Carbera manghas Leaf Extract as Larvacide in Controlling *Aedes aegypti*

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Abstract. One method to control A.aegypti is by using synthetic larvacide (temephos); however it can cause larval resistance. To impact and environmentally-friendly control larvae, Carbera manghas leaf extract can be used. The study assesses the effectiveness of the extract against A. aegypti larvae based on LC50 and LT50 to inhibit pupa growth. 20 instar larvae were used for each of 2500, 4000, 5500 and 7000 ppm concentrations with positive/negative control, 6 treatments and 4 repetitions, observed every 6 hours within 72 hours. One Way Anova and Probit Analysis were applied. The highest larval mortality was in 7000 ppm, valued at 83.75%. With Kruskall Wallis test, P-value 0.000 < 0.05. Mann-Whitney test, it is suggested that the extract affects larval mortality and inhibits the growth of pupae. The LC50 value is in 3971 ppm concentration within 72 hours. The LT50 values in 2500, 4000, 5500 and 7000 ppm concentrations are 10.8, 8.50, 7.10 and 6.11 hours respectively. 7000 ppm concentration is the highest in inhibiting pupa growth, with 69.23%. The extract can be used as natural larvacide, yet further researches on effects on human body and water purification treatments are needed.

Keywords: Carbera manghas, larvacide, A. aegypti

1. Introduction

Aedes spp is a vector of Dengue Fever (DD) and Dengue Hemorrhagic Fever (DHF). *Aedes spp* is spread throughout tropical countries. In Indonesia there are two species of dengue vectors, namely *Aedes aegypti* as the main vector and *Aedes albopictus* as potential vectors. Both are scattered throughout the country, except those whose height is more than 1000 m above sea level [1].

Dengue Hemorrhagic Fever (DHF) is one of the infectious diseases that the incidence is still high in Indonesia. The results show that *Aedes spp* mosquitoes are not only able to live in clear water, but can also be found in water that gets stuck and clear. With the increasing number of breeding places from *A. aegypti* mosquitoes, there needs to be more precise and effective control [1]. According to Utami [2] the most popular control nowadays is chemical control by using insecticides that work more effectively and the results are faster than biological control. However, excessive and unwise use of insecticides leads to pest resistance, pest resurgence, secondary pest explosions, inhospitable and killing of non-target organism.

*Aegypt*i mosquito control which is often done so far is by fogging. According to Wahyudin [3], fogging using ordinary insecticides is not effective in eradicating dengue vector mosquitoes, because it can cause mutations that affect the resistance of *A. aegypti* mosquitoes. "So even if smoked or fogging, these mosquitoes do not die but *A. aegypti* is even immune to insecticides are sprayed. He also considered, fogging is not effective in combating the mosquito vector DHF. Judarwanto [4] explains, prevention by fumigation is actually less effective, only dispels or kills adult female mosquitoes but can not kill the larvae.

Exposure to LC_{50} active synthetic insecticides transflutrin and d-allethrin in adult mosquitoes, will strengthen mosquitoes in the generation of filial 1 (children), which increases the fecundity rate and prolong the life of mosquitoes. The non-dead mosquitoes due to synthetic insecticidal exposure are potentially more potent and able to multiply more. Longer life of mosquitoes will increase the chance of mosquitoes to breed [5].

Besides controlling the adult mosquito, it is often done by using synthetic larvicida that is abate powder (temephos), but the use of abate powder in Indonesia from 1976 until now which has more than 30 years.it is not impossible it will cause resistance from various species of mosquitoes become a vector of disease [6]. *A. aegypti* larvae resistance report to abate has been found in several countries such as Brazil, Bolivia, Argentina, Cuba, Caribbean and Thailand according Felix in Nugroho [6]. It has also been reported resistance of *A. Aegypti* larvae to abate in Surabaya [6]. Therefore, it needs to be made another alternative that is more environmentally friendly by using natural ingredients. Plant-based insecticides are one of the greenest solutions to minimize the negative impacts caused by excessive use of non-biological insecticides. Vegetable insecticides are not too toxic so they are safe for the environment and safe for humans because the residues are easy to decompose [7].

