# NANOPARTICLES OF ABATEAS AEDES AEGYPTI MOSQUITO LARVAE EXTERMINATOR USING EXTRACTION OFBROTOWALI STEM (TINOSPORA CRISPA) ABSORBED BENTONIT

**[1]WAHYU LESTARI, [2]HILMA EKA MASITOH, [3]DRA. CORNELIA BUDIMARWANTI, M.SI**

[1,2]Jurusan Pendidikan Fisika, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Yogyakarta
[3]Jurusan Pendidikan Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Yogyakarta
E-mail: hilma.ekamasitoh@gmail.com, w.fisika@yahoo.com

**Abstract-** Controlling dengue hemorrhagic fever vector would be better when the people could choose own choice of method such as spraying synthetics insecticide, using abate synthetics as synthetics larvacide, using fish as biological controller, and using electrical racket as a mechanical controller. Besides, using poisonous plant as alternative controlling method was being encouraged. Brotowali (Tinospora crispa) is a wild plant from a tropical area, which could not be eaten, easy to grow, cheap, safe and environmental friendly. Using extract of brotowali stem (Tinospora crispa) mixed by bentonit soil to be abate concerned with nature. Lethal concentration CL50 towards larvae is 400 mg/100 ml or 4000 ppm for well water and 300 mg/100 lm or 3000 ppm for PAM water. The particle morphology of sonication methods of abate mosquito in general are mesoporous nanoparticles.

**Keywords-** Brotowalistem, Aydes aygypti, Natural, Larvacide, safe.

## I. INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by denguevirus with certain signs and spread through the bite of the Aedesaegypti. Dengue cases in Indonesia increase and even more rampant with global warmingeach year. Pambudi (2009) in his research said since 1968 the number of dengue cases in Indonesia tends to increase and the spread is expanding. Information center from Ministry of Health recorded, that the number of dengue cases in Indonesia on January 2008 reached 8765 cases with 68 deaths.

In 2000, WHO estimates that 2.5 to 3 billion people at risk for infection with dengue virus and every year there are 50-100 million people worldwide are infected with dengue virus, 500 thousand of them require intensive care in a health care facility. Each year in Indonesia reported 21,000 children die because of dengue (Widarto, 2009). Bell saidDengue Hemorrhagic Fever (DHF) is a severe viral fever that occurs sporadically and epidemics that are transmitted to humans and other primates by mosquito bites (Wardani, 2009). The disease is not only found accidentally in urban areas, but also found in rural areas. Transmissions of dengue fever occur by dengue virus multiply their self in the body of Aedesaegypti.

The exact eradication of dengue disease is to control the vector mosquitoes as transmitters. At this time has not found some drugs and vaccines of denguedisease. Satari(2004) declared the eradication of Aedesaegypti can be done by providing insecticide or without insecticide. With the provision of insecticide repeatedly and excessively can cause poisoning in humans or animals, and occur environmental pollution that affected the life cycle (Widarto, 2009).

Eradication of the Aedesaegypti mosquito chemically is often found, such as fogging and abate mosquito, but they are not effective enough. Eradication of mosquitoes using fogging only exterminates mosquitoes, while the larvae of mosquito still can hatch. Moreover, it can lead to shortness of breath for people who breathe it.

To reduce the adverse effects of chemicals should be developed drugs of mosquito from materials found in nature that are safer for humans and the environment, and its source is available in large quantities. Utilization of natural larvaicides in the eradication of the vector is expected to reduce dengue cases. In addition because it is made from natural ingredients, it is expected that this larvicides will be easier to decompose (biodegradable) in nature so it does not pollute the environment and relatively, safe for humans and animals because the residue is easily lost (Agriculture and Forestry Department, 2004).

From the phenomenon, it needs to research what would be solution of the problem by making abate mosquito with brotowali (Tinosporacrispa) that can purify water. As we know that brotowali (Tinosporacrispa) is a plant that can be easily found and easily grown in Indonesia. Brotowali (Tinosporacrispa) can be used to break the life cycle of mosquitoes because it contains tinokrisposid. These substances can be used to make abate mosquito in order to prevent the spread of AedesAegepti.

