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Effectiveness Lime Shells of *Anodonta anatina* for *Larvae of Anopheles* and *Aedes aegypti* mosquitoes in Jayapura District, Indonesia.

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ABSTRACT

Abate usually uses as an insecticide effective against the larvae of certain aquatic insects, its relative safety to mammals and birds, it has been widely used for control of larvae and pupae for *Anopheles* and *A. aegypti* mosquito. To determine effectiveness dose between abate and lime shells *A. anatina* and differ larvae and pupae of *Anopheles spp* and *A.aegypti* for 48 hours. The water has been treated which can still be used as drinking water. This was as an experimented, controlled, and comparative study with two different materials.The materials test were lime shells and abate with dose 0.5 mg/25 liters drinking water and mosquitoes larvae in instars III and IV. The larvae and pupae were used in this study still active. The study showed that larvacides from lime shells *A.anatina*, the number of larvae were dead in 60 minutes, which were totally *Anopheles* and *A. aegypti* mosquitoes (59.4%) whereas for 24 hours (40.2%) and 48 hours (21.4%), but for abate, the larvae were dead up to 60 minutes, total for both mosquitoes (88.7%), whereas for 24 hours (0.0%). There were differences significantly period of two mosquitoes which are *Anopheles* and *A. aegypti* using abate and lime shells. *A. anatina* with same dose was 15 minutes using abate and 30 minutes for lime shells killed both larvae but abate can be replaced with lime shells *A. anatina* because it was environmental friendly as local material which can be cultivated and made by local community and more effectiveness for larvae than pupae.

Keywords: Abate, Lime shells of *A.anatina*, *Anopheles spp, Aedes aegypti, Larvae.*

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INTRODUCTION

The world malaria 2014 reported that information received from 97 malaria endemic countries. Globally, malaria transmission occurs in all six WHO region, an estimated 3,3 billion people are at risk of being infected with malaria and developing disease and 1.2 billion are at high risk[1]

Malaria is still found throughout the province in Indonesia. Based on annual parasite incidence (API), and stratification conducted at the eastern of Indonesia regions which the parasite malaria stratification incoming high. Based on report per province of the years 2008-2009 was found annual parasite incidence (API) that higher cases in West Papua, east Nusa Tenggara and Papua Provinces [2]

Malaria caused by *Plasmodium* through bitten Anopheles mosquitoes which are asexual reproduction. Species of *Anopheles* will be varied in each area depending on geographical factors, spreading, climate and bleeding place[2]. Malaria poses as a major public health problem in Papua[3]. It eradicates larvae and pupae are still using abate as larviciding. Majority all people in Papua are using abate to kill larvae of *Anopheles* and *A.aegypti*. Moreover, Abate can destroy the human cell based on research of Raharja 2011. That's way we tried to conduct study to find resources which can be used to replace abate without side effect to human and environment [4]

Larviciding is a common term for killing immature mosquitoes through applying agent, collectively called larvicides in order to control mosquito larvae and/ or pupae. Larvae sources management includes both modification of water habits, often referred to as source decreased, and directly applicator of larvicides to control mosquito's breed. Majority of mosquito species spend much their life cycle in the larva. The Most stage when they are highly susceptible both predation and control efforts. They often concentrated within defined water boundaries, immobile with little ability to disperse, and accessible. Adult mosquitoes, in contract, fly in search of mates, blood widely distributed. Therefore, effective larviciding can reduce, create a nuisance, and lay eggs which leads to more mosquitoes [5].

The effective control of larvae and pupae are basic principle of integrated pest management include understanding local mosquito ecologies and patterns of selecting appropriate mosquito control tools. The most general methods of integrated pest management involve environment, or source reduction, larviciding, and adulticiding [6].

Abate (temephos) is an organophosphate pesticide registered by environmental protection agency in 1965 to control mosquitoes larvae and is the only organophosphate with larvacidal used [7].

It is a potent larviciding based on the active ingredient temephos that effectively manages a broad spectrum of nuisance and diseases that causing insects, such as mosquitoes, before they hatched. While long lasting insecticidal nets and indoor residual spraying focus on the individual and the household. Abate larviciding takes vector control to community level [8]

According to Martanto in Raharja (2011) abate are containing harmful chemicals so it can cause cancer. If uses continuously so can be lead to carcinogenic in organs, for example when having tooth brush or drinking water containing abate. In Jayapura municipality according public health that abate less effective for inhibit or kill larvae and pupae *Anopheles* mosquitoes and *A.aegypti* than in central Java [4].

