

# Effectiveness of Betel Leaves Extract (*Piper betle*, Linn) as Mosquito *Anopheles spp* Repellent

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**ABSTRACT:** Mosquito control by using natural repellent is one of alternatives to control malaria vector. Betel (*Piper betle*, Linn) which contains essential oils, alkaloids, flavonoids, saponins, tannins, eugenol, and chavicol is one of the plants that has the power of antibacterial, fungicidal, insecticidal and antioxidant. The aim of the research was to analyse the protection power of betel leaves extract in the concentrations of 20%, 40%, and 80% against *Anopheles spp*. The research method used is an experiment in the laboratory. *Anopheles spp* mosquitoes are obtained from egg hatching. Betel leaves extract concentrations of 20%, 40% and 80% were tested for resistance or protection against *Anopheles spp*. Observation of the number of mosquitoes that perch on the arm is carried out every hour starting from the 1<sup>st</sup> hour (immediately after basting) until the 6th hour. The effective protection of betel leaves extract is if the protection power is  $\geq 90\%$ . The results showed that the protection of betel leaves extract against *Anopheles spp* at a concentration of 20% was 50%, the concentration of 40% was 68.75% and the concentration of 80% was 93.75%. Effective protection power is 80% at concentration of 93.75% protection power. This is in accordance with the Pesticide Commission (1995, h.2) which states that repellent is considered effective if the protection power is  $\geq 90\%$ . People can use betel leaves extract with a minimum concentration of 80% as a repellent against mosquitoes, especially *Anopheles spp*. Other researchers can conduct similar research using extracts from other types of plants.

**KEYWORDS:** *Anopheles spp*, Betel Leaves, Concentration, Mosquito Repellent, Protection Power

## I. INTRODUCTION

Malaria is a disease that has a wide spread. Up to now, malaria is still a health problem in Indonesia. Thus, it is an actual problem that does exist in society and is decent to be inspected [1]. Malaria as an infectious disease is caused by an infection of the protozoa of the genus Plasmodium, which is transmitted from person to person through the bite of a female *Anopheles* mosquito [2]. *Anopheles* mosquitoes can breed in clean freshwater pools, dirty water, brackish water, and stagnant water on the seafront [3][4]. Based on WHO data in 2010, there were 544,470 positive malaria cases in Indonesia. In 2009, there were 1,100,000 cases of clinical malaria, and in 2010 it increased again to 1,800,000 cases of clinical malaria and had received treatment. Malaria can make people are vulnerable, can't work and leads to a big loss because they lose revenue. Fight for malaria is one of Millennium Development Goals (MDG) [5].

Various control efforts have been made such as fumigation, the use of chemical insecticides, but the results have not been satisfactory. Besides being able to cause resistance to insects, it can also provide side effects to other control environments that can be used to repel mosquitoes using anti-mosquito lotion and cream. To reduce the impact caused by the use of chemical insecticides, it is needed to make control effort which is safer and cheaper using natural materials such as the use several types of plants extract that are known to contain compounds such as alkaloids, terpenoids, steroids, and tannins which can be larvicidal and insecticidal. In addition, botanical insecticides can also be used as repellent against mosquitoes so that they can prevent mosquito bites.

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One of the botanical insecticides that can be used is betel leaves extract (*Piper Betle, Linn*) which contains 4.2% essential oil, alkaloids, flavonoids, saponins, tannins, eugenol, and chavicol. Betel (*Piper betle*) is a type of vine and rests on another tree trunk [6]. When the betel leaves extract is applied to the skin, the essential oil will seep into the pores and then evaporate into the air. The active ingredient in betel leaves extract has antibacterial, antioxidant and fungicidal properties, and is toxic to mosquitoes. Betel leaves are usually used as a mouth freshener after meal [7]. Betel leaves are rich in nutrients, antioxidant, phytochemical and in many nutraceuticals properties yet only few people who know it [8][9].