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## II. METHOD

### 1. Materials and Method

The materials used are stems of brotowali (Tinosporacrispa) 5 kilos and ground bentonite. Stemsare dried using an oven with a temprature $40^0$ C for 36-48 hours until its mass change into constant, then stems which have been dried are crushed with a tool glinding R to be powder. Then sieved (sieve size 48 mesh). Powder is extracted using maceration

method which is mixed with 96% ethanol solution as much as 2 liters and stored in a box covered with alumunium foil and then leave them for 24 hours and then filter theith paper strain. The filtrate 1 obtained then are evaporated using an evaporator so it gained as much as 4,25 grams of pure extract. Furthermore filtrate 2 is sonicated using ultrasonicator during 120 second at a temperature of $30^0$C. Then mix it with bentonite which is already activated in a temperature of $50^0$C so it obtain 266,94 grams of abate powder.

**2. Efficacy of Brotowali Stem Extract**

The observation of Aedes aegypti treated with brotowali stems extract (Tinosporacrispa) to see larval mortality at 1000-5000 ppm or 100-500 grams/100 ml with 2 typesnof water, water wells and piped water and is performed 3 times repetitions on each type of water and is obseved for 48 hours. In the tests performed, the concentration of <1000 ppm or 100 grams/ 100 ml up on the threerd day yet can be dedly larvae up to 100%.

**3. Efficacy Test Against Larvae Larvacide**

To test the efficacy of plant ekstracts brotowali (Tinosporacrispa.) against larvae, the size of the glass countiner used 30 300 ml whit 15 glass of whel water samples and 25 larvaes of Aedes aegypti. Various levels of the brotowali stems extract concentration tested was 100 g/ 100 ml or1000 ppm, 200 g/100ml or 2000 ppm, 300 g / 100 ml or 3000 ppm, 400 g / 100 ml or 4000 ppm, and 500 g/ 100 ml or 5000 ppm into each glass and any type of water performed three repetitions. The observation performed for 48 hours by watching larvae which can move clearly and does not responds to stimuli.

**4. Determination of Size and Morphology of Nanoparticles with Scanning Electron Microscopic (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS)**

The materials are made to be conductive and photo taken at 20 kV electron voltages with a magnification of x100, x 500, x1000 and X2000 and x5000.

## III. RESULT AND DISCUSSIONS

Produced by one kind of abate powder that made from pure extracts of brotowali stems(Tinosporacrispa) which absorbed by bentonite.

A sample ofbrotowali stems (Tinoporacrispa) that obtained from household plants in Kulonprogo, Yogyakarta. At the beginning, it was standardized by measuring the water content therefore that it could be seen the weight of free sample which contain of extracted water. From research that had been conducted, it was acquired 13.63% water content. Brotowali stems (Tinosporacrispa) which were approximately 5 kgextracted with 96% ethanol using maceration method on temperature room ($25^0$C) for 24 hours. The extraction were evaporated using an evaporator, then those wereconcentrated by water batch, therefore those were acquired dark green viscous liquid that had been concentrated and soluble-free as the mass of 4.25 grams.

The result of pure concentrated extract in sonication within 2 minutes with a temperature of $30^0$ C. Then, the result of sonication was absorbed by bentonite soil which had been activated at a temperature of 300 degrees Celsius for later sonication.

The results of effectiveness test can be seen in the following tables.

