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Effectiveness of Papaya (*Carica papaya* L.) Seed Extract and Alum on *Aedes aegypti* Larvae Death

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A B S T R A C T

People generally use larvicide to kill mosquito larvae. Synthetic chemical larvicides leave residues that have a negative impact on the environment. Therefore, research on natural larvicides has been developed to suppress the negative effects of chemical larvicides. Microparticle preparations can speed up the compound delivery system so that it will be effective in its use as a larvicide. The important composition in making microparticle preparations is polymer. The polymer used in this research is chitosan. The formulation of papaya seed extract and alum microparticles using chitosan polymer with the spray drying method was made into three formulas with different concentrations of extract and alum, namely formula 1 (0.1% : 0.05%), formula 2 (0.15% : 0.075%). %), and formula 3 (0.2% : 0.1%). The results obtained show that formula 3 with a concentration of 0.2% papaya seed extract and 0.1% alum has the best quality characteristics based on the polydispersity index parameters, namely 0.181, particle size 1233.67 nm, zeta potential value 72.6 mV. The number of samples was 500 test larvae consisting of 5 treatment groups. Each group contained 25 larvae and 4 replicates. The test larvae were observed after 24 hours and then analyzed. The test analysis carried out was the Probit test. The LC_{50} value obtained in this study was 95.5 ppm. These results indicate that the microparticle powder preparation has larvicidal efficacy against third/IV instar *Aedes aegypti* larvae.

1. Introduction

Indonesia is an archipelagic country consisting of 17,504 islands located between two continents and two oceans. It has a heterogeneous tropical climate and is rich in fauna and flora, including various mosquito-borne diseases such as dengue hemorrhagic fever (DHF). Until now, dengue hemorrhagic fever has been a problem for public health. In recent years, along with the increasing frequency and outbreaks of dengue hemorrhagic fever, the clinical manifestations of this disease have become more serious. (Karyanti and Hadinegoro, 2016).

DBD vector control can be done through chemical methods and environmental management, one of which is through Pemberantasan Sarang Nyamuk (PSN). Chemical vector control (such as fumigation or fogging) can kill infectious adult mosquitoes but not larvae. If the larva is still alive, it will grow back into an adult mosquito. By using larvicides, the control will be easier because, at this stage, the movement and activity of the larvae are still in the water and are limited (Husna et al., 2020). The use of chemical larvicides in dengue vector control is like a double-edged sword, which means it is both beneficial and detrimental. Using chemicals to kill larvae over a period of time can lead to vector resistance.

One of the natural ingredients that can be used as a natural larvicide is papaya seeds. According to research by Asmira and Sulasmi (2019), papaya seeds can be used as an ingredient in controlling dengue hemorrhagic fever vectors. This is because papaya seeds contain chemicals, one of which is karpain alkaloids, which can be used as contact poisons.

Based on research conducted by Preet and Sneha (2011), alum can be used as a larvicide against *Aedes aegypti* mosquito larvae. Alum works as a contact poison and stomach poison, inhibits energy production processes, and causes biochemical changes in larvae. The use of alum is also one way to reduce turbidity because it is useful as a coagulant in the water purification process.

In the research results of Iskandar et al. (2017), papaya seed powder is effective in killing larvae, but when dissolved in water, it releases natural pigments that affect the water and turn the water cloudy. Making preparations in the form of microparticles is expected to reduce water turbidity and make the powder quickly dissolve in water, as according to Salim et al. (2018), who stated that the smaller the powder, the wider the surface area and the easier it will dissolve so that the water added to papaya seed powder remains clear.

One method of making microparticles is spray drying. The principle of drying in a spray dryer is that all the liquid in the material to be dried is converted into water droplets by using a nebulizer to dry it. The liquid material that is already in the form of a mist is then flown by hot steam. This contact event causes the liquid in the form of a mist to dry and turn into powder. Then the separation between the hot steam and powder is carried out by means of a cyclone or filter. After the powder is separated, the temperature is lowered to meet research and production needs (Rosidah et al. 2012). According to Yunilawati R. et al. 2018 the results of spray drying technology depend on a number of variables, such as the velocity of the liquid flow, the temperature of the hot air entering the drying tank, and the type of atomizer.