One of plant that has the potential as a source of vegetable larvacide is bintaro plant. Bintaro is known as one of the annual crops widely used for reforestation, city decoration, medicinal plants and vegetable pesticides. This plant can be used among others as a laxative and fight cancer. All parts of bintaro plant are toxic because they contain alkaloid compounds, which are repellent and antifeedant [8]. Bintaro leaf extract contains flavonoid compounds, steroids, saponins and tannins that have toxic effects on insects [9]. There have been several studies on bintaro plants. Utami [2] reported that bintar seed methanol extract caused the highest mortality by 90% compared to fruit and leaf meat, respectively by 83.33% and 80%. The results showed that bintaro extract had a significant effect on mortality and inhibition of insect development of Eurema spp. Tarmadi [10] reported that skin and bintaro leaf extracts had a mortality effect on termites (Captotermes sp)

Research on bintaro leaf has been done by Kristiana [11] who studied the effect of bintaro leaf extract (Cerbera odollam) on mortality of *A. aegypti* mosquito larvae describes the effect of bintaro leaf extract on mortality of *A. aegypti* mosquito larvae. Test concentration 0.4%; 0.6%; 0.8% and 1.0% and 0% as controls with observational mortality every 24 hours for 3 days. The results showed that bintaro leaf extract significantly affected the mortality of *A. aegypti* larvae at 24, 48 or 72 hours after treatment and LC_{50} and LC_{90}, ie: 0.660% and 1.338% at 24 h after treatment, 0,572% and 1,130% at 48 hours after treatment, 0.439% and 0.998% at 72 hours after treatment. The optimal concentration is 1.0%. But in research conducted by Kristiana did not see the ability of bintaro leaf extract inhibited the development of larvae into pupa. Therefore we tested bintaro leaf extract as larvae *A. aegypti* larvae with concentration 2500 ppm, 4000 ppm, 5500 ppm and 7000 ppm, with observation every 6 hours for 72 hours, with the aim of research to see the effect of natural larvasida of bintaro leaf against larvae *A. aegypti* based on LC_{50} and LT_{50} and inhibit the development of pupa.

This study aims to see the effect of natural larvasida bintaro leaf extract on *A.aegypti* larvae mortality based on LC_{50} and LT_{50} and inhibit the development of larvae into pupa.

2. Method

2.1. *Location and Time of Study*

This research was conducted at Microbiology & Parasitology Laboratory of Health Analyst Academy (AAK) Yayasan Fajar Universitas Abdurrab Pekanbaru and Research Laboratory of Sekolah Tinggi Pharmacy Riau Pekanbaru in March - June 2017.

2.2. *Insect Test*

The population in this study is *A. aegypti* mosquito larva obtained from breeding. Maintenance of *A. aegypti* mosquitoes was performed at the Aak Fajar Microbiology & Parasitology Laboratory of AAK Fajar University Abdurrab Pekanbaru University until it reaches the initial 3 instar larvae used as test insects.

2.3. *Plant Source Extract*
Bintaro leaf used in this study was obtained in the vicinity of the environment outside the campus Binawidya Riau University Pekanbaru

2.4. *Making bintaro Leaf Extract*
For the manufacture of bintaro leaf extract taken as much as 3000 grams of leaves, washed with flowing tap water then dried in room temperature protected from direct sunlight. Bintaro leaf drying also uses an oven drying cup in the laboratory to make the bintaro leaf dry perfectly. The dried bintaro leaves are blended and then soaked with 96% ethanol until completely immersed (maserase). After 3 days, the solution was filtered with filter paper and the filter result was concentrated using Rotary Vaccum Evavorator tool until it was obtained a viscous extract with 100% concentration of bintaro leaf that was blackish. The extracts that have been obtained are stored in cabinets until they are used.