This study was the first research found changing of abates to the natural resources in Indonesia. Those natural resources come from *A. anatine* which found in Sentani lake, Bukisi, Nafri, and Biak District in Papua. The composition of powder made from Lime Shells *A. anatina* are Calcium carbonate, Calcium hypochlorite, Phosphate, Sulfate, Flour, Peroxide, and Free chlorine. This study was very interested to change the chemist material into natural resources. For how many years all people were using abate to kill the larvae of *Anopheles* and *A. aegypti*. That was the reason why this study conducted by researchers. Another benefits of this study that found the material which can kill the larvae without side effect to the human, animals, and plants. The water with lime shells can be drunk without side effect to the human and all environment around.

Lime shells from *A. anatina* is material that is made by burning shells at 100^{o}C and charcoal in shear zones and be made limestone powder that is ready to be used as ingredients to turn off larvae or pupae in

Jayapura Municipality, Papua, Indonesia. The materials should be used directly after making and stored at long time because if its stored for long time so power of lime shells activity will be reduced (see fig 1 and fig 2) [9.]

The *A. anatina* are alive in fresh water mussels occupied a wide range of habitats, but most often occupy lot waters, flowing water such as rivers, streams and creeks [9.]

It is important to select the appropriate control agent and formulation based on performances and other factors. It is critical to have a conscientious knowledge of the biology of the targeted species in order to determine the appropriate larvicides, timing of the application, amount of product to be applied. The larvae of *A. farauti* are unevenly distributed and the density where they do happen much higher than other times in their development when they tend to be more evenly dispersed in salt marsh pools. This situation may call for an application rate higher what is normally used, but never exceeding the maximum allowed on the label [10.]

Anopheles species and *A. aegypti* mosquitoes very important and for medical and most frequency was found in tropical and subtropical areas of the world. *A. aegypti* historically is considered to be a primary vector of viral diseases [8] such as the dengue fever in Indonesia and especially in Jayapura Municipality and Jayapura district. Therefore we need larviciding that could be made from local materials by local people as change abate to turn off the larva and pupa of *A.aegypti* and *Anopheles spp* in Papua and west Papua Provinces. The *A.farauti* and *A.punctulatus* mosquitoes are vectors in those areas that have long been known to be vectors of human diseases such as malaria and dengue fever [11,12.]

This research was the first study conducted in Papua. This study found another substance to change abates killed parasites to be grown. The substances come from lime cells of *A. anatine* which can found around Papua area. *A.anatina* which was used in this research found from Bukisi, Sentani lake, Nafri and Biak District as part of Papua province. The powder of lime cells can kill larvae and pupae of *Anopheles* and *A. aegypti*. The powder of lime cell will not harm the human cells environment.

There are several methods that have been used to control mosquitoes population. The chemical methods (pesticides) still the main and the most effective one, especially in cases with more over populations. Mosquitoes have developed resistance for almost all classes of insecticides [13].

METHODS STUDY DESIGN AND SITES

Description Study Side. This study was conducted at Abepura and Sentani areas in Jayapura District and laboratory of Polytechnic of Health, Ministry of Health Jayapura. It is located in Altitute 2°31'58,8" North and longitute 140°43'1,2" East. The habitants of Jayapura Municipality is mainly Papuan and migrant from Java, Sulawesi, Mollucas, and other parts of Indonesia. Jayapura Municipality is a wide territory covering 442,540 km^2 and it is divided into mainland, swamp, river areas, and large heading to the Pacific Ocean. The Municipality is bordered in the north by Pacific ocean and the east with Papua New Guinea.

The climate is typically tropical with average temperature between 25-35^oC.The difference between rainy season and dry season as cause of wind effect. May to November, the wind is blowing from South-east with less amount of water vapor, whereas in December to April the westerly wind is blowing sea and causes rainfall. The range of rainfall is between 1,500 - 6,000 mm per year. The majority of mosquito in Papua Province are *Anopheles farauti*, *An*. *punctuates* and *An. bancrofti* whereas *An*. *koliensis* and *An. kowari* are secondary vectors [14].

In this study, we report on toxicity of one commercial abate (temephos) and one conventional from lime shells of *A.anatina* used from Biak District, Nafri village in Jayapura Municipal and Sentani municipal also Bukisi village in Jayapura district, Papua Province, Indonesia. Both confound above were used for inhibit growth and killing mosquitoes larvae such as *A.aegypti* and *Anopheles,spp* and could be for Culex also.