Spray drying technology is also used to convert reactive materials into more stable materials, thereby extending the shelf life of the product. The spray drying method for the manufacture of nanoparticles is the most commonly used method because of its low cost, fast drying process, high quality dry particles, and easy scale-up (Rosidah et al. 2012 and Yunilawati, R. et al. 2018).

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This research aims to make microparticles from papaya seed powder that can kill larvae while still maintaining water clarity. The characteristics of the microparticle powder obtained were tested, including water content, particle size, zeta potential, particle morphology, determination of percent recovery, as well as testing the effectiveness and clarity of the water added to the papaya seed microparticle powder.

2. Materials and Methods

Instruments

Spray dryer (BUCHI 190 Mini Spray Dryer), Malvern particle size analyzer (PSA), Scanning electron microscope (SEM) Zeiss EVO MA10, chocolate bottle, disposable cup, magnetic stirrer IKA HS7, dropper pipette, moisture balance, turbidimeter, Pyrex measuring cup, Maspion oven, Philips blender, 30 mesh sieve, analytical balance, crucible, crucible and test tube.

Materials

Papaya seeds, instar III/IV larvae, 1% acetic acid, aquades, chitosan, alum, abate, 96% ethanol, 10% gelatin solution, 3% FeCl3, Mg powder, concentrated HCl, HCl 2 N, Wagner, Dragendorff, and Hager reagent.

Experimental

1. Preparation of microparticles using the spray dry method

The research of Arimaswati et al. (2017) which used papaya seed ethanol extract as a larvicide has a recommended LC_{50} value to kill *Aedes aegypti* larvae, which is 0.154%. Then in the study of Preet and Sneha (2011) which used alum as a larvicide at a concentration of 100 ppm, it killed all 20 larvae. So that the two studies become a reference in determining the formulation of microparticle preparations to be made.

Atomized droplet drying in a hot air stream is the basis of spray drying, which is the method used. In this method, chitosan is first dissolved or dispersed in a dilute acetic acid solution (Agnihotri et al., 2004). Then added alum into the solution of chitosan and acetic acid. Then the papaya seed extract is dissolved with acetic acid. The two solutions were mixed and then homogenized using a magnetic stirrer hot plate for 30 minutes and continued at a speed of 8000 rpm for 5 minutes to reduce the particle size. The mixture that has been formed is tested for viscosity. This solution is atomized in a stream of hot air to form small droplets. From this process, the solvent instantly evaporates and produces free-moving particles. (Jayanudin et al., 2017). The particle size depends on the nozzle size, spray flow rate, atomization pressure, inlet air temperature and degree of cross-linking.

$$\%\text{Recovery} = \frac{\text{The obtained microcapsule weight}}{\text{Microcapsule material weight}} \times 100\% \qquad (1)$$

2. Microparticle characterization

Determination of water content

The moisture content of the papaya seed extract microparticles was determined by means of a moisture balance. Microparticle powder was weighed approximately 1 gram and placed on an aluminum container evenly in the tool at a temperature of 105˚C for 1 minute.

Determination of particle distribution

A particle size analyzer (PSA) was used to determine the particle size. The microparticle sample was dissolved using a suitable solvent and then put into a cuvette. Insert the cuvette into the PSA tool holder. The cuvette is then fired with visible light so that diffraction occurs. Particle size measurements take advantage of this principle of visible light scattering. PDI requirements for particles are in the range of 0.01-0.7 (Yuwono et al., 2015).