2.5. *Testing Method*
The research design was a complete random design (RAL) design with 4 concentrations of 2500 ppm, 4000 ppm, 5500 ppm and 7000 ppm and K (+) using temefos 1%, K (-) using aquades performed 4 repetitions. To get 2500 ppm concentration taken 2.5 gr bintaro leaf extract and then dissolved with 1 L aquadest. In the test execution, the diluted test solution for 2500 ppm concentration is divided into 4 parts and then put into 4 beaker glass each of 250 ml. The same is done for concentrations of 4000 ppm, 5500 ppm and 7000 ppm. At K (-) each uses 4 beaker glass of 250 ml without giving bintaro leaf extract. For K (+) put as much as 0.025 ppm temephos into 250 ml of distilled water on each beaker glass. In each beaker glass was put 20 head instar larvae 3 beginning from mosquito A. aegypti which still move active.

Observation of larval mortality was performed every 6 hours for 72 hours. Death data is calculated in percent mortality by the following formula:

$$\text{Persen Kematian } (\%) = \frac{\sum \text{Larva yang mati}}{\sum \text{Total Larva}} \times 100\%$$

According to Prijono (1998) in Utami (2010), extract insecticide activity is classified into several categories: (1) strong activity: mortality (m)> 95%, (2) somewhat strong: 75% <m <95%, (3) strong enough: 60% <m <75%, (4) moderate: 40% <m <60%, (5) weak: 25% <m <40%, (6) weak: 5% <m <25% , (7) inactive: m <5%.

The surviving larvae continue to be observed as a pupa. The percentage of success of pupa formation is calculated by using the following formula:

$$\text{Proses pembentukan pupa} = \frac{\sum \text{pupa yang terbentuk}}{\sum \text{larva yang hidup}} \times 100\%$$

2.6. *Data analysis*
Data analysis using statistical test of variance analysis with RAL followed by one way ANOVA test. After the data is processed the one way ANOVA test can not be done because it does not meet the requirements, then the alternative test is tested Non Parametric Test Kruskal-Wallis and Whitney Man test. Probit analysis is done to see LC_{50} and LT_{50}.

3. Results and Discussion

3.1. *Larval Mortality*
The results of observation of *A. aegypti* larvae mortality process at various concentration of treatment is: early indication seen more active larvae movement by doing ups and downs, larvae that experienced the death of the body is getting smaller, thinner, and the movement is getting slower and stiffer. The number of deaths increases with increasing treatment concentration. At K (+) all larvae die during the first 6 hours and at K (-) all larvae survive. Bintaro leaf extract gave insecticide effect was slightly weaker at 35% at 2500 to slightly strong concentration at 7000 ppm concentration of 83.75%.

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The results of observation on mortality percentage of *A. aegypti* larvae every 6 hours for 72 hours can be seen in figure 1 below.

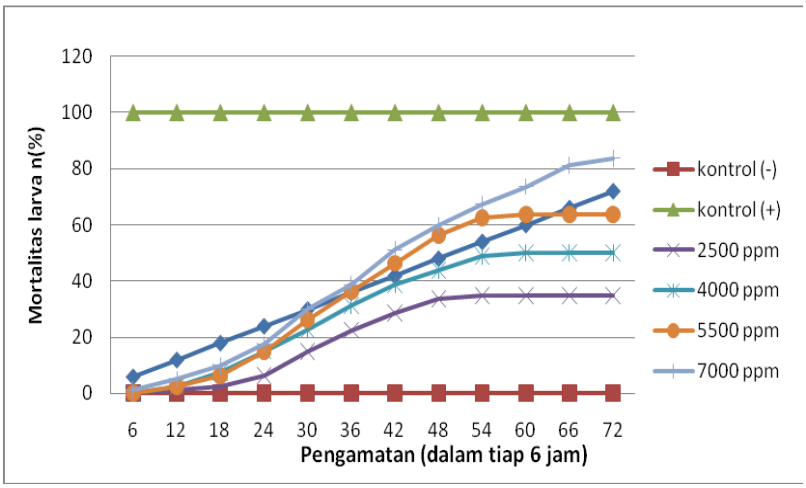

Figure 1. Percentage mortality of *A. aegypti* larvae at various concentrations of bintaro leaf extract

