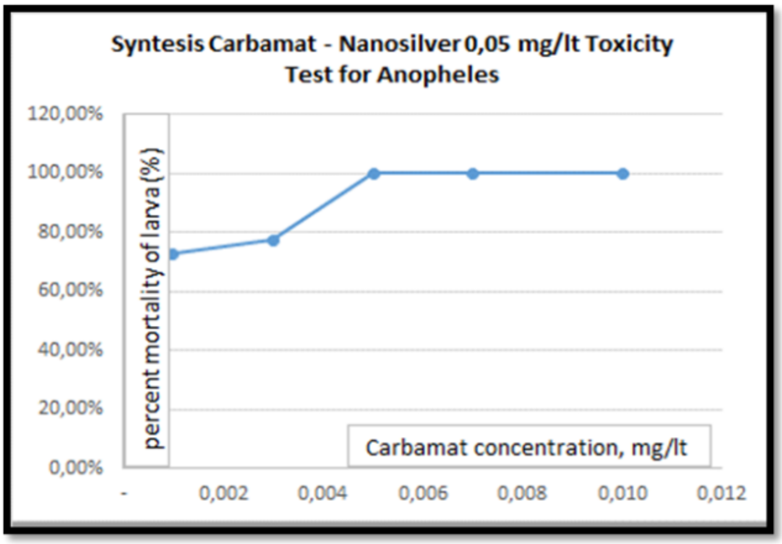

Fig. 3. Graphic Synthesis of Nano Silver nitrate (Ag2NO3) and Carbamate Toxicity Test

The graphic above (figure 3) shows that the increase of death animals test numbers along with the increase in concentration. The death all of test animals (100%) begins at concentration of 0.005 mg / l carbamate + 0.05 mg / l nanosilver.

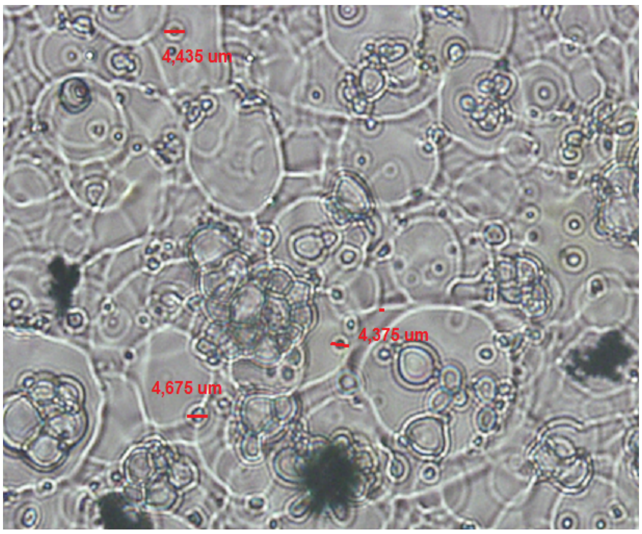

Fig. 4. Size of Carbamate Particle

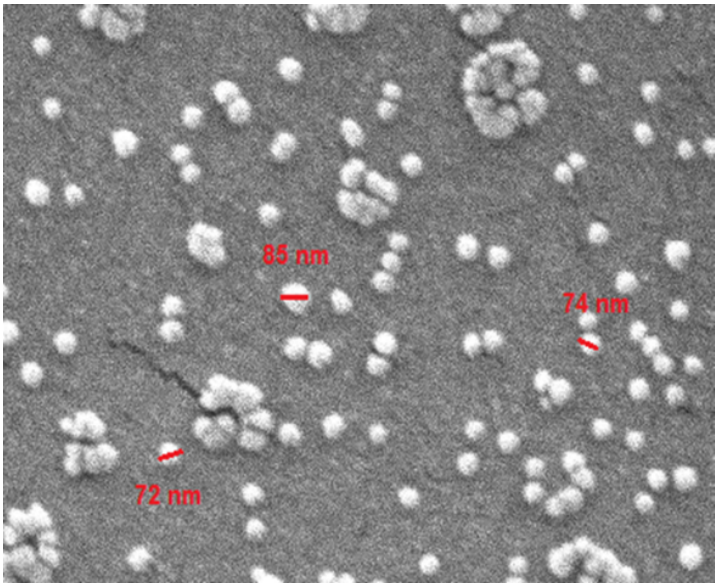

Fig. 5. Size of Nanosilvernitrate (Ag_2NO_3)

4 Discussion

Chemical control using various 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 3-4 of *Anopheles* in 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 life cycle of larvae. Biochemical mechanisms in the body of the larva are used to choose the form of intervention so that the larvae are easily destroyed and are not allowed to become adult mosquitoes.

The results of the nanosilver particles (Ag_2NO_3) toxicity test 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, which encourage the activeness of the larvae. The Acetylcholinesterase (AChE) enzyme can grow optimally. It is consistent with Leibig's theory that the growth of organisms is controlled by limiting factors [10].