Zeta potential

Particle size analyzer (PSA) was used to measure the zeta potential. The zeta potential value was carried out to see the stability and quality of the obtained microparticles. To prevent aggregation between particles to remain stable, the zeta potential value of the microparticles must be higher than ± 30 mV (Pertiwi et al., 2019).

Determination of recovery percentage

Determination of the recovery test (1) or process yield (% w/w) was determined by comparing the weight of the obtained microparticles to the weight of the microparticle-forming material used (Rosidah et al., 2012).

Morphology

A scanning electron microscope (SEM) was used to read the morphology of the formed microparticles. The microparticles were coated with gold and palladium metal using a finecoater under vacuum and samples were examined using SEM (Rosidah et al., 2012). Then the sample is observed. Morphological results can be spherical (round) and amorphous.

Water turbidity test

This test uses a tool that is a turbidimeter. The samples used were the three microparticle formulations of a combination of papaya seed extract and alum dissolved in water. Then each sample was put into a small bottle and then put into a turbidimeter. Wait until the sample turbidity value appears on the screen. However, before the turbidimeter is used, calibration is carried out first to ensure the level of sample measurement using the standard solution available on the turbidimeter (Parera et al., 2013). According to the Minister of Health (2017) in Permenkes No. 37, the standard of turbidity of water used for daily purposes is 25 NTU.

3. Effectiveness test

The effectiveness test was carried out by 2 kinds of preparations, namely by using a mixture of thick papaya seed extract with alum and using microparticle preparations that had been made. Based on the guidelines issued by WHO (2005), 5 clean disposable cups of ±150 mL were prepared and labeled according to their concentration and formulation.

A mixture of thick extract of papaya seeds and alum then each disposable cup is put in 100 mL of distilled water, added thick extract of papaya seeds and alum, with a concentration of thick papaya seed extract of 0.1%; 0.15%; and 0.2% and alum is 0.05%; 0.075%; and 0.1%. Then put 25 *Aedes aegypti* larvae.

The preparation of microparticles was first made a 1% solution for each formula by dissolving 0.1 g of microparticle powder into 10 mL of water. The reference used is based on the WHO Guidelines for Laboratory and Field Teating of Mosquito Larvasides (2005) where the maximum percentage of the most effective concentration in larvicide research is 1%. Then the disposable cup was put in 100 mL of aquadest and added a 1% sample solution, then 25 *Aedes aegypti* larvae were added. The control test for both types of preparations in this study used 2 controls, namely positive control, by adding 100 mL of distilled water and 25 larvae (larvae) with the addition of abate. And negative control, by adding 100 mL of aquadest and 25 larvae (larvae) of *Aedes aegypti* without adding anything. Observations were made to count how many larvae died. All treatments were repeated 4 times (WHO, 2005).

The number of deaths of *Aedes aegypti* larvae was observed after the addition of papaya seed extract microparticles. According to WHO (2005), the minimum time for larvicide testing on a laboratory scale is 24 hours, so this test will be carried out for 24 hours and observed every 1 hour. Larvae can be said to have died if when given mechanical treatment such as being touched using a pipette or spatula it did not move. Data obtained from observations during the experiment were presented in the form of tables,

percentages and graphs (number of larvae deaths in the form of percent against time). And calculated LC_{50} using the probit analysis method to determine the effective concentration that produces a response in the form of death by 50% of the population.

3. Result and Discussion

The mixed chitosan-(extract+Alum) solution was made into microparticles using the spray drying method. Spray drying is a drying process using hot gas to produce a dry powder or a uniform fine powder. The chitosan-extract solution was put into spray drying with a 1.0 mm standard nozzle with a pressure of 6ml/min, an inlet temperature of 160˚C, an outlet temperature of 70-75˚C and an atomization air flow rate of 6m3/min. The results obtained are in the form of powder or white powder. The results of the microparticle powder can be seen in **Figure 1**.