Based on figure 1 above the mortality of *A. aegypti* larvae after 72 hours of observation at 2500 ppm, 4000 ppm, 5500 ppm and 7000 ppm respectively were 35%, 50%, 64% and 84% respectively. Bintaro leaf extract is able to work from 1 HSP and most effective on day 2 of HSP and then on day 3 of HSP decrease. In the Kruskall Wallis test results, it was obtained P-value 0.000 <0.05. This score indicates that there are significant differences in mortality of *A. aegypti* larvae with different concentrations of bintaro leaf extract.

From the Mann-Whitney test results, it can be concluded that the groups that have very significant differences affecting mortality of *A. aegypti* larvae are: group K (+) with concentration of 7000 ppm with p = 0.001^b, followed by K (+) group with concentration of 4000 ppm and 5500 ppm with p = $0,01^b$ have real difference which influence to mortality of *A. aegypti* larvae.

In the non-Parametric correlation test, Spearman analysis obtained a Sig (2-tailed) score of 0.000 (less than 0.05), meaning there is a correlation between increasing concentration of bintaro leaf extract to mortality of *A. aegypti* larvae. Correlation strength is denoted by "strong" interpretation with a value of 0.642 **.

3.2. *Score of Lethal concentate 50 (LC_{50}) Bintaro leaf vegetable larvae*

Table 1. Graph Data Probit Value Mortality Larvasida vegetable bintaro leaves Against Larva *A. Aegypti*

Concentration (ppm)	Percentage of death (%))	Concentration Log	Probit score
2500	35	3,39	4,61
4000	50	3,60	5,00
5500	64	3,74	5,36
7000	84	3,84	5,99

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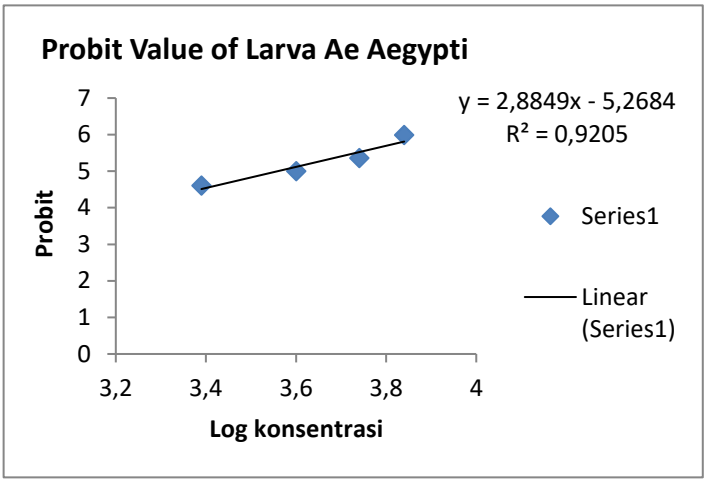

Figure 2. Graph of Probit Value of Larva *A. aegypti* Mortality Against Bintaro Leaf Extract

Based on the graph of figure 2, the probit score of bintaro vegetable larvicidal mortality on *A. aegypti* larvae can be seen clearly the increase in larval mortality from the smallest extract concentration to the largest concentration of extract. In addition, the LC_{50} score of the mortality graph of the larvae was 3971 ppm in dedah for 72 hours. This means that the concentration of 3971 ppm of bintaro leaf extract effectively gives a mortality effect on *A.aegypti* larvae based on LC_{50}.