The concentration that the researchers used in this study was 20%, 40% and 80%. This is based on previous studies that tested betel leaves extract against *Aedes aegypti* mosquitoes with concentrations of 5%, 10% and 20%. From the concentration of 20% it can be used to resist *Aedes aegypti* mosquitoes with the percentage of mosquitoes that do not perch around 58% -80% [10].

The work in this paper is divided in two stages. 1) Betel Leaves Extract Making 2) Mosquito Repellent Scoring. Betel Leaves Extract Making is done step by step starting from cutting betel leaves into pieces, chemical process and end up as repellent. Afterwards, there is available the score which shows the number of mosquitos with various treatments. Paper is organized as follows. Section II describes related work containing theoretical review about betel leaves, *Anopheles sp* mosquito and extraction to resist *Anopheles*. Respectively, the scheme to make extraction is shown in Section III. Section IV provides experimental results showing results of repellent test. Finally, Section V presents conclusion.

## II. RELATED WORK

Betel leaves that have been known since 600 BC contain antiseptic substances that can kill bacteria so that it is widely used as an antibacterial and antifungal agent. Some literature mentions that betel leaf which is the main ingredient of this betel has styptic properties (resist bleeding), vulnerary (heals skin wounds), stomachic (digestive tract medicine), strengthens teeth, and cleanses the throat. There are also those who say that the betel leaf besides having the ability of antiseptic, have the strength as an antioxidant and fungicide. Betel leaf extract contains substances that can function as *larvasida* and *pupasida* of *Aedes aegypti* mosquito.

Mosquitoes are included in [11] subfamily Culicinae, Family Culicidae (Nematocera: Diptera), is the main vector or contagious agent of arbovirus. *Anopheles* mosquito activity is strongly influenced by air humidity and temperature. Basically, *Anopheles* mosquitoes require a temperature between 21°C-32°C, but the optimum temperature is 28°C for its growth while the optimum humidity for mosquito breeding is above 60%. Female *Anopheles* mosquitoes bite humans or animals for the growth of their eggs. Generally, *Anopheles* actively forages at night usually starts to bite in the evening until early morning. The flight distance of *Anopheles* mosquitoes is usually 0.5-3 km, but can reach tens of kilometres because it is influenced by transportation and fast winds. The age of *Anopheles* mosquitoes is around 1-2 weeks outside but, in a laboratory, they can reach 3-5 weeks [12]. *Anopheles* female mosquitoes have an average age of 25.6 days, according to the results of Horsfall's (1955) study that *An. aconitus* female mosquitoes can reach ages 8-41 days with an average of 24 days. Male and female mosquitoes can survive around 25 days, of which 50% of male mosquitoes live more than 13 days and female mosquitoes more than 12 days [4]. The behaviour of *Anopheles* mosquitoes in their breeding always requires three types of places, namely breeding places, places to look for blood (feeding places) and places to rest (resting places) [13]. Environmental control is the best way to control arthropods because the result is permanent [14].

Betel leaves contain essential oils where the main component consists of phenols and their derivatives such as chavicol which has a powerful antiseptic properties [15], chavibetol, carvacrol, eugenol, and Alli pyrocatechol. In addition to essential oils, betel leaf also contains carotene, thiamine, riboflavin, nicotinic acid, vitamin C, tannins, sugar, starch and amino acids. The use of betel leaves for medicine is caused by the essential oils they contain. In this case Prof.J.F.Eykman, a chemist during the Dutch colonial period separated the essential oils from betel leaves. The effort was carried out in the Bogor Botanical Gardens, Indonesia in 1885. After being separated it turned out that one third of the essential oil consisted of phenol and most of Chavicol. It is this chavicol that gives a distinctive smell of betel leaves and has the ability to kill bacteria five times that of ordinary phenols and can repel / expel various insects.