**Tabel 1. Effectiveness Test in AedesaegyptiLarvae at First Well Water**

| Concentration (ppm) | 2h | 4h | 6h | 12h | 24h | 48h |
|---|---|---|---|---|---|---|
| $10^3$ | 0 | 0 | 1 | 2 | 13 | 23 |
| $2x10^3$ | 0 | 0 | 0 | 3 | 15 | 24 |
| $3x10^3$ | 0 | 0 | 0 | 4 | 14 | 25 |
| $4x10^3$ | 0 | 0 | 1 | 6 | 17 | 25 |
| $5x10^3$ | 0 | 0 | 0 | 4 | 10 | 25 |

**Tabel 2. Effectiveness Test in AedesaegyptiLarvae at Second Well Water**

| Concentration (ppm) | 2h | 4h | 6h | 12h | 24h | 48h |
|---|---|---|---|---|---|---|
| $10^3$ | 0 | 0 | 0 | 1 | 14 | 25 |
| $2x10^3$ | 0 | 0 | 0 | 2 | 15 | 25 |
| $3x10^3$ | 0 | 0 | 0 | 2 | 16 | 25 |
| $4x10^3$ | 0 | 0 | 2 | 5 | 18 | 25 |
| $5x10^3$ | 0 | 0 | 1 | 3 | 12 | 25 |

**Tabel 3. Effectiveness Test in AedesaegyptiLarvae at Third Well Water**

| Concentration (ppm) | 2h | 4h | 6h | 12h | 24h | 48h |
|---|---|---|---|---|---|---|
| $10^3$ | 0 | 0 | 0 | 2 | 13 | 25 |
| $2x10^3$ | 0 | 0 | 1 | 1 | 14 | 25 |
| $3x10^3$ | 0 | 0 | 1 | 2 | 17 | 25 |
| $4x10^3$ | 0 | 0 | 0 | 6 | 20 | 25 |
| $5x10^3$ | 0 | 0 | 0 | 3 | 11 | 25 |

**Tabel 4. Effectiveness Test in AedesaegyptiLarvae at First PAM Water**

| Konsentrasi (ppm) | 2h | 4h | 6h | 12h | 24h | 48h |
|---|---|---|---|---|---|---|
| $10^3$ | 0 | 0 | 1 | 2 | 11 | 25 |
| $2x10^3$ | 0 | 0 | 0 | 3 | 18 | 25 |
| $3x10^3$ | 0 | 0 | 0 | 4 | 20 | 25 |
| $4x10^3$ | 0 | 0 | 0 | 4 | 15 | 25 |
| $5x10^3$ | 0 | 0 | 0 | 3 | 15 | 25 |

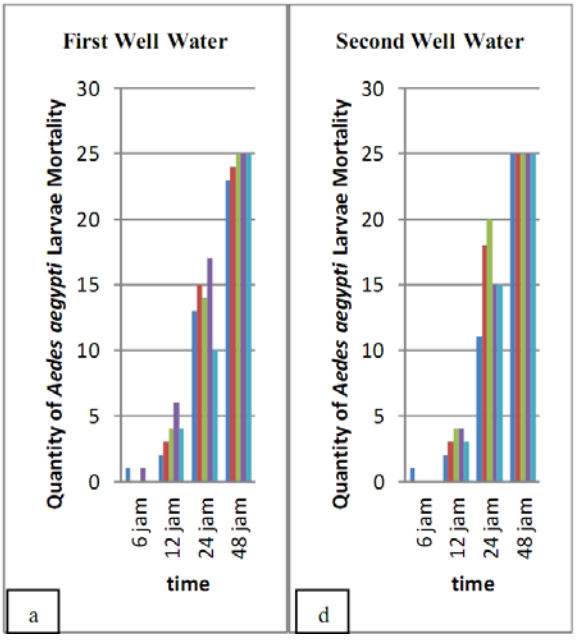
**Tabel 5. Effectiveness Test in AedesaegyptiLarvae at Second PAM Water**

| Concentration (ppm) | 2h | 4h | 6h | 12h | 24h | 48h |
|---|---|---|---|---|---|---|
| $10^3$ | 0 | 0 | 1 | 2 | 13 | 25 |
| $2x10^3$ | 0 | 0 | 1 | 1 | 18 | 25 |
| $3x10^3$ | 0 | 0 | 2 | 4 | 19 | 25 |
| $4x10^3$ | 0 | 0 | 1 | 3 | 14 | 25 |
| $5x10^3$ | 0 | 0 | 1 | 2 | 16 | 25 |