The three powders were tested to measure their water content using a moisture balance tool. The results of the three formulas, respectively, have an average moisture content for F1, F2 and F3 which is 3.28%; 3.56%; and 3.42%. The three formulas meet the requirements of the water content of the powder preparation <10% (BPOM RI, 2019).

Formula 3 gives PSA results with the smallest size of 1233.67 nm and a polydispersity index (PDI) of 0.181. Formula 3 has the highest viscosity, which is 132.2 cps. The results of the polydispersity index test showed that formula 3 had the best polydispersity index that met the requirements of 0.181. PDI values in the range of less than 0.5 indicate uniform dispersion. The lower the PDI value, the smaller the particle size distribution. This means that the microparticles have a more uniform diameter (Dewantari et al., 2013). Zeta potential of microparticles was measured with a zetasizer. The measurement of the zeta potential value is intended to determine the stability of the compound so as not to cause coagulation or aggregation. This is also related to what was stated by (Pertiwi et al., 2019) where microparticles with a potential value greater than ± 30 mV will be more stable. The three resulting formulas show that the zeta value obtained is stable. Microparticles with a zeta potential value greater than (+/-) 30 mV have stable properties to prevent agglomeration between particles. The higher the zeta potential value, the more stable the particle is.

Percent recovery

Determination of the recovery test is carried out by comparing the weight of the microparticles obtained with the weight of the microparticle-forming material used. Based on the results of the three formulations in **Table 3**, the highest yield of microparticles was found in formula 1 of 27.88%, while in formulas 2 and 3, the yields were 25.51 and 22.78%, respectively. The difference in the recovery results is due to the difference in the viscosity of each medium.

SEM

The particle morphology was observed with a Scanning Electron Microscope (SEM). SEM testing was carried out with the best formulation, namely formula 3. The selection of the best formulation was based on the polydispersity index value of 0.181 and the zeta potential value of 72.6 mV with a particle size of 1233.67 nm. Microparticle SEM results can be seen in **Figure 2**.

The magnification of 30.000x in **Figure 2** shows the surface morphology of the spherical microparticles, and there is a shrinkage on the surface of the particles. The factors that affect the morphology of the microparticles are due to the influence of temperature and pressure on the spray drying process which causes the particles to shrink (Ningsih et al., 2017)

Water turbidity test

The turbidity test was carried out in UPTD LABKESDA Bogor City. The test results can be seen in **Table 4**. The test is carried out using the method SNI 06 6989.25-2005 the turbidity test with a nephelometer. The principle of the test is that the light intensity of the sample that is absorbed and refracted is compared to the light intensity of the standard suspension. The standard suspension used is a standard turbidity suspension solution with a turbidity value of 40 NTU. From the description above, it can be concluded that all the variations of the formula used are optimum and can be utilized in the community because they have met the clean water standards by the standards Permenkes RI Nomor 32 Tahun 2017 for hygiene and sanitation, namely 25 NTU (Nephelometric Turbidity Units).

Table 1. Microparticle formula

Material	FI (%w/v)	FII (%w/v)	FIII (%w/v)
Papaya seed extract	0,1	0,15	0,2
Alum	0,05	0,075	0,1
Chitosan	1,5	1,5	1,5
Ethanol 96%	5	5	5
Acetic acid ad	100	100	100

