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AN EFFECTIVENESS TEST ANALYSIS OF SEA MANGO SEEDS EXTRACT (CERBERA MANGHAS) ANDPAPAYA LEAVES EXTRACT (CARICA PAPAYA)IN CONTROLLING THE VECTOR OF AEDES AEGYPTY MOSQUITOS

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ABSTRACT

The aim of the research is to determine the effectiveness of sea mango *(CerberraManghas)* seeds extract and Papaya (*Carica papaya)* leaves extract as vegetal insecticide in controlling the vector of *Aedesaegypti*mosquitos. The experiment was done in the Entomology Laboratory of Health Research and Development Center of Disease Vector and Reservoir (B2P2VRP) Salatiga with 600 adult *Aiedesaegypti*mosquitos being 3-5 years old with 3 times repetitions. The research design is experimental, using the design of the posttest only controlled group design. The data analysis used was computer (SPSS software) with the probit analysis of one way ANOVA and Least Significance Difference (LSD). The value of LC50 sea mango seeds extract obtained was 1.995% (19.950 ppm) and LC95 of 23.659 (236.590 ppm), while that of the papaya leaves was 3.168% (31.680 ppm) in 100ml of distilled water and 122.202% (1222.020 ppm) in 100 ml distilled water respectively. The one way ANOVA of the sea mango seeds extract showed that it had a significant value of 0.001 ($p < 0.05$). The result of the LSD showed that there were two groups having significant differences namely the group which concentration was 2.5% with 10% and 2.5% with 20%. There was a significant difference between each concentration of sea mango seeds extract toward the death of *Aedesaegypti*mosquitos.

KEYWORDS: Sea Mango Seeds, Papaya Leaves, *Aedesaegypti*, Effectivity.

INTRODUCTION

WHO (*Word Health Organization*) 2012, reports that Asia-Pacific area have the death rate due to dengue fever (DBD) of 75% compared to the other areas. For the last five decades, the case of dengue fever have increased up to 30 times (Murray *et al*., 2013).

Dengue fever is a quite popular disease in Indonesia. The incidence rate per 100,000 residents is 50.75, while the number of regencies infected by dengue fever in 2015 is 86.7% from the total regencies in Indonesia (Indonesian (Nene *et al*., 2007). *Aedesaegypti* is the main vector of arbovirus diseases, particularly dengue fever.

In several part of the world, it has been reported that *Aedesaegypti*has been resistant toward several classes of insecticide. In 2001, it was reported in Brazil that *Aedesaegypti*had been resistant toward Temephos (Braga *et al*., 2004). The same thing happens in Thailand, Ponlawat*et al*., (2005) reported that *Aedesaegypti*have been resistant toward Permethrin and Temephos, but they are still susceptible toward Malathion. Brengues*et al.*, (2003) report that *Aedesaegypti*from Semarang has been resistant 296 times toward Permethrin. Resistance cases of *Aedesaegypti* toward Pyrethroid and the resistance mechanism occurred are also reported by Ahmad *et al.* (2007) stating that *Aedesaegypti*from Bandung has been resistant 79.3 times toward Permethrin and that from Palembang have been resistant 23.7 times toward Deltamethrin.

A concern that *Aedesaegypti*in Indonesia has been resistant toward Malathion and Temephos is a quite reasonable thing because those two insecticides have been used in many places in Indonesia for more than 32 years. The insects' resistance toward insecticide, regardless its type will arise after 2-20 years of continuous usage (Georghio, 1983). The usage of insecticide can act as a natural population selection agent that will make insects resistant gene that will stay alive and will be passed to the next generation. Consequently, the percentage of resistant insects will keep increasing, while the susceptible insects will be eliminated because of the insecticide. In the end, the usage of insecticide will be ineffective because the number of resistant insect is much greater than the susceptible ones.

Vegetal pesticide contains not only single active ingredient, but multiple active ingredient. The result of the research shows that several types of vegetal pesticide are quite effective toward several types of pest, whether they are in a field, in a household (mosquito and fly), or in a warehouse (Kardinan and Iskandar 1999a, 1999c).

Sea mango plants *(Cerberamanghas)* is known poisonous because its seeds contain cerberin that can obstruct the calcium ion channel inside the cardiovascular muscle, so that it may result in death (Rohimatun, 2011). The chemical substances are as follows: steroid, triterpenoid, saponin, and alkaloid. Alkaloid consists of cerberin (0.6%), cerebroside, neerifolin, and thevatin. The alkaloid compound has toxic, repellent, and antifeedant properties on insects, while saponin has the property like dissolved soap in the water leading to the decline of digestive enzymes and the inhibit of food absorption (Utami, 2010).