**Tabel 6. Effectiveness Test in AedesaegyptiLarvae at Third PAM Water**

| Concentration (ppm) | 2h | 4h | 6h | 12h | 24h | 48h |
|---|---|---|---|---|---|---|
| $10^3$ | 0 | 0 | 1 | 1 | 11 | 25 |
| $2x10^3$ | 0 | 0 | 0 | 2 | 17 | 25 |
| $3x10^3$ | 0 | 0 | 1 | 5 | 20 | 25 |
| $4x10^3$ | 0 | 0 | 1 | 3 | 16 | 25 |
| $5x10^3$ | 0 | 0 | 0 | 2 | 18 | 25 |

Brotowali (Tinosporacrispa) can be used to break the life cycle of mosquitoes because it contains tinokrisposid. From the results of effectiveness test in Aedes aegypti larvae are shown in some tables above show that for 48 hours with an interval time of 2 hours, 4 hours, 6 hours, 12 hours, and 48 hours and the number of larvae in each test sample is 25 with two variations from two types of water that are frequently used by the society are (well water) and PAM/PDAM water (tap water) which each variation contain 5 concentration of 100 g/100 ml or $10^3$ ppm, 200 g/100 ml or $2x10^3$ ppm, 300g/100 ml or $3x10^3$ ppm, 400 g/100 ml or $4x10^3$ ppm, and 500 g/100 ml or $5x10^3$ ppm with 3 repetitions.
The results of effectiveness test can be seen in the following graphic.



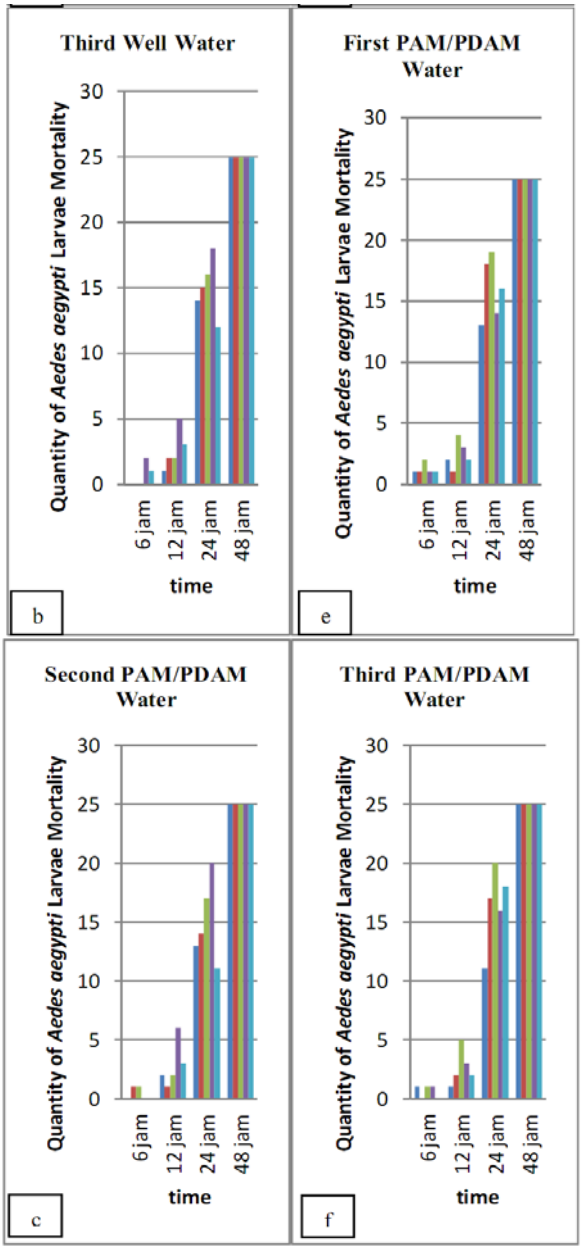




**Figure 1.Results on graph of effectiveness abate test in Aedes aegypti larvae (a) repeating 1st at well water, (b) repeating 2st at well water, (c) repeating 3st at well water, (d) repeating 1st at PAM water, (e) repeating 2st at PAM water, (f) repeating 1st at PAM water**