The concentration of 0.35 mg / lt showed that all of *Anopheles* larvae (100%) died. This concentration is bigger than carbamate. It needs 0.01 mg / lt of carbamate concentration to kill all of the test animals. In the test, by synthesizing carbamate and nanosilver material, the following results were obtained. At carbamate doses of 0.001 and 0.05 nanosilver, 73% of larvae died. Carbamate concentration and synthesis of nanosilver needed to kill 100% of test animals, as much as 0.005 mg / lt, was synthesized with 0.05 mg / lt of nanosilver. The concentration is smaller than when carbamate is used singly, which requires a concentration of 0.01 mg / lt (2 times greater). Nanosilver Synthesis 0.05 mg / lt can increase the effectiveness of carbamates up to 200%.

Test results using the UV-VIS Spectrophotometer Test and Particle Size Analyzer (PSA) show the size of the Nanosilver is 72-86 nanometer, while for Carbamate has a size of 4,376 - 4,675 µm. The abdominal mosquito larvae size is 0.5 mm. The 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 the silver nitrate particles. The potential for the occurrence of nanomaterial 's intrusion material through the abdominal pores of the larvae is very large. This intrusion encourages more effective reactions between nanoparticles and carbamates to react with the enzyme Acetyl Chloniesterase (AcHe).

The size of the nanoparticles is 1-100 nm. It has high absorption properties to all of the media around them. Anopheles larvae breathe through a funnel on their tail. Food sources enter through the gastrointestinal tract and the pores of the abdomen [11]. Nanosilver with a size of 72-86 nanometer 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) have a size of 10,000 nm, and a bacterium has a size of 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 [12].

Ag_2NO_3 nanoparticles with size 72-861-100 nm have a high absorption capacity in Anopheles larvae. Ag_2NO_3 nanoparticles are dispersions of solid particles or colloidal structures ranging in diameter from 1 - 1000 nm. Nanosilver's compounds consist of the synthetic of polymers, semi-synthetic, and natural active therapeutic molecules that can be trapped, encapsulated, dissolved, absorbed, or chemically attached [13]. The properties and characteristics possessed in biodegradability, biocompatibility, and ease of use [14][15].

The size of nanosilver nitrate has greater potential to enter through the pore and foodways (gastrointestinal). It causes the increase of particles' speed to interact with the enzyme system in the larval body so that in low concentrations, it has accumulated in the larval body that can inhibit the working system of the enzyme Acetylcholinesterase (AChE).

Synthesis of Nanosilver and Carbamate. Every material mixed will mix and react with each other to form new compounds. Ag_2NO_3 compound is a salt compound, clear in color, also very toxic and corrosive. Brief exposure will not cause side effects immediately other than purple, brown, or black spots on the skin, but at constant exposure to high concentrations, side effects will appear, including burns. Long-term exposure can cause eye damage. Silver nitrate is known as a substance that irritates the skin and eyes [16]. Carbamate (CO_2NH_3) is a compound that leaves little residue [17]. Carbamate is a carbon compound containing carbamic acid. The synthesis of both materials will cause a chemical reaction. Based on the best HKSA equation model, a new compound of carbamate derivative obtained with predictive insecticide activity is better than the existing carbamate insecticide compound, which is 3-ethyl-2-isopropoxyphenyl methylcarbamate. [18].

Results showed that the synthesis of carbamate and silver nitrate could increase the effectiveness of carbamate use from 0.01 mg / lt to 0.005 mg / lt with the addition of nanosilver nitrate 0.05 mg / lt. The efficiency increases 2 times (200%), than only using a single carbamate compound. This research shows that the synthesis resulted in a new compound. In previous research, conducted by Agus using silica ions, it produced 3-ethyl-2-isopropoxyphenyl methylcarbamate. Substituting silica with silver nitrate is strongly suspected also to produce compounds with the same properties.

This research reinforces the previous research. There are better benefits, especially Ag_2NO_3 nanoparticles to other organisms, compared to the use of other Nano Ag_2NO_3

insecticides, which are more environmentally friendly. Silver nitrate nanoparticles have been known not to affect 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]. The potential of silver nanoparticles (AgNP) used in mosquito control is interesting. 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 detoxification process of insecticides [4].

Target mosquitoes absorb the enzyme's performance as a determinant of the insecticides. Specific site targets are usually in the form of enzymes or proteins that act as effectors of insecticides [24]. Another study has shown that the high metabolic activity of the Esterases enzymes in *Anopheles*, in Sri Lanka, causes Potential Resistance to malathion [25][26][27].

Isopropyl carbamate as a compound of synthesis works to inhibit the enzyme acetylcholinesterase (AChE), which results in the accumulation of acetylcholine (ACh) Acetylcholine. It 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. However, none of these compounds show selectivity that is useful for the inhibition of AgAChE to AChE [28].

High concentration of carbamate syntheses to kill Anopheles larvae caused the process of material entry only through the digestive tract (gastrointestinal). High concentrations are needed to achieve the disruption of the enzyme system in Anopheles larvae. The enzyme Acetylcholinesterase (AChE), as a controlling metabolism of Anopheles larvae, is incomplete with a carbamate concentration of 0.001 mg / lt.

5 Conclusion

The synthesis of nano Ag_2NO_3 particles at concentration 0.05 mg / lt was able to reduce the concentration of carbamate (CO_2NH_3) CO_2NH_3 200% (from 0.01 mg / lt to 0.005 mg / lt) to kill 100% of the test larvae.

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