3.3. *Lethal Time 50 (LT_{50}) value of vegetable larvacide bintaro leaf extract*

Table 2. LT_{50} test results

Concentration (ppm)	LT 50 (6 hours)	Interval (%)
2500	10.815	9.539 – 12.998
4000	8.503	7.628 – 9.586
5500	7.097	6.367 – 7.843
7000	6.111	5.829 – 6.400

In Table 2, the LT_{50} test results on mortality of *A. aegypti* larvae were respectively is: at concentrations of 2500 ppm, 4000 ppm, 5500 ppm and 7000 ppm of 10,815 hours, 8,503 hours, 7.097 hours and 6,111 hours.

3.4. *Effect of Bintaro Leaf Extract on Larva A. aegypti Development Become a pupa*

Table 3. Percentage of pupa formation and inhibit the development of *A. aegypti* larvae after 72 hours of observation

No	Concentration (ppm)	Total live larvae	Total larvae become pupa (%)		Ability to inhibit the development of pupa (%)
1	2500	52	36	69,23	30,77
2	4000	40	21	52,50	47,50
3	5500	29	12	41,38	58,62
4	7000	13	4	30,77	69,23
5	K+	0	0	0	100
6	K-	80	80	100	0

Based on Table 3, the percentage of pupa formation at concentrations of 2500 ppm, 4000 ppm, 5500 ppm and 7000 ppm was 69.23%. 52.5%, 41.38% and 30.77% respectively. Ability of bintaro leaf extract inhibited the development of pupa at concentration 2500 ppm 4000 ppm, 5500 ppm and 7000 ppm

respectively were 30,77%, 47,5%, 58,62% and 69,23%. Ability to inhibit the development of the highest pupa occurred at 7000 ppm concentration of 69.3%.

Based on statistical test, on the alternative of Non Parametric Test of Kruskall Wallis, it was obtained P-value 0.000 <0.05 This score means significant difference of pupa *A. aegypti* formation between group of treatment group concentration 2500 ppm, 4000 ppm, 5500 ppm 7000 ppm negative control and positive control . From Man Whitney Test, it is concluded that the groups that have very significant differences that affect the length of time of pupa *A. aegypti* formation are: positive and negative control group with p = 0,001[b]; Positive control group and concentration 2500 with p = 0.001[b]; The groups that had significant differences were the positive control group and the concentration of 5500 with p = 0.014[b.]

The result of observation of *A. aegypti* larvae mortality process at various treatment concentration, based on graph in picture 1, there was an increase in larval mortality as the concentration of bintaro leaf extract increased. The higher the concentration the greater the effect of insecticide given, the higher the mortality of *A. aegypti* larvae. The results of this study are in accordance with previous research that has been done by Utami,et al. [9], that in general the concentration of bintaro leaf extract has a positive correlation with percentage mortality of Spodoptera litura larvae, the higher the concentration the higher the percentage of larval mortality. This is because the higher the concentration, the more toxic compounds are absorbed by the body of the larvae, both as contact poison, respiratory toxin and stomach poison so that akumulaifly faster and more toxic effect in the body of the larvae, and ultimately result in death. Susanto [12] explains that the level of larvacid toxicity to kill larvae is highly dependent on the form of larvacide, the way the compound enters the body, the concentration and amount of compounds in the body as well as the size, structure, stage and habitat of the larvae. From the results of this study, the level of toxicity gives the larvasidal effect increases with increasing concentration of bintaro leaf extract.

In addition, the length of time exposed to insecticides, it will also increase the toxicity of bintaro leaf larvae. Because the more absorbing compounds that are toxic, it will affect the body's metabolism and cause mortality in *A. aegypti* larvae. This is consistent with the results of the study of Sa'diyah, et al. [13] entitled the effect of bintaro leaf extract on the development of Grayak caterpillars, that the more absorbing compounds that are toxic from bintaro leaf extract will affect the caterpillar's metabolism and will cause death.