If we observe form of curve in order to see the change of larvicides test results, it can be seen as follows:

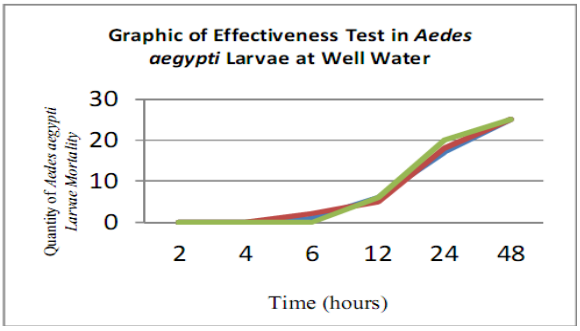



**Figure 2.Graph of Concentration Effektiviness 400 grams/100 ml or $4x10^3$ ppm for Well Water**

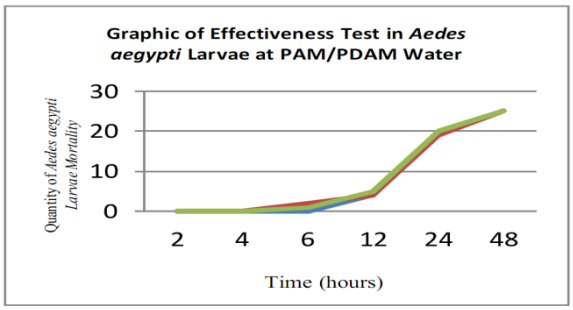



**Figure 3.Graph of Concentration Effektiviness 400 grams/100 ml or 4x10$^3$ ppm for PAM/PDAM Water**

The chart and graphic above shows the effectiveness of abate with each concentration in the interval time of Aedes aegypti larvae. Dark blue color indicates a concentration of 100 mg/100 ml or 103 ppm, brown color indicates a concentration of 200 mg/100 ml or 2x103 ppm, light green color indicates a concentration of 300 mg/100 ml or 3x103 ppm, purple indicates a concentration of 400 mg/100 ml or 4x103 ppm, and light blue color indicates a concentration of 500 mg/100 ml or 5x103 ppm. It is clearly seen that the suitable concentration for well water is 400 mg/100 ml or 4x10$^3$ ppm marked with purple color chart, and a suitable concentration for tap water is 300 mg/100 ml or 3x10$^3$ ppm marked with brown color chart.

As we know that the brotowali stems (Tinosporacrispa). Is the plants which were encountered and easily planted in indonesia. Brotowali (Tinosporacrispa) can be used to break mosquito life cycle because it contains tinokrisposid.

Abateusedin the testAedesaegypti larvaealsowere characterizedusingSEMandEDSphoto.