Figure 1. Microparticle powder from spray-dry

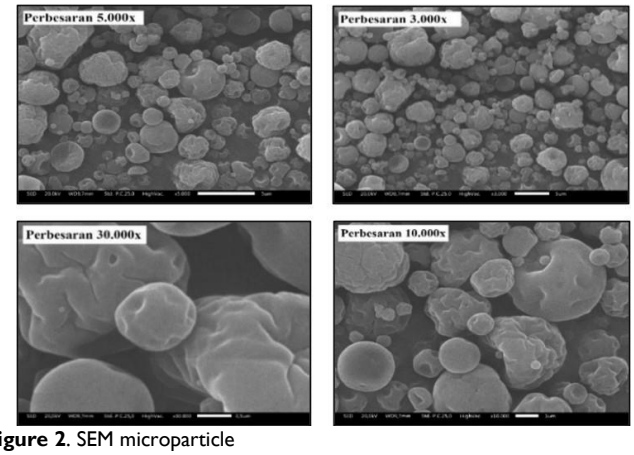

Figure 2. SEM microparticle

Table 2. Particle size, and zeta potential

Formulation	PDI	Particle	Zeta potential (mV)
1	0,691 ± 0,291	1692,67 ± 165,66	45,35 ± 1,0606
2	0,201 ± 0,028	1399,67 ± 45,65	57,1 ± 1,4142
3	0,181 ± 0,031	1233,67 ± 20,59	72,6 ± 0

Table 3. Microparticle recovery results and viscosity

Formulation	Viscosity (cps)	Recovery (%)
1	118	27,88
2	126	25,51
3	132,2	22,78

Table 4. Water turbidity test results

Formulation	Unit	Result	Standart
1		7,16	25
2	NTU	10,12	25
3		14,16	25

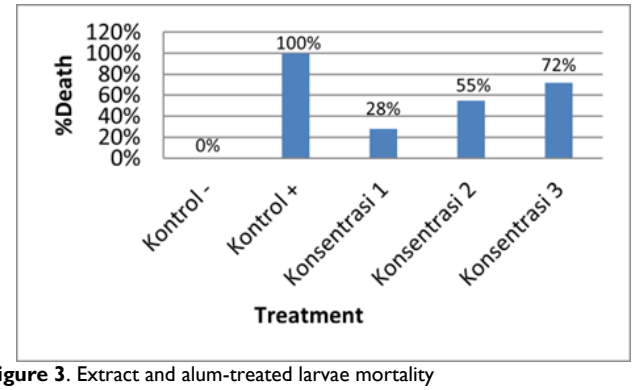

Figure 3. Extract and alum-treated larvae mortality

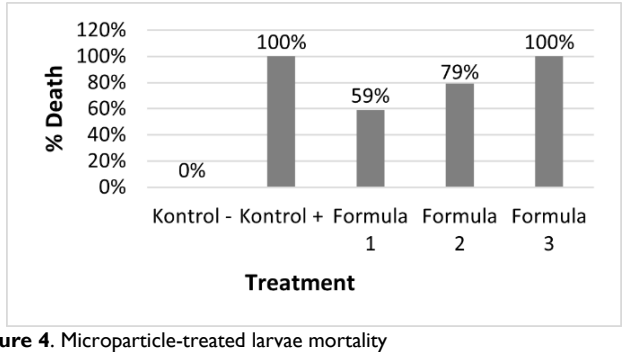

Figure 4. Microparticle-treated larvae mortality

Effectiveness test

Before this research begins, the first step is to conduct an ethical review of the test animals that will be used as research subjects. The main purpose of conducting this ethical review is to ensure the safety and welfare of the test animals during the research process.

Aedes aegypti mosquito larvae were obtained from the Entomology Laboratory of the Faculty of Veterinary Medicine, IPB. The inclusion criteria were the last instar III *Aedes aegypti* larvae to the early IV instar larvae that were still active. The exclusion criteria set were *Aedes aegypti* larvae instar I and II, dead larvae, and larvae that have become pupae. According to WHO (2005), 25 larvae are needed for each mosquito larvicide laboratory test. So it takes 500 mosquito larvae for this research.

Preparation of test larvae is carried out through mosquito larvae rearing activities. The larvae obtained were stored in a cloth-covered place. Observe and feed the larvae once a day with crushed fish feed (Utami et al., 2016). If there are larvae that become pupae, immediately put them in alcohol so they don't become mosquitoes. The larvae were reared until they became the last III instar larvae to the early IV instar larvae, which will be used for testing. The larvae used in the study were healthy and agile, all of which were evenly selected (Wahyuni, 2016).