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Our theoretical framework is shown as Figure 1. as follows

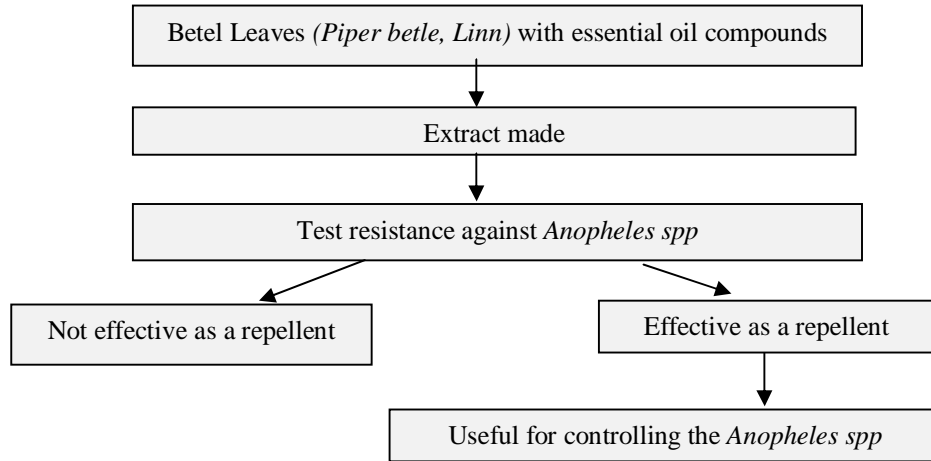


Fig 1. System Model of Betel Leaves Extract

Figure 1 shows the theoretical framework of this research. The betel leaves are mixed with essential oil and through the process of drying and chemical test until the extract is obtained. After all the processes are done, then the test resistance against *Anopheles spp* is executed. In the test itself, there is in-depth analysis to take the conclusion whether the test resistance is effective as a repellent or not. The effective result is then used for controlling the *Anopheles spp*.

### III. METHODOLOGY

The methodology in this research was involving some components such as variables, relation among variables as well as operational definition. Not to mention, we also used instruments to help operational procedure in preparing to finishing the betel leaves extraction. Firstly, the variables and their relation structure are expressed in Figure 2. below

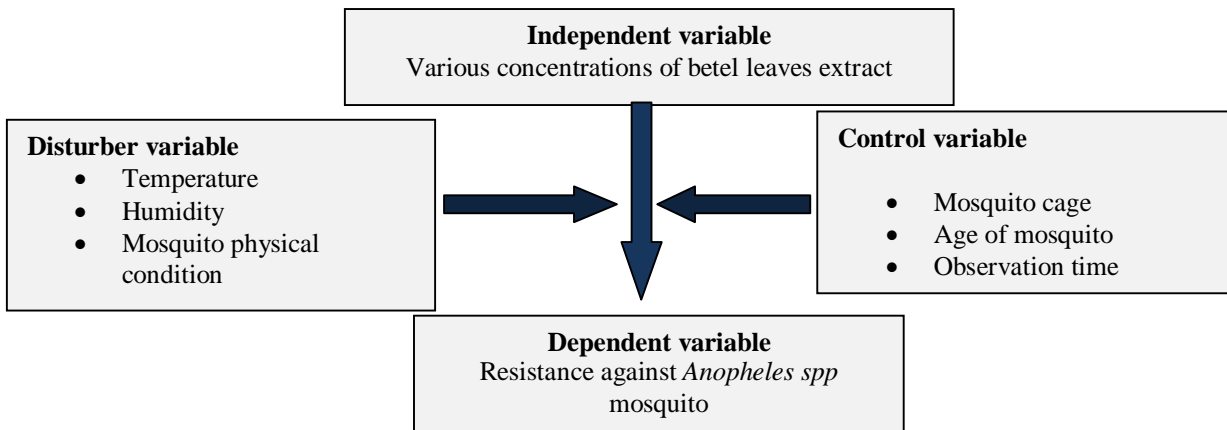


Fig. 2. Structure relation among variable

Based on the Figure 2, the independent variable is the concentration of betel leaves extract which is divided into 20%, 40% and 80%. While the disturber variable is using three items namely temperature, humidity as well as the mosquito