The process of death of *A. aegypti* larvae at various treatment concentrations: a). The larvae that die of the body look stiff, this is because the flavonoids contained in bintaro leaves can cause the loss of chitin and abnormal stretching of the larvae, which enter the mouth and the respiratory tract / spiracles. This study is in line with the study of Gautam [14] in *Anopheles* and *A. aegypti* larvae given Vitex negundo plant extracts containing flavonoids showing integral disentegration features associated with loss of chitin and abnormal stretching of the larval body. This is due to the neurotoxic effects of Vitex negundo plant extract containing flavonoids. Utami [9] explains that flavonoids in bintaro leaves have an effect on mortality of S litura larvae. Flavonoid compounds are a group of the largest phenol compounds found in nature that contain toxic, antimicrobial / protective effects of plants from pathogens and antifeedants. Hollingworth in Utami [9] rotenon is a flavonoid group compound that has a lethal effect on insects. He thinks the rotenon acts as a cellular respiratory toxin, which inhibits electron transfer in NADH-coenzyme ubiquinone reductase (complex 1) of the transfor electron system in the mitochondria.

In the process of death of *A. aegypti* larvae: b). It can be seen body of the larvae getting smaller, thinner and the movement slowed down and eventually died. Saponins contained in bintaro leaf are known to be highly toxic to A. aegypti larvae and it can inhibit larval eating activity. Because of damage to the cell membrane of the gastrointestinal tract and it will affect the absorption of food that is likely to cause the body of the larvae to shrink and the movement progressively slow down so that it affects the mortality of larvae. Chaieb [15] reported that saponin compounds can be insecticidal by altering their eating behavior by inhibiting food (uptake) in the gastrointestinal tract and inhibiting larval stage growth by interfering with the larval molting stage. Furthermore adds that saponins can be larvicidal by

lowering the surface tension of the mucous membrane tractus digestifus larvae so that the tract wall becomes corrosive [16]. Kristiana [11] describes the consequences of cell damage is allowing the transfer of vital components of the cell to the outlet or vice versa, thus it will affect cell metabolism.

The ability of bintaro leaf extract inhibited the development of pupa at concentration 2500 ppm 4000 ppm, 5500 ppm and 7000 ppm were 30,77%, 47,5%, 58,62% and 69,23%. The higher concentration of bintaro leaf extract the less pupa formed and the higher the ability to inhibit the development of larvae into pupa and the longer time it takes to turn into pupa [2]. This is because bintaro leaves also contain saponins and tannins.

In addition to saponins contained bintaro leaf extract also contained tannins. Tanin also affects the mortality of *A. aegypti* larvae, as it also interferes with the digestive system of larvae in the absorption of foodstuffs which results in the body of the larvae is getting smaller and thinner. The reduced percentage of larvae to pupae at high concentrations due to the content of bioactive compounds contained in bintaro leaf extract can inhibit the growth of larvae, such as saponin and tannin compounds. Yunita [17] explains that tannins may interfere with insects in the digestive process of food because tannins will bind proteins in the digestive system that insects need for growth so that the process of protein absorption in the digestive system becomes disrupted. The same thing, according to Hopkins and Hiiner in Yunita [17] that tannin suppresses eating intake, growth rate and survival ability. Tannins, saponins and quinones have a bitter taste that can cause a feeding mechanism in the test larvae, and the larvae will not eat, it will starve and die eventually. This research is similar to the research [2] that the compounds in bintaro are strongly suspected to have a significant effect on the mortality of Eurema larvae. Besides causing mortality, it also has a significant effect on the inhibition of growth and the success of pupa formation.

According to research, bioactive compounds that enter through the digestive system will disrupt the physiological process of Grayak caterpillars, which disturb the working system of enzymes and hormones [13]. Ekdison hormone triggers skin turnover and if there is interference which will disrupt the process of development in insects. The compound disrupts the ecstasy process is saponin. Saponins can bind sterols in the food ducts so that there is a decrease in the rate of sterols in hemolimfa. Sterol acts as a precursor (stimulant) for the formation of ekdison hormone. The decrease of sterol supply, then th skin change will also be disrupted, there is disruption to the growth and development.