Papaya (*Carica papaya*) is one of the most important sources of new chemical compounds that can be made as medicine or as model compound to obtain new active compound. Papaya leaves contain alkaloid carpaine, caricaxanthin, violaxanthin, papain, saponin, flavonoid, and tannin (Soranta, 2009). The alkaloid compound can be made as a vegetal insecticide compound. Alkaloid contained in papaya leaves also has been examined and proven to be able to inhibit the growth of myeloma cells in mice. Papaya leaves can also be used as natural insecticide toward the growth controlling effort of *Sitophiluszeamais*, namely warehouse pest / rice weevil (Setiawati, 2009).

So far, the influence of sea mango seeds (*Cerberamanghas*.) and papaya leaves (*Carica papaya*) extracted with ethanol solvent for adult *Aedesaegypti* mosquitoes has not been conducted. Therefore, it is necessary to conduct further research on the toxicity of sea mango seeds and papaya leaves extracted with ethanol toward *Aedesaegypti* mosquitoes on a laboratory scale.

MATERIALS AND METHODS

The type of this research is the post-test only controlled group design with 2 controls. This research used concentration of sea mango seeds (*Cerberamanghas*) and papaya leaves (*Carica papaya*) in 4 levels (2.5%, 5%, 10% and 20%). Preliminary tests were conducted with 6 treatments and 2 controls in 3 repetitions. Further test were conducted with 8 treatments and 2 controls in 3 repetitions. The subjects used in this research were 3-5 days old female/male adult mosquitoes which live and actively move obtained from the Entomology Laboratory of Health Research and Development Center of Disease Vector and Reservoir (B2P2VRP) in Salatiga, Central Java. The research method of insecticides was done by spraying the extracts of sea mango seeds (*Cerberamanghas*) and papaya leaves (*Carica papaya*) toward *Aedesaegypti* mosquitoes.

Tools and materials

Tools

The tools used in this study were square Glass Chamber in size of 70x70x70 cm^3, stopwatch, measuring cylinder, sprayer (mini compressor with a particle size of 0.3 MM), aspirator, pipette, indoor thermometer, and hygrometer.

Material

The materials used in this study were the extracts of sea mango seeds (*Cerberamanghas*) and papaya leaves (*Carica papaya*) in 4 levels (2.5%, 5%, 10% and 20%), the solvent ingredient namely ethanol 96%, sterile aqua des, adult *Aedesaegypti* mosquitoes which were alive and actively moving.

Mechanism

a. Pre-test

Glass chamber was cleaned in order not to be contaminated by other insecticides, by: (1) the inside of the glass chamber was washed thoroughly with a wet rag containing detergent liquid, (2) glass chamber was rinsed with wet rag without detergent, and (3) glass chamber was dried with wet rag without detergent then it was stretched for one hour.

b. Preliminary test

1) 20 *Aedesaegypti* mosquitoes were released in the glass chamber and the room temperature of the glass chamber was recorded.
2) The extract of sea mango seeds (*Cerberamanghas*) and papaya leaves (*Carica papaya*) were sprayed at a determined concentration in which the amount of spray was suitable with the application result of spray levels (Y times of spray). The control was sprayed with the solvent (aqua des) as much as Y times of spray.

1. The sprayer which had been filled with the extracts of sea mango seeds (*Cerberamanghas*) and papaya leaves (*Carica papaya*) are weighed, for example is A gram.
2. Sprayed the sprayer maximally 10 times.
3. The sprayer and the extracts of sea mango seeds (*Cerberamanghas*) and papaya leaves (*Carica papaya)* were re-weighed (B gram).
4. The spraying was conducted and repeated 3 times (C gram and D gram), then the weight difference for each repetition was averaged.

$$\frac{(B.I.A - B.P1) + (B.P2) + (B.P3)}{3\ Repetitions\ X\ 10\ Sprays}$$

Where
B.I.A = Initial Insecticide Weight
B.P1 = Insecticide Weight of 1st Spraying
B.P2 = Insecticide Weight of 2nd Spraying
B.P3 = Insecticide Weight of 3rd Spraying

It was observed for 20 minutes, the number of fainting or dying mosquitoes every specified time period were counted and recorded.