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physical condition. In the same time, it is also used the control variable that is affecting dependent variable but not being a priority yet the portion is equalized. The control variable in this research is mosquito cage, age of mosquito and also observation time. In the end, the dependent variable that the value is prone to change then observed. The dependent variable is the resistance against *Anopheles spp.* While the operational definition done in this research is shown by Table 2. as follows

Table 1. Operational definition

No.	Variable	Operational Definition	Measuring Instrument	Measuring Method	Unit	Scale
1	Betel leave concentration	Percentage of betel leave extraction in 20%, 40%, 80%	Measuring cylinder	Dissolving betel leaves extract into water with various concentrations	ml	Ratio
2	Mosquito cage	Place used to put the mosquito that would be observed in the experiment.	Ruler	Measuring by using ruler	cm <sup>3</sup>	Ratio
3	Age of mosquito	How long <i>Anopheles</i> lives during the research	Manual calculation	Calculate the age from hatching until it's ready to bite	Day	Ratio
4	Observation time	Time of conducting the research	Watch	Observe directly by keeping eyes on the watch	Minute	Ratio
5	Mosquito physical condition	The state of the body and the body's resistance to mosquito used during the research period	Vision sense	Observing the mosquito condition directly	Bad / Good	Nominal
6	Temperature	The size of the cold heat of the research site measured by units of degrees (°C)	Indoor thermometer	Observe the device and measure the room temperature directly	°C	Interval
7	Humidity	Measure the level of water content in the research air measured by the hygrometer in %	Hygrometer	Observe the device and measure the room humidity directly	Percentage (%)	Ratio
8	Resistance	Extract concentration to resist mosquito	Visual and calculation formula	Expose the hand that has been smeared with extract, then count the number of mosquitoes that perch / bite	%	Ratio

The operational definition is hints for the researchers on how to measure a variable. It is informed about each variable definition of term as well as the instrument used for measuring. In the operational definition is also informed about the unit and scale of the variable. Not to mention, in the operational definition is also explain the measuring method which shows each instrument function in the research. This is intended to help the researchers obtaining the result.

Talking about the methodology, in this research, the sample taken was female *Anopheles sp.* Every treatment (concentration) needed 25 mosquitos in 3 concentrations (20%, 40%, 80%) and 1 control. Each concentration is replicated 3 times and each replication uses new mosquitoes. The total number of mosquitoes used were 300. The research was done in Laboratory of EntomologiLokaLitbang P2B2 Ciamis, West Java, Indonesia.

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The steps were done in extracting the betel leaves are as follows:

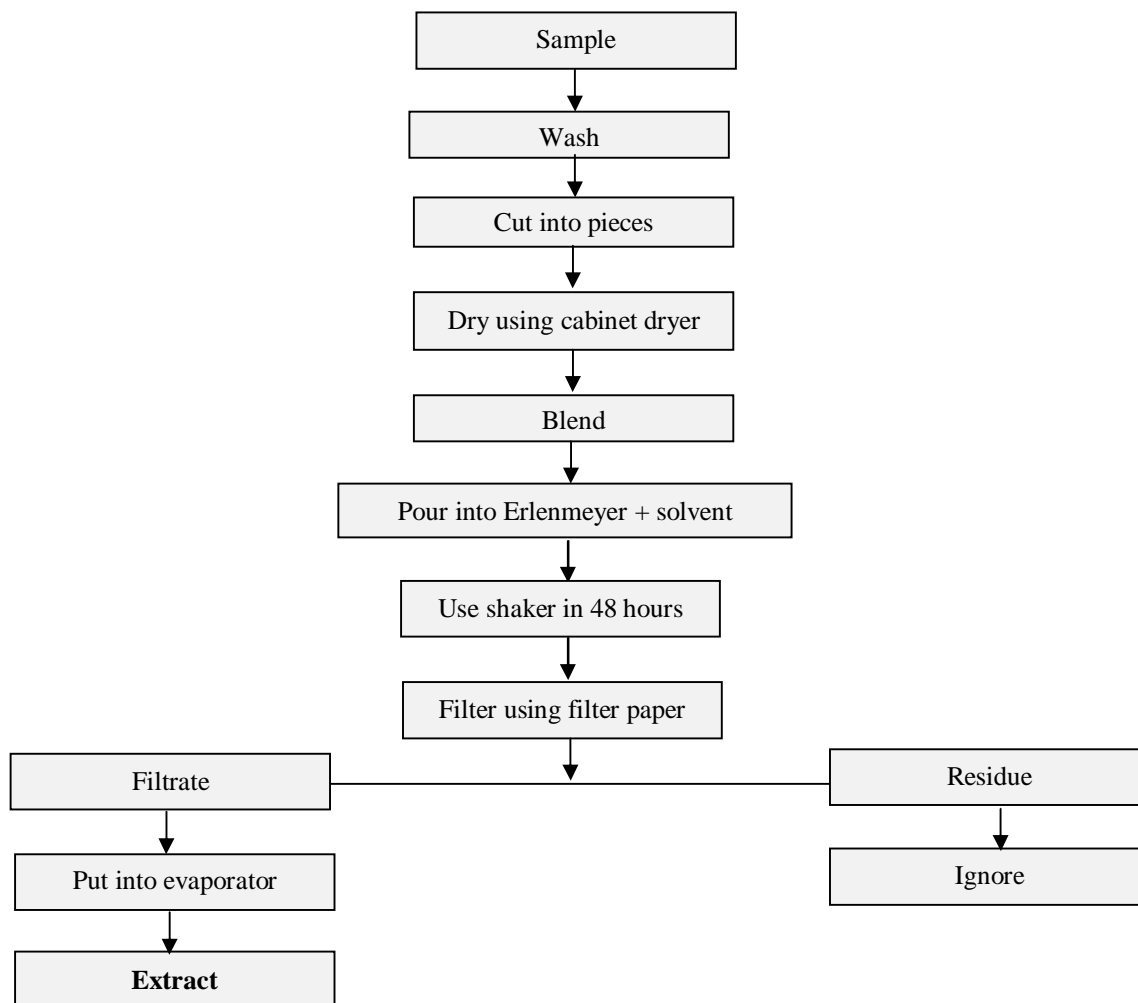


Fig. 3Steps in making betel leaves extract

The making of extract is by way of drying the sample (betel leaves extract) using cabinet dryer or can also be done under the sun light. The dried sample is then processed in the blender. After that, it is put into Erlenmeyer and add the solvent with the comparison of 1 : 4 which is 1 is for sample and 4 is for solvent. Then, cover it with the aluminium foil paper to protect it from light. Use shaker for 48 hours so that the compound is dissolved in methanol or another solvent. Next, filter it and put the filtrate into evaporator and set the temperature to 40°. Let the sample and residue separate.

## IV. EXPERIMENTAL RESULTS

The mosquito of *Anopheles spp* that were used for this research are from the egg hatching. The results of measurement of temperature and humidity in the research location are shown as follows:

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Table 2. Measurement of temperature and humidity at location

No.	Parameter	Result of Measurement
1.	Minimum temperature	27°C
2.	Maximum temperature	29°C
3.	Average temperature	28°C
4.	Average humidity	82 %

According to Table 3 it is known that the air temperature at the research site is quite hot. This is because the location of the laboratory is near Pangandaran beach. The average air temperature of 28°C that occurs during the research is the optimal temperature for the growth of *Anopheles spp* whose habitat is near the coast. Optimal air temperature conditions at the study site strongly supported mosquito activity during the research. This means that the air temperature at the research site is not a limiting factor that is detrimental to the growth of *Anopheles spp*, but as a positive supporting factor. Based on the trials had been done, the repellent results towards *Anopheles spp* are shown below:

Table 3. Number of mosquitos perch/bite on repellent test at 1<sup>st</sup> replication

Concentration (%)	Number of mosquitos	Mosquito perch/bite on 5 minutes at <i>x</i> hour						Average number of mosquitos
		1	2	3	4	5	6	
0 % (C)	25	15	16	16	16	17	16	16
20 %	25	7	7	7	7	8	9	7
40 %	25	4	4	5	5	5	5	4.6
80 %	25	1	1	1	1	1	1	0.5

Table 4 shows the number of mosquitos that perch/bite on repellent test at first replication. In the concentration of 20%, average number of mosquitos is 7, while in the concentration of 40%, the average number of mosquitos is 4.6 while in the concentration of 80%, it shows 0.5 average number of mosquitos.

Table 4. Number of mosquitos perch/bite on repellent test at 2<sup>nd</sup> replication

Concentration (%)	Number of mosquitos	Mosquito perch/bite on 5 minutes at <i>x</i> hour						Average number of mosquitos
		1	2	3	4	5	6	
0 % (C)	25	15	15	15	16	16	16	15.5
20 %	25	8	8	9	9	9	9	8.6
40 %	25	5	5	5	6	5	4	5
80 %	25	0	1	1	1	1	1	0.8

Table 5 shows the number of mosquitos that perch/bite on repellent test at the second replication. In the concentration of 20%, average number of mosquitos is 8.6, more than that in Table 4. While in the concentration of 40%, the average number of mosquitos is 5 while in the concentration of 80%, it shows 0.8 average number of mosquitos.

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Table 5. Number of mosquitos perch/bite on repellent test at 3rd replication

Concentration (%)	Number of mosquitos	Mosquito perch/bite on 5 minutes at x hour						Average number of mosquitos
		1	2	3	4	5	6	
0 % (C)	25	17	17	18	17	18	18	17.5
20 %	25	7	8	8	8	8	9	8
40 %	25	4	4	5	4	4	5	4.3
80 %	25	0	1	1	1	1	2	1

In table 6, the concentration of 20% obtained the average number of mosquitos in as much as 8. While in the concentration of 40%, it shows 4.3 average number of mosquitos, lesser than that in Table 5. Lastly, in 80% of concentration, the average number of mosquitos is 1. Based on Table 4,5 and 6, it is obtained the average number of mosquito on the repellent test using betel leaves extract :

Table 6. Average of mosquitos perch/bite on repellent test

Concentration (%)	Number of mosquito	Average of mosquitos perch/bite on the 5 minutes at 1 <sup>st</sup> to 6 <sup>th</sup> hour			Average number of mosquito
		Replication I	Replication II	Replication III	
0 % (C)	25	16	15.5	17.5	16
20 %	25	7	8.6	8	8
40 %	25	4.6	5	4.3	5
80 %	25	1	0.8	1	1

Based on table 7 it is known that on average the mosquitoes perched at a concentration of 0% (as a control) were 16, the concentration of 20% as many as 8, the concentration of 40% as many as 5 and the concentration of 80% as many as 1. The higher the concentration of betel leaves extract, the fewer *Anopheles* spp mosquitoes that perch or bite. This is because the higher the concentration of the extract means the higher the content of the secondary metabolites, resulting in the fewer *Anopheles* spp mosquitoes perching or biting hands. The protective power of *Anopheles* spp from each betel leaf extract concentration can be calculated. Calculation of protection power uses the formula as follows:

$$\text{Protection Power} = \frac{K - R}{K} \times 100 \%$$

Remarks :

R = perched mosquitos in treatment

K = perched mosquitos in control

From the calculation using formula above it is obtained the numbers as follows:

Table 7. Protection power of betel leaves extract towards *Anopheles* spp

Concentration (%)	Number of tested mosquitos	Average number of mosquitos perch/bite	Protection power (%)
0 % (C)	25	16	-
20 %	25	8	50
40 %	25	5	68,75
80 %	25	1	93,75



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It can be concluded that the higher the concentration level of fragrant lemongrass extract, the higher the protection against *Anopheles* spp. Based on Table 8 it is also known that the effective protection of betel leaves extract against *Anopheles* spp mosquitoes is 80% with 93.75% protection power. This is in accordance with the Ministry of Agriculture's Pesticide Commission (1995, h.2) that repellent is considered effective if the protection power is  $\geq 90\%$ .