Larvicidal activity: Fifty mosquitoes larvae (*Anopheles spp* and *A. aegypti*) (late 3rd and early 4th instars) were placed in a 30 liter container contained 25 liters of water. Solutions of required concentrations were in container containing abate or lime shell 0.5 gram and in to 25 liter water. Each concentration was replicated three times of Anopheles spp and three times of A. aegypti and pupae and one control received only the solvent were maintain during the test without larvae and pupae. Dead larvae were counted till 48 h after

treatment; larvae that did not move when touched with a thin needle were considered dead shown in (table 1 and 2) [15]

Larvicidal and Pupicidal activity of abate and lime shells *A.anatina*. Laboratory tests were conducted in container with 25 liters of water. Fifty larvae and twenty-five pupae were placed in each container before treatment. Area of surface water was estimated as 160 cm^2, Abate or lime shells *A.anatina* as much as 0.5 gram was added to surface of water to give the required rates of application showed in (table 3). For example, to apply a rate of 25 liter water, 0.5 gram and 1gram of abate or lime shells (*A. anatina*) put in each container, three containers of larvae and three containers of pupae and one container as control that containing 25 liter water to give 0.5 gram abate and lime shells (*A. anatina*) of each container were run under same conditions [16.]

Inclusion criteria of this sample. The lime shells *A. anatina* which is used in this study such lime shells that still fresh and the time of lime shells *A. anatina* powder should not be stored more than one month.

Exclusion criteria of this sample. The lime shells that used not from rock or the other *Anodonta* species.

RESULTS

The result shown table 1, that the average of larvae of Anopheles mosquito were dead at 30 minutes as much as 15%, in 45 minutes as much as 21.3, in 60 minutes 28.7%, in 24 hours as much as 21.3% and in 48 hours as much as 10.7%, respectively. Based on the statistic used Anova test indicated that there was different significantly effectiveness time used abate which were the dead number of *Anopheles larvae* (*P value* = 0.001< 0.05, CI= 95%, α = 5%).

The result shown table 2, that the average larvae of *A. aegypti* mosquito were dead at 30 minutes as much as 20.7%, in 45 minutes as much as 20%, in 60 minutes as much as 30.7%, in 24 hours as much as 18.7% and in 48 hours as much as 10.7%, respectively. Based on the statistic Anova test indicated that there was different significantly effectiveness time used lime shells which where the dead number of *A.aegypti* larvae (*P value* = 0.0005 < 0.05, CI= 95%, α = 5%).

The result shown table 3, that the average larvae of *Anopheles mosquito* were dead at 15 minutes as much as 10.7%, in 30 minutes as much as 19.3%, in 45 minutes as much as 30.0%, in 60 minutes as much as 40.0%, in 24 hours as much as 0.00% and in 48 hours as much as (0.00%), respectively. Based on the statistic Anova test indicated that there was different significantly effectiveness time used lime shells which were the dead number of *Anopheles* larvae (*P value* = 0.001 < 0.05, CI= 95%, α = 5%).

The result shown table 4, that the average larvae of *Anopheles mosquito* were dead at 15 minutes as much as 8.0%, in 30 minutes time as much as 18.7%, in 45 min 24.7%, in 60 minutes as much as 48.7%, in 24 hours as much as 0.00% and 48 hours as much as 0.00%, respectively.
Based on the statistic Anova test indicated that there was different significantly effectiveness time used lime shells which were the dead number of *A.aegypti* larvae (*P value* = 0.0005 < 0.05, CI= 95%, α = 5%).

The result was indicated table 5, that larviciding lime shells of *A.anatina* more effectively working and killing pupae after 48 hours. But abate can be kill pupae at 24 hours.

This observation of table 6 was conducted for 24 hours and 48 hours for lime shells *A. anatine* larvacide. The number of sample were 10 mosquitoes larvae for each Erlen meyer whereas conducted for abate which was 1 hour and 6 minutes. Lime shells with concentration 0.008 ppm larvae faster dead than 0.001ppm, in contrast using abate was quickly killed lower than 1 hour.

The result shown table 7, that the parameters examined water chemistry for drinking water. Therefore the water was still in normal standard but in contrast phosphate and chlorine free which were slightly higher concentration of lime shells *A.anatina*.

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DISCUSSION

Based on the result in our laboratory test shown that affectivity dose of abate and lime shells *A.anatina* that used for the larvae were 0.5 gr/25 ml water for 48 h and abate more lethal or more toxic to larvae in 15 min to 60 min time than the lime shells of *A. anatina* in 30 min to 48 h whereas the pupae with the same dose not all death because the form of the pupae can be resistant at this level concentration of abate and lime shells a high. Based on epidemiology of malaria which were three keys component of larval control by environmental management, biological control and chemical control [17].

Chemical control are used to control mosquito larvae and pupae to now in Papua or generally in Indonesia was using abate (organophosphate) or temephos that abate (temephos) can be safely and effectively used to treat temporary water or highly polluted water where were few non target organisms and / or live stock were not access allowed. The efficacy of temephos maybe up to 30 days depending on the formulation. Due to small amount needed and the fast rate that temephos breaks down in water, this type of larvicide did not pose an unreasonable health risk to humans, but at large doses it can cause nausea or dizziness but can cause mosquitoes larva resistance again it. This product was toxic to birds and fish. Fish and other aquatic organism in water tread with abate maybe killed. It did not contaminate water by cleaning of equipment or disposing of wastes [18.].