1) All the mosquitoes were transferred with an aspirator into a paper cup and stored in a holding for 24 hours.
2) The mortality rate of the mosquitoes was calculated after 24 hours. The calculation results were inserted in the table.
3) If the mortality rate of the mosquitoes in the negative control group was less than 5%, it would be ignored, but if the mortality rate of the mosquitoes in the negative control group was more than 20%, the test must be repeated. Whereas, if the mortality rate of the mosquitoes in the negative control group was 5-20%, so the *Abot* formula was conducted to calculate the percentage of mosquitoes' mortality rate in each concentration as follows:

$$A1 \frac{(A - C)}{(100 - C)} X\ 100$$

Where
A1 = Paralysis rate / the mortality after correction
A = Paralysis rate / the mortality in treatment
C = Paralysis rate / the mortality in control
c. After getting the proper concentration from the preliminary test, it can be continued for further testing in the same way.

RESULT

The effect of insecticides on the mortality of mosquitoes was determined by the 24 hours of mortality rate after contacting to the walls sprayed with insecticides based on the standard testing carried out by World Health Organization (WHO, 2006).

Table 1: Knockdown (fainted) of *Aedesaegypti* mosquito in the treatment of Bintaro Fruit Seed extract and Papaya Leaves.

Ingredient	Concentration (%)	Total	*Knockdown* (Minute)						Mortality (Hour)		
			1	3	8	10	15	20	24	Average	%
Sea mango seeds	2,5	60	0	0	0	0	0	0	35	11,67	58,33
	5	60	0	0	0	0	0	0	42	14,00	70,00
	10	60	0	0	0	0	0	0	51	17,00	85,00
	20	60	0	0	0	0	0	0	57	19,00	95,00
Papaya leaves	2,5	60	0	0	0	0	0	0	27	9,00	45,00
	5	60	0	0	0	0	0	0	36	12,00	60,00
	10	60	0	0	0	0	0	0	41	13,67	68,33
	20	60	0	0	0	0	0	0	48	16,00	80,00
Control (-)		60	0	0	0	0	0	0	0	0,00	0,00
Control (+)		60	40	60	60	60	60	60	60	20,00	100

The test results in table 1 showed that sea mango seeds seed extract spray, papaya leaves, and on negative control (distilled water) the number of mosquitoes that knockdown from minute 1 to minute 20 were not found.

Table. 2 Mortality of mosquito *Aedesaegypti* in the extract treatment of Sea Mango Seeds and Papaya Leaves.

Material	Concentration (%)	total	Number of dead mosquitoes in replication						Mortality (hour)		
			1	%	2	%	3	%	24	Average	%
Sea Mango Seeds	2.5	20	10	50	12	60	13	65	35	11.67	58.33
	5	20	13	65	14	70	15	75	42	14,00	70.00
	10	20	17	85	16	80	18	90	51	17.00	85.00
	20	20	20	100	17	85	20	100	57	19.00	95.00
Papaya leaves	2.5	20	7	35	9	45	11	55	27	9.00	45.00
	5	20	7	35	15	75	14	70	36	12.00	60.00
	10	20	9	45	17	85	15	75	41	13.67	68.33
	20	20	17	85	16	80	15	75	48	16.00	80.00
Control (-)		20	0	0	0	0	0	0	0	0,00	0.00
Control (+)		20	20	100	20	100	20	100	60	20.00	100

In every increase of concentration (2.5%, 5%, 10% and 20%), it would be followed by an increase of average mortality from both extracts. The death of mosquitoes on the negative control treatment did not occur, whereas in the positive control the death of *Aedesaegypti* mosquitos was always 100%.

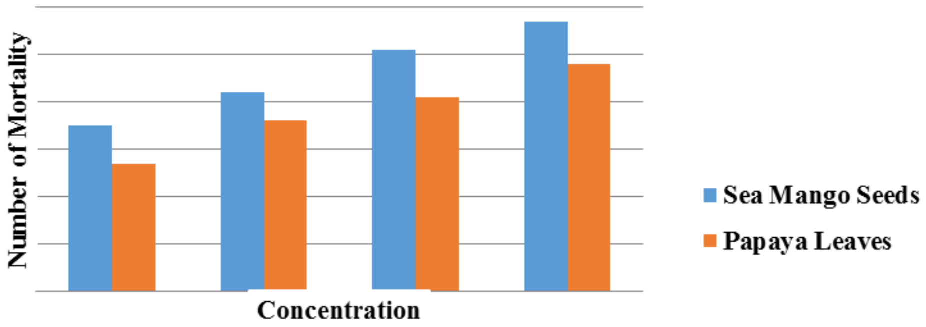

Figure 1. The mortality of mosquito toward both Sea Mango seeds and Papaya Leaf

The extract with different concentrations would have a different effect on the death of the mosquitoes. The higher concentration of the extract was, the higher number of death on the mosquito would be. The data was presented in Figure 1.