In addition to saponin,the bintaro leaf also contains steroid. steroid has toxic effects and inhibit the development of insects. According to Kristiana [11] steroid compounds will affect the central nerves system produce and secrete the eco-hormones and jouvenil hormones responsible for the larval skin replacement.If there is no steroid in hemolymph then the larvae will not change skin. Yunita [17] explains that steroids can inhibit the development of *A. aegypti* mosquitoes. Besides steroids contained in plants have protective functions, such as photoecdison so that steroids can inhibit the process of skin turnover in insects.

Based on LC_{50} obtained at 3971 ppm within 72 hours, this means that the concentration of 3971 ppm of bintaro leaf extract effectively gives a 50% mortality effect on *A.aegypti* larvae. The bioactive compound in bintaro leaf extract is the cause of larval death because it can act as toxicant. The death of the larvae is due to the inability of the larva to detoxify the toxic compound that enters the body of the larvae. The LC_{50} score in this study were lower than those of with concentrations of 0.4% (4000 ppm), 0.6% (6000 ppm), 0.8% (8000 ppm) and 1 % (10000 ppm) and observation of *A.aegypti* larvae mortality performed every 24 hours, 48 hours and 72 hours. From the LC_{50} results observed after 24 hours, 48 hours and 72 consecutive hours were 6600 ppm, 5720 ppm and 5390 ppm and the optimal concentration of bintaro leaf extract was 10000 ppm [11].

In LT_{50} test results at the highest concentration (7000 ppm) it takes 6.11 hours to get 50% mortality of larvae. This is because the greater the concentration of bintaro leaf extract given to *A. aegypti* larvae the faster the time it takes to kill the larvae.

The utilization of bintaro leaf extract in this study at low concentrations as larvae of *A. aegypti* mosquito can be used because it causes mortality and inhibits the development of larvae into pupa. From the results of this study allegedly bintaro leaf extract (*Carbera manghas*) can be used as a vegetable larvacide in the community, because bintaro leaf is also used as a laxative and fight cancer. Which means bintaro leaves in low concentrations are suspected to have a low toxic risk to humans so itwill not affect the body. This opinion is based on Rohimatun's opinion [8], that young leaves of bintaro have efficacy as soft laxative, which for Ambon tribe of young leaves is cooked as vegetable. In addition, bintaro leaf methanol containing 17 Beta H-neerifolin can fight breast and ovarian cancer cells, so it is potentially for further development.

However, it is suspected that bintaro leaf extract can be utilized as a vegetable larvicide, it is necessary to further test to the extent of its effect on the human body, as one of the requirements of a plant-based insecticide is relatively safe for humans and animals as well as for the environment. Besides, based on LC_{50} of 3971 ppm, the concentration is still very high when compared with the use of abate powder (temephos). At the time of implementation of this study the use of bintaro leaf extract affects the water color, so the water is not clear. This needs further testing of the eff that can be done so that the water can be clear, because one of the water conditions that can be used is not colored.

In terms of quantity, bintaro leaves abundantly compared to the fruit and bark of the stem, making it is easy to be obtained. Utami [2] explains, in the utilization of plants that have potential as insecticides / vegetable larvasida, there are several things to note that is: 1). Plants that are potential as bioactive insecticides are easily available in nature and are ubiquitous, 2). Biomass can be obtained in abundance, 3). Easy to decompose in nature so as not to pollute the environment and relatively safe for humans and pets because the residue is easily lost. Therefore, in terms of the number of parts of bintaro leaves can be used as a vegetable insecticide in *A aegypti* mosquito considering the leaves are abundant compared to the fruit and bark.

4. Conclusions

Bintaro leaf extract had a significant effect on larval mortality and it inhibited the development of pupa. LC_{50}3971 ppm for 72 hours. LT_{50} at 2500 ppm, 4000 ppm, 5500 ppm and 7000 ppm of 10.82 hours, 8.50 hours, 7.10 hours and 6.11 hours. Ability to inhibit the development of the highest pupa at 7000 ppm concentration of 69.23%. Bintaro leaf extract can be used as a vegetable larvacide. However it is necessary to test its effect on the human body and the purification efforts of water.

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