

THE EFFECTIVENESS OF NEEM LEAF EXTRACT (*Azadirachta Indica Juss*) IN ETHANOL AS REPELLENT FOR THE BITES OF *Aedes Aegypti*

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Background : *Aedes aegypti* mosquito is the primary vector of dengue fever is common in tropical countries, including Indonesia. Control measures with eradication of vector / adult mosquitoes using plant neem (*Azadirachta indica Juss*) containing compound azadirachtin, salanin, nimbin and nimbidin which can inhibit the appetite of adult mosquito *Aedes aegypti*. The purpose of this study to determine the effect of neem leaf extract (*Azadirachta indica Juss*) on the growth and development of the mosquito *Aedes aegypti*.

Methods : This research method using descriptive experimental (purely experimental test). Samples of neem leaf extract and as an indicator is adult mosquito *Aedes aegypti*. The concentration of neem leaf extract used 0% (g/ml) (control), 10% (g/ml), 20% (g/ml), 30% (g/ml), 40% (g/ml), 50% (g/ml), 60% (g/ml), and excuted the experiment twice.

Results : Frequency bite of *Aedes aegypti* was 210 at concentration of 10%, 195 at concentration of 0%, 164 at concentration of 30%, 138 at concentration of 40%, 129 at concentration of 50%, 88 at concentration 60%.

Keywords: *Aedes aegypti*, *Azadirachta indica Juss*

In general, the *Aedes aegypti* mosquito is a big enough problem that concerns public health in countries with tropical climates, including Indonesia. *Aedes aegypti* is a vector of several serious diseases that can infect humans, such as malaria, encephalitis, "yellow fever", dengue fever, dengue, filariasis, and arboviruses. One of the major problems in Indonesia caused by the mosquito *Aedes aegypti* is dengue fever and dengue hemorrhagic fever.

One of the major problems posed in Indonesia by the *Aedes aegypti* mosquito is dengue fever and dengue hemorrhagic fever. Dengue Hemorrhagic Fever (DHF) is one of the diseases that has no cure or vaccine. The treatment is supportive only in the form of bed rest and fluids intravena. Eradicate action, by wiping out mosquito lair and killing larva and adult mosquitoes, is the best course of action (Aradilla, 2009).

This disease go into Indonesia in 1968 through the port of Surabaya. In 1980 DHF has been reported spread in all provinces in Indonesia. DHF clinical symptoms such as high fever which lasts continuously for 2-7 days and bleeding manifestations are usually preceded by seeing the typical signs such as

red spots (petechia) on patient's body. The patients can experience shock and death. Until now the disease is still a public health problem. The main vector of DHF is garden mosquito called *Aedes aegypti*, while the potential vector is *Aedes albopictus* (Soegijanto, 2004).

Thrombocyte and endothelial cells are estimated to have important role in the pathogenesis of dengue fever and dengue hemorrhagic fever, based on the fact that dengue fever caused thrombocytopenia accompanied by increased capillary permeability. The two components have long been known to be an unity of function in maintaining homeostasis, if one component injuries, it will affect the activity of other components. Thrombocytopenia (thrombocyte count $<100,000/\text{mm}^3$) is one of laboratoric criteria in addition to increased hemotrokrit $>20\%$ of the criteria for dengue diagnosis, according to WHO (Soegijanto, 2006).

In Indonesia, dengue disease is a health problem because there are many endemic areas. DHF endemic area in general is a source of spreading the disease to other areas. Every extraordinary events (KLB) dengue usually begin with an increasing

number of cases in that region. To limit the spreading of dengue disease eradicating mosquito lair movements, fogging, and larvatiditation are needed continuously (Widoyono, 2011). One of the plants that contain botanical insecticide (naturally) is the neem leaves (*Azadirachta indica* Juss). Plant neem (*Azadirachta indica* Juss) belonging to familia Meliaceae. Neem, especially the seeds and leaves, contain several components from secondary metabolic production allegedly very useful in agriculture field (pesticides and fertilizers) and in pharmaceutical field (cosmetics and medicines). Among others are Azadirachtin, salanin, meliantriol, Nimbin and Nimbidin which are the best essence in agricultural (pesticides and fertilizers) and pharmaceutical (cosmetics and medicines). Based on research conducted by RD Ndione, O Faye, M Ndiaye, A DIEYE, and JM Afoutou in 2007, using seeds of neem leaves against *Aedes aegypti* Linnaeus 1762, which also contains azadirachtin, salalinin, meliantriol, Nimbin and Nimbidin, capable to kill *Aedes aegypti* larva. The effect of neem leaf extract insecticides against *Aedes aegypti* larva (Aradilla, 2009).