Table 3. *Lethal Concentration* and *Confidence Limits* value of Sea Mango seeds Extract against *Aedesaegypti* After 24 Hours.

LC	Concentration	95% Confidence Limits for Concentration	
		Lower	Upper
LC50	1.995	. 953	2.906
LC90	13.701	9.646	26.759
LC95	23.659	14.784	63.152

The obtained *Lethal Concentration* value in Sea Mango seed LC50 was *1.995%* (19.950 ppm) in 100 ml of distilled water, LC90 13.701% (137.010 ppm) in 100 ml of distilled water and LC95 23 659% (236, 590 ppm) in 100 ml of distilled water.

Table 4: Lethal Concentration and Confidence Limits value of Papaya Leaf Extract against Aedesaegypti After 24 Hours.

LC	Concentration	95% Confidence Limits For Concentration	
		Lower	Upper
LC_{50}	3.168	1.356	4.733
LC_{90}	54.540	25.680	420.312
LC_{95}	122.202	45.010	1969.337

The result of the analysis probit LC_{50} and LC_{95} showed that the mortality could reach 50%, 90%, and 95% in *Aedesaegypti* mosquitos which was on the concentration of 3.168% (31.680 ppm) in distilled water, 54.540% (545.400 ppm) in 100 ml distilled water, and 122.202% (1222.020 ppm) in 100 ml distilled water at the 24^{th} hour after the treatment.

The measurement of room temperature at the beginning and the end of the research on average was 27^{0}C. The air humidity at the beginning and the end of research showed the average number of 80%.

Table 5: Test Result *One way* ANOVA Sea Mango Seeds and Papaya Leaves.

Extract		Sum of Squares	df	Mean Square	F	Sig.
Sea Mango Seeds	Between Groups	94.250	3	31.417	17.136	.001
	Within Groups	14.667	8	1.833		
	Total	108.917	11			
Papaya Leaves	Between Groups	78.000	3	26.000	2.516	.132
	Within Groups	82.667	8	10.333		
	Total	160.667	11			

Based on the *one way* ANOVA test result on sea mango seeds, it was discovered that the existence of meaningful differences. Therefore, Least Significance Difference (LSD) Test was done. Based on the result of the LSD test, it could be found that the two groups had significant difference namely between the group with the concentration of 2.5% and 10% and the group with concentration of 2.5% and 20%. On the other groups between 2.5% and 5%, the difference was not significant because the value of $p > 0.05$.

DISCUSSION

The biological activity of the natural ingredients could influence the behavior of insect like feeding inhibitor, feeding refusal, or spawn activity inhibitor. Besides that, it also could influence the physiological activity of insects such as the inhibitor of growth, ovicidal, larvicidal, or death.

The effect of insecticide toward the death of *Aedesaegypti*mosquitos was determined by the mortality rate of 24 hours after the exposure in accordance with the standardized test done by World Health Organization (WHO 2006).

The temperature and humidity were important factors in the survival of mosquitos. According to the research (Simoy, *et al*, 2015) studying about the temperature condition toward population dynamics of *Aedesaegypti*in every growth phase, adult mosquitos phase could live at the temperature between 11^0C and 35^0C. Under the temperature of 11^0C, the activity of mosquitos would be lowered. If it was over 35^0C, the lifetime of mosquitos would be shorter. While the optimal humidity required for the growth of mosquitos was between 60% and 80%. The temperature and humidity in the research was still in the optimum condition which supported the survival of mosquitos, so the occurrence of mosquitos' death was caused by the existence of insecticide exposure toward the mosquitos.

The concentration enhancement of sea mango seeds extract and papaya leaves caused the increase of death on the 1^{st} hour until the 24^{th} hours. The contents of the secondary metabolite compound existing in sea mango seeds extract and papaya leaves could cause the increase of the number of mosquitos' death in 24 hours observation.

Based on the result of the study, sea mango seed extract and papaya leaves have different level of insecticide activity. Sea mango seed extract has more potential to kill mosquitoes compared to papaya leaves extract. The different ability in insecticide activity is probably due to the difference of the concentration of metabolic compound as well as the presence of other compounds contained in sea mango seeds causing the death of mosquitoes.

Sea mango also contained steroids (Kuddus, *et al.*, 2011). The ripe and fresh core of sea mango contain cerberin alkaloids, a substance that tastes bitter and poisonous. Sea mango bark also contains alkaloid compounds that function as antifungal (Singh, et al., 2012).