Larviciding was a general term for killing immature mosquitoes by applying agents, collectively called larvicides, to control mosquito larva and/ or pupae. Larval source management involves both the modification of water habitats, often referred to as source reduction and the direct application of larvicides to control mosquito production [18.]

In Central Java, they were using of abate as drug killer mosquito larvae and pupae less effective because it contained harmful side effects to the human body such as skin irritation and itching on the skin [12].

Abate (temephos) classified as organo-phosphates when these compounds ingested or inhaled will bind to the enzyme acetyl-cholinesterase. So that it cannot be the outlines of acetylcholine became acetyl and choline to maintain the balance between production and degradation acetylcholine which was a neurotransmitter in the autonomic nervous (parasympathetic and somatic (skeletal muscle) and its receptor. Organophosphate was hazardous pesticides because of the nature of these compounds can enter the body through the skin, inhalation and ingestion. Otherwise it may organophosphate affected acetyl-cholinesterase that there exist in erythrocytes, blood plasma and other body parts [15].

Lime shells of materials which made from *A.anatina* shells highly effective for inhibit and kill of mosquito growth of larvae instars I to IV of *Anopheles mosquitoes* and *A.aegypty* because the active ingredient contained in the lime shells *A. anatina* was Calcium. Calcium hypoclorite and peroksida. The lime shells is still life, it's have been peroksidase enzyme that could be killing *E.coli* in the fresh water [9] but not for pupae. It maybe small concentration so that pupae can survive for more than two days and die. Excess of these shells was the nature soluble and settles in water, so it can be ensured nor interfere with human health and environmentally friendly materials unlike abate (temephos) that was water soluble. At these times, larviciding has a high impact on local population number with minimal application efforts. At other times, larviciding may be less rewarding because small number of larvae and pupae are widely and unevenly distributed [5,9.]

Environmental management such as eliminating potential mosquito breeding sources for reduction in mosquito populations. There were three levels of environmental management; 1) source elimination. This strategy was generally limited to artificial habitats created by urbanization. For examples of sources elimination include emptying or turning over containers holding water and reservoir, 2) source reduction, this strategy intend to alter and sometimes eliminate available habitat for larvae and pupae which substantially reduced mosquito breeding and the need for repeatedly applying pesticides. For example of source reduction include limiting the growth of emergent vegetation in wetlands and ponds, constructing drainage ditches to remove water from areas prone to flooding, and clearing storm water channels of silt and debris, 3) source maintenance that consists of water management, vegetation management, wetland infrastructure maintenance and wetland restoration [5.]

Biological control uses predators to reduce populations of mosquitos' larvae and it was often combined with environmental management to enhance results. The mosquitoes fish that was used as *Gambusia affinish*, *Panchas-panchas* and *Tilapia mosambica* have been used to control mosquitoes in Jayapura Municipal and Jayapura District. The small fish were effective against mosquito larvae because they growth and reproduce rapidly, feed at the water surface where mosquito larvae are found, and tolerate a wide range of temperature and water quality. The small fishes were most effective in permanent ponds and wetlands, but they were also used in fields and storm water canals with permanent water as the lake water in Sentani. The same procedure also was reported from California [3,6,18.]

The control of adult mosquitoes through spraying the walls of house was with insecticide (*indoors residual spraying*) or use of insecticide treatment nets. But it should be emphasized that the vector control must be done REESAA (*rational, effective, efficient, sustainable, affective and affordable*) given geographical condition of Indonesia's vast and diverse bionomics of the vectors so that the mapping breeding places and mosquito behavior becomes very important. It was necessary for the role of local governments, all stakeholders and the public in malaria vector control. The same procedure also was reported from California [6,18.]

The result of the water was after being given treatment with lime shells *A. anatina* show that the level of phosphate and chlorine free slightly higher maybe due to the storage in long time at room temperature and officers working in the laboratory was reported [16.]

CONCLUSSION

The effectiveness time of abate and lime shells *A.anatina* which were more kill than larvae was 60 min. There was difference significant time was 15 minutes of abate and 30 minutes of lime shells *A. anatina* for kill the larvae of *Anopheles* and *A. Aegypti* mosquitoes between abate and lime shells of *A. anatina* with the same concentration in a container of drinking water, but abate can be replaced with lime shells from *A. anatina* because the lime shells was environ- mentally friendly materials and a local material that can be cultivated and made by the local community.

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