It is known that Azadirachtin is food appetite derivation and ecdyson blocker (insect growth hormone blockers). Salanin is one of food appetite derivation. Meliantriol act as a deterrent (repellent) so that insects are reluctant to approach these plants. Nimbin and Nimbidin have antimicrobial activity, antifungal and antiviral in humans and animals (Aradilla, 2009). Because of it, the research wants to know the effectiveness of neem leaves (*Azadirachta indica* Juss) known as natural reject test against *Aedes aegypti* mosquito.

Objective

1. To determine the effectiveness of neem leaf extract (*Azadirachta indica* Juss) as a reject test of *Aedes aegypti* to break the chain of dengue fever spreading.
2. To know the effectiveness reject power test of neem leaf extract (*Azadirachta indica* Juss) in ethanol on the bite activities of *Aedes aegypti*.
3. To determine the concentration of neem leaf extract (*Azadirachta indica* Juss) as a reject power test against *Aedes aegypti*.

Research Methods

This study is a descriptive experimental study (experimental test pure) because researchers conducted experiments the effectiveness reject power test of neem leaf extract (*Azadirachta indica* Juss) in ethanol on the bite activity of *Aedes aegypti* mosquito and then described the results in article.

Indicators in this study is *Aedes aegypti* mosquito adults because it has a better defense system o than larval instar III and IV, so that it can be assumed that the dose is able to avoid the adult mosquito.

Equipment and Materials

- a. The container in the form of gauze confinement to put mosquito,
- b. glass funnel,
- c. measuring 5 ml pipette,
- d. measuring 10 ml pipette,
- e. pipette,
- f. glass,
- g. filter paper,
- h. paper cab,
- i. Aspirator,
- j. plastic cups,
- k. gauze,
- l. plastic bottles.

Materials :

- a. Raw foods used for mosquito breeding such are rabbits or guinea pigs,
- b. Food ingredients used for breeding larva are floured chicken liver or fish pellets,
- c. Alcohol 70 %,
- d. Distilled water,
- e. Neem leaf powder.

Procedures

Aedes aegypti Rearing Procedures

Breeding mosquitoes from egg bank of *Aedes aegypti* mosquito or catching it in the wild by using an aspirator; *Aedes aegypti* mosquitoes were caught incorporated into gauze cages measuring 50x50x50 cm; Mosquitoes in the confinement fed (guinea pig blood) for 45 minutes and 10% sugar water and then enter the water compartment, inside the container circled filter paper with 1/3 position filter paper into the water where mosquitoes lay their eggs.

Every day the container is examined for the presence of eggs. If found, the next is the hatching eggs process is by way of filter paper containing the eggs, transferred to plastic trays measuring 20 x 30 cm or 30 x 40 cm and add water until 2/3 trays; Eggs that hatch into larvae (larvae) are given in the form of flour eat chicken livers and water nam; ve changed every 3 days to avoid deposition of food / in plastic cups filled with water ¼ section, then a plastic cup is inserted into the cage (cage) mosquito grown up.

Neem Leaf Extract Production Procedures

- a. The neem leaves cleaned first with clean water and then drained at room temperature for a week.
- b. The results of dried neem leaves are blended to be ready in the experiment. Do immersion by putting the blended neem leaves earlier into the jar and add the alcohol 70%.
- c. Leave it for 7 days and at the same time shake it if sediment occurred.
- d. After 7 days separate the wasted with the filtrate.
- e. Return the container / jar open.
- f. Make a dilution with the addition of distilled water.
- g. The dilution result is ready for experiments.

Experimental Procedures

- a. Prepare the eggs of *Aedes aegypti* rearing results from the egg banks; let it stand for a week to happen ekslskion eggs into adult mosquitoes,
- b. Apply the extract on hand with neem leaf extract dilution, respectively - each 5% - 12.5%; on Testing repelan power required to close the glove fingers so as not to be subjected test mosquito bites; stopwatch to calculate the duration of contact between mosquitoes to test arm;

In one test cages supplied 25 female mosquitoes in a state of hunger;

- c. The arms are used as bait to be equated first condition that is by washing with running water, then dried until it is completely dry;
- d. Each arm will be used as bait smeared repelan solution 0.1 ml to 30 cm² surface area; For each treatment tested concentrations used one arm in one cage containing 25 mosquitoes in a state of hunger;
- e. Observations were made every 5 minutes in every hour up to six hours; Calculated number of mosquitoes that land and or biting the arm;
- f. power calculated by the formula: $DR = (K - P) / K \times 100\%$. Where DR is a repellent power, while K is the number of mosquitoes landed on the control arm, and P is the number of mosquitoes landed on the arm smeared repelan. Neem leaves can be deemed to have power when the value repelan repellent Power (DR) can reach 95%.