The eggs of the *Aedes aegypti* L. mosquito were obtained from Laboratorium Entomologi Fakultas Kedokteran Hewan, IPB. The number of larvae used for each treatment was 25 larvae instar III or IV. Based on the results and data analysis, the higher the concentration of papaya seed extract and alum used in the treatment, the higher the number of larval deaths. The number of larvae used for each treatment was 25 larvae instar III or IV.

In the test of papaya and alum seed extract using a concentration of 1 (0.15%) the results obtained were 28 larvae mortality (28%). In testing the extract of papaya seeds and alum using a concentration of 2 (0.225%) the larvae mortality was 55 (55%). And in the test of papaya and alum seed extract using a concentration of 3 (0.3%) the results obtained were 72 larvae mortality (72%). Based on the graph above, the highest larval mortality was found at the 3rd concentration (0.3%). From the percentage of mortality above, it can be concluded that the higher the concentration, the higher the percentage of larval mortality. In addition, based on the results of the analysis using the probit analysis method in Microsoft Excel, the LC_{50} value was 2089.3 ppm or 0.208%.

Based on the results and data analysis, the higher the concentration of papaya seed extract and alum in the microparticle powder used in the treatment, the higher the number of larval deaths. In testing the microparticles of papaya seed extract and alum, formula 1 resulted in the mortality of 59 larvae (59%). In testing the microparticles of papaya and alum seed extract using formula 2, the results obtained were 79 larvae mortality (79%). And in testing the microparticles of papaya and alum seed extract using formula 3, the results obtained were 100 larvae mortality (100%). Based on the graph above, the highest larval mortality was found at the 3rd concentration (0.3%). From the percentage of mortality above, it can be concluded that the higher the concentration, the higher the percentage of larval mortality. In addition, based on the results of the analysis using the probit analysis method in Microsoft Excel, the LC_{50} value obtained is 95.5 ppm or 0.00955%. Positive control abate 100 ppm used in the 2 tests above can kill 100% of larvae in 24 hours. This is because temefos inhibits the cholinesterase enzyme so that the larvae cannot hydrolyze acetylcholine, then acetylcholine will accumulate in nerve endings causing nerve activity to stop. This causes continuous muscle contractions that lead to the death of the larvae (Atmosoehardjo, 1991). Negative control did not cause larval death.

Based on the results and analysis of the two data, the relationship between concentration and larval mortality was that the higher the concentration used in the treatment, the higher the number of larval deaths. The death of *Aedes aegypti* larvae was caused by the increasing number of active compounds contained in the extract of papaya fruit seeds namely alkaloids and saponins that entered the larvae body with higher concentrations. Asmira and Sulasmi (2019) stated that the active compounds contained in papaya seeds can cause chemical reactions in the metabolic processes of the larval body, such as carpaine alkaloids which can interfere with the work of the larval nervous system by inhibiting the work of acetylcholinesterase, causing the death of the larvae. The addition of alum can also be the cause of death of *Aedes agypti* test larvae because when alum continues to enter the digestive system, it will inhibit taste receptors on the larval mouth wall and inhibit larval digestive enzymes. *Aedes aegypti* larvae are said to be dead if the larvae are immobile when touched and are at the bottom of the water, and do not appear again to the surface of the water. Dead larvae look pale.

Based on the research that has been done, it can be concluded that the preparation of microparticles with an LC_{50} of 95.5 ppm (0.00955%) is more effectively used as a larvicide. This is based on the magnitude of mortality in the larvae of the *Aedes aegypti* mosquito.

4. Conclusions

Formula 3 has good microparticle characteristics with a polydispersity index parameter of 0.181, a particle size of 1233.67 nm, a zeta potential value of 72.6 mV and effectively kills larvae of 100%. The LC_{50} value of the combination of papaya seed extract and alum as a larvacide against *Aedes aegypti* larvae was 95.5 ppm.

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