In the sea mango seeds there are 6 new types of cardenolide glycoside that had been isolated namely, 3β-O-(2'-O-acetyl-α-L-thevetosyl)-14β-hydroxy-7-en-5β-card-20(22)-enolide, (7,8-dehydrocereberin), 17β-neriifolin, deasetiltahnginin, tanghinin, cerebrin, and 2'-O-acetyl-cerleaside. Among those six substances, cerberin had the cardioxity potential (Chinpracha, et al., 2004).

Cerberine was a monoacetyneriifolin compound (Gaillard, et al, 2004; Chang, et a., 2000). Cerberin also had toxic nature so that it could cause anorexia in larvae (Murray, et al., 2013; Dono, et al., 2008). Several substances were also found in sea mango namely alkaloids, tannins and saponins having antibacterial, cytotoxic nature and as central nervous system depressants because of the presence of two alkaloids and saponins (Ahmed, et al., 2008).

The data from the research result showed that the ethanol extract of sea mango seeds (*Cerberamanghas*) and papaya leaves (*Carica papaya*) had an insecticidal effect that could kill *Aedesaegypti* mosquitoes. The higher the concentration of ethanol extract of sea mango seeds and papaya leaves were, the higher the presentation of mosquito death would be.

The effect was caused by the components of the active compounds contained in papaya leaves, namely alkaloids, saponin, flavonoids, and the enzyme papain. The alkaloid compounds found in papaya leaves were carpaine alkaloids. Alkaloid compounds worked by inhibiting the activity of the enzyme acetylcholinesterase which affected the transmission of nerve impulses, causing the enzyme to undergo phosphorylation and became inactive. This would result in the inhibition of the degradation process of acetylcholine so that acetylcholine accumulation occurred in the synaptic cleft. This condition caused transmission problems that could lead to impaired muscle coordination, convulsions, respiratory failure and death (Chidozie, et al., 2014).

The Papain enzyme was a proteolytic enzyme which played a role in breaking down connective tissue, and had a high capacity to hydrolyze exoskeleton proteins by breaking peptide bonds in proteins so that the protein would be broken off (Junkum et al., 2004).

Carica papaya contained bioactive compounds namely alkaloids, saponin, phenolic, flavonoid, and tannin (Baskaran, et al., 2012). Papaya leaves also had proteolytic activity because of the content of the enzyme papain they have. Alkaloids, terpenoids, phenolic, flavonoid and tannin had the ability to inhibit microbes through various mechanisms. Based on the research conducted by Vuong, et al., 2013, it was shown that saponin was a bioactive compound mostly contained in papaya leaves extract.

Other compounds such as tannin and steroid became the synergistic compounds in larvicidal activity. Tannin was known to inhibit bacterial growth by damaging bacterial cells (Lim 2006). Secondary metabolite compound in the alkaloid group found in sea mango seed extract was cerberin. Cerberin was a toxic and repellent substance, and it causes anorexia in larvae (Dono, et al., 2010).

Flavonoid acts as antimicrobial and antifungal, and it has cytotoxic effects on larvae (Samantha, et al., 2011; Andersen and Markham, 2006). Saponin as detergent-like materials had the ability to damage cell membranes (Turk, 2006).

Saponin could inhibit the action of the acetylcholinesterase enzyme so that there was an accumulation of acetylcholine which caused damage to the delivery system of impulses to muscles leading to seizures and paralysis of the muscles. It also ended in death. Saponin also had the function as antifungal, antibacterial, antiviral and antiprotozoal (Francis et al. 2002; Turk 2006).

Tannin could suppress the feed consumption, the body growth, and the survival abilities of insects. The component of tannin prevented insects in digesting the food since it bounded protein in digestive tracts which was required by insects for growing. Therefore, the absorption of protein in digestive tract was disturbed (Yunita, *et al.*, 2009). The clinical symptoms for individuals who tannin-poisoned including anorexia, depression, ulcer in digestive tract. It depended on how much tannin entering the body (Frutos, *et al*., 2004).

The research result of Utami (2010), mortality was direct effect of plant bioactive substance in insects. Rapid mortality effects could be observed in insects with exposure of vegetal insecticide working as nerve poison.

CONCLUSION

The higher the concentrate of bintaro seed extract and papaya leave exposed to adult *Aedesaegypti* mosquito, the higher the mortality percentage on preliminary and advanced tests. In addition, the value of LC_{50} and LC_{95} in sea mango seed extract (*Cerberamanghas*) and papaya leaves (*Carica papaya*) showed in probit test for 24 hours are 1,995% (19,950 ppm) for LC_{50} and 23.659% (236, 590 ppm) for LC_{95}. In papaya leaves, the value of LC_{50} is 3,168% (31,680 ppm), and the value of LC_{95} is 122,202% (1222020 ppm).

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