## V. CONCLUSION

We have done doing the research on effectiveness of betel leaves extract as *Anopheles* spp repellent. From all the results, we can take conclusion that the protection of betel leaves extract against *Anopheles* spp at a concentration of 20% is got 50%, concentration of 40% is got 68.75% and concentration of 80% is got 93.75%. In addition, there are differences in the protection of betel leaves extract against *Anopheles* spp mosquitoes at concentrations of 20%, 40%, and 80%. Lastly, the most effective protection of betel leaves extract against *Anopheles* spp mosquito is 80% concentration that is resulting 93.75% protection power. This is in accordance with the Ministry of Agriculture's Pesticide Commission (1995, h.2) which states that repellent is considered effective if the protection power is  $\geq 90\%$ .

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## BIOGRAPHY



**Khomsatun** was born in Pati, Indonesia, in 1962. He received Bachelor of Education degree from UM Purwokerto, Indonesia, in 1998, and the Master of Health (M.Kes) from Diponegoro University, Semarang, Indonesia in 2013. He has joined Department of Environmental Health in Health Polytechnic of Health Ministry, Semarang as a Lecturer since 2007. He teaches the lecture of profession ethic, people empowerment, sanitation clinic, housing sanitation and also people health counseling. His current research interests include river pollution, Larva Destroyer with electrical voltage to control *Aedes Aegypti* mosquitos as well as environmental sanitation in scabies disease that was done in 2007, 2008, 2010 respectively. He was the speaker in General Stadium seminar 2007 conducted in Health Polytechnic of Health Ministry Semarang. Besides, he was the General Chair of Writing for Publication Workshop in 2009 in Health Polytechnic of Health Ministry Semarang and now he serves as a lecturer in Environmental Health Study Program in Health Polytechnic of Health Ministry Semarang.



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**Gustomo Yamistada** was born in Bengkulu, Indonesia, in 1976. He received the Bachelor of Education degree in FKIP Jambi University Jambi, Indonesia in 2003, and M.Sc (Master of Science) degree in Health Environment from Gadjah Mada University, Yogyakarta, Indonesia, in 2009. His current interests research is about the topics in Parasitology, Microbiology dan Environment. The author has experiences in compiling AMDAL and UKL UPL documents for various environmental management efforts such as Environmental Documents for hospitals, environmental management documents for coal mining businesses and electricity generation (PT. PLN) from 2005 until now. The author also conducted research, several among them are in the field of environment health, Research on Effectiveness of Use of Dual Filter Media in Reducing Iron and Manganese Content in Water in 2011, Research on Difference in Washing Techniques in Reducing Germ Numbers in Cutlery in 2012. Research on Factors Associated with Lung Disorders in Wood Craftsmen in Jambi City in 2013. Research Study of Hygiene of Operators and Sanitation on Bacteriological Quality of Drinking Water Refill in Jambi City in 2015. Research on Individual Hygiene Overview and Worm Egg Contamination in Nail Impurities of Primary School Students in the Seberang Region of Jambi City in 2016. Research on Individual Hygiene Overview and Contamination of Worm Eggs on Nails Cleaning Officers of the Environmental Office of Jambi City in 2017. Research on Individual Hygiene Overview and Worm Egg Contamination at Waste Transport Officers at the Public Works Agency and Jambi City Spatial Planning in 2018. Currently in 2019 the author serves as the Chair of the Diploma III Environmental Health Study Program of the Health Polytechnic of Health Ministry of Jambi.