Hypothesis :

There is a difference in the reject power test of neem leaf extracts against *Aedes aegypti* mosquito bites with a concentration of 10%, 20%, 30%, 40%, 50%, and 60%.

Data Analysis

Used the analysis of one direction variance to determine whether there are differences between treatments 1, 2, and so on with more than one repetition (Sudjana, 1994). In this study, it will tested whether there are average differences in reject power of neem leaf extracts against *Aedes aegypti* bites at concentrations of 10%, 20%, 30%, 40%, 50%, and 60%.

Tabell. The procedures for making neem leaf extract

No	Filtrate	Aquadest	Concentration (%)
1	0 mL (control)	100 mL	0
2	10 MI	90 mL	10
3	20 mL	80 mL	20
4	30 mL	70 mL	30
5	40 mL	60 mL	40
6	50 mL	50 mL	50
7	60 mL	40 mL	60

Results and Discussion

The results of the research that has been done on the effect of repellent Neem leaf extract on *Aedes aegypti* as follows :

Table 2. Effect of Neem Leaf Extracts Against *Aedes Aegypti* Within 2 Hours

	The concentration of neem leaf extract						
	0%	10%	20%	30%	40%	50%	60%
Experiment 1	375	82	74	62	57	53	31
Experiment 2	375	128	121	102	81	76	57
Total	750	210	195	164	138	129	88

After two experiments, there is a difference between the experiment 1 and the experiment 2. At concentration of 10%, there is a difference between experiment 1 (82 mosquitoes bite the arm) and experiment 2 (128 mosquitoes bite the arm). At concentration of 20%, there is a difference between experiment 1 (74 mosquitoes bite the arm) and experiment 2 (121 mosquitoes bite the arm). At concentration of 30%, there is a difference between experiment 1 (62 mosquitoes bite the arm) and experiment 2 (102 mosquitoes bite the arm). At

concentration of 40%, there is a difference between experiment 1 (57 mosquitoes bite the arm) and experiment 2 (81 mosquitoes bite the arm). At concentration of 50%, there is a difference between experiment 1 (53 mosquitoes bite the arm) and experiment 2 (76 mosquitoes bite the arm). At concentration of 60%, there is a difference between experiment 1 (31 mosquitoes bite the arm) and experiment 2 (57 mosquitoes bite the arm).

Table 3. Effect of neem leaf extract in 15 minutes (in 2 attempts)

The frequency of mosquito bites (in minute)	10%	20%	30%	40%	50%	60%
1	9	22	6	2	3	3
2	12	26	7	6	9	3
3	17	21	14	7	9	3
4	17	16	13	11	10	4
5	21	16	13	11	10	4
6	18	10	18	13	9	7
7	15	7	16	11	8	3
8	18	11	14	16	7	8
9	13	12	14	19	14	3
10	11	11	6	14	14	9
11	15	6	7	6	7	9
12	11	8	8	4	9	9
13	11	6	8	4	7	9
14	13	10	7	8	9	8
15	9	13	7	7	4	6
Total	210	195	164	138	129	88

Concentration of 10 % from first minute 9 bites, in the second minutes 12 bites, the third minutes 17 bites, in the fourth minutes 17 bites, in the fifth minutes 21 bites, in the sixth minutes 18 bites, in the seventh minutes 15 bites, in the eighth minutes 18 bites, in the ninth minutes 13 bites, in the tenth minutes 11 bites, in the eleventh minutes 15 bites, in the twelfth minutes 11 bites, in the thirteenth minutes 11 bites, in the fourteenth minutes 13 bites, and in the fifteenth minutes 9 bites, the number of mosquitoes that bite the arm at a concentration of 10 % are 210 bites.

At a concentration of 20 % from the first minute 22 bites, in the second minutes 26 bites, in the third minutes 21 bites, in the fourth minutes 16 bites, in the fifth minutes 16 bites, in the sixth minutes 10 bites, in the seventh minutes 7