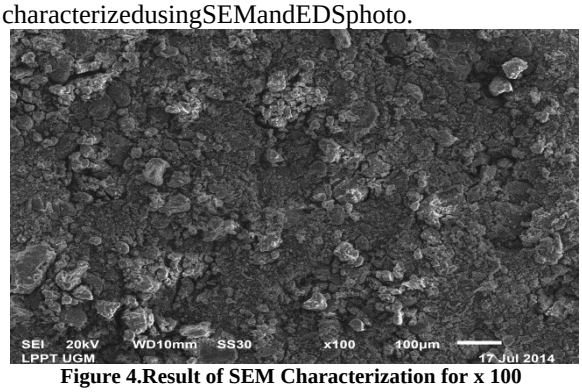



**Figure 4.Result of SEM Characterization for x 100 Magnifications**

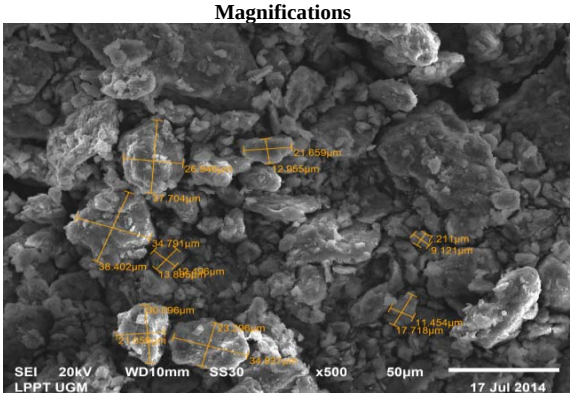



**Figure 5.Result of SEM Characterization for x 500 Magnifications**

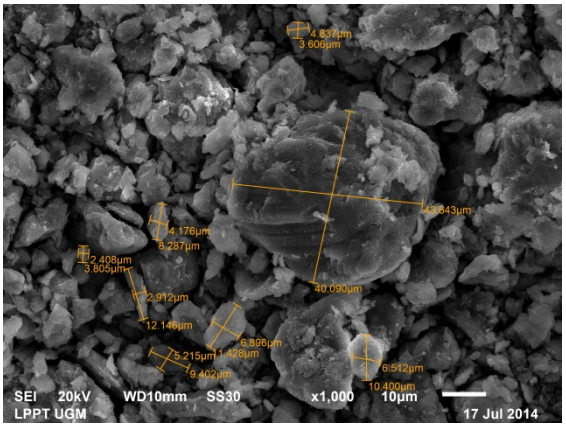



**Figure 6.Result of SEM Characterization for x 1000 Magnifications**

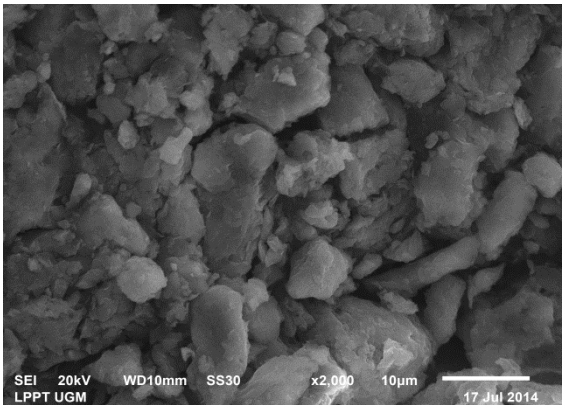



**Figure 7.Result of SEM Characterization for x 2000 Magnifications**

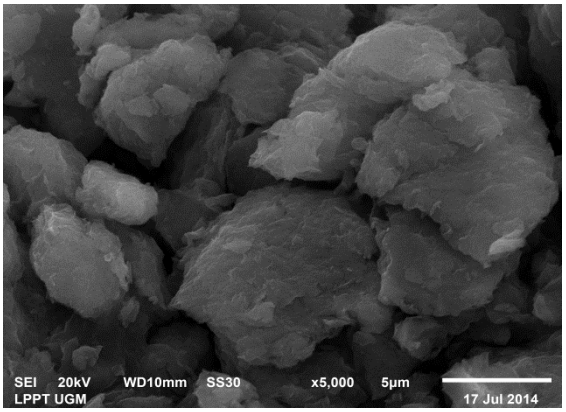



**Figure 8.Result of SEM Characterization for x 5000 Magnifications**

Figures 4, 5, 6, 7, and8arerecordedimageshownofcharacterizationanalysisScanning Electron Microscope(SEM). Figure 4shows theresults ofSEM(Scanning Electron Microscope) with x100 magnification, figure 5shows the results ofSEM(Scanning Electron Microscope) withx500 magnification, figure 6shows the results ofSEM(Scanning Electron Microscope) withx1000 magnification, figure 7shows the results ofSEM(Scanning Electronmicroscope) with x2000 magnification, figure 8shows the results ofSEM(Scanning Electron Microscope) withx5000magnification.

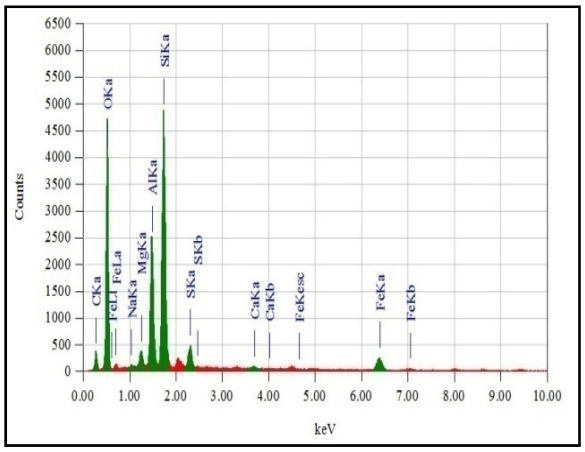



**Figure 9.Result of EDS (Energy Dispersive X-ray Spectroscopy) Characterization with x500 Magnification**

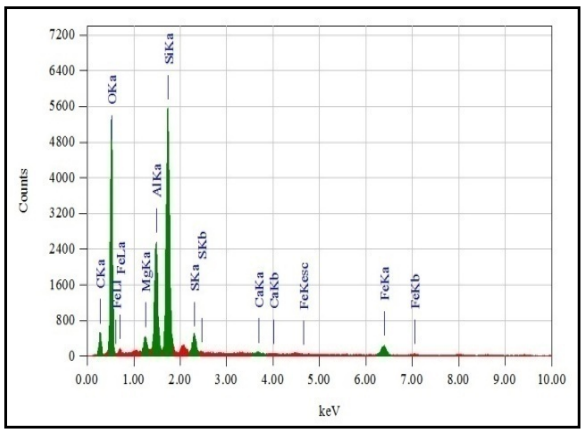



**Figure10.Result of EDS (Energy Dispersive X-ray Spectroscopy) Characterization with x1000 Magnification**

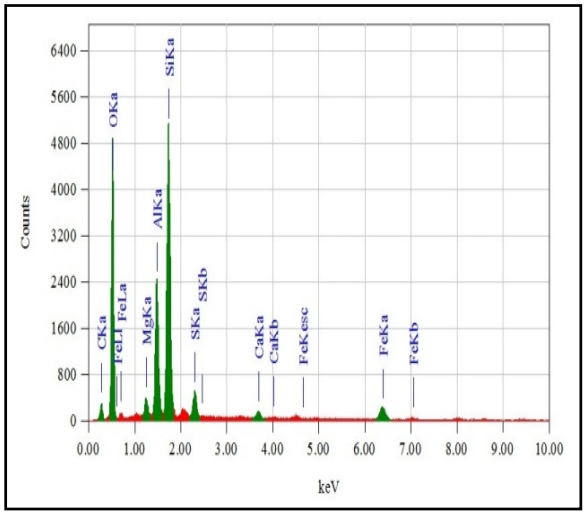



**Figure11.Result of EDS (Energy Dispersive X-ray Spectroscopy) Characterization with x500 Magnification**

Figure 9, 10, and11arerecordedphotoimageshownofcharacterizationanalyzesEDS(Energy Dispersive X-ray Spectroscopy). Figures 9 and11show the results ofEDS(Energy Dispersive X-ray Spectroscopy) with a magnification ofx500, Figure 10shows the resultsof EDS(Energy Dispersive X-ray Spectroscopy) with a magnificationx1000.

## CONCLUSION

The test of the effectiveness of using ensemble with three systems in one time. The data obtained is calculated by the statistics method. Larvacide test showed that to make abate actively working as losing Aedes aegypti larvae in 6-48 hours and the concentration suitable for water wells is 400 mg / 100 ml or 4 $x10^3$ ppm and the one suitable for tap water is 300 mg / 100 ml or 3 x $10^3$ ppm.

The result particle morphology of this sonication methods are mesoporous nanoparticles. The particle size distribution looks good for most of the samples although looks a little clumping occurs when the absorption of bentonite. Morphology and particle size distribution are formed from the results of sonication for 8.3 hours to 250 ml of pure brotowali stem extract with a temperature of $30^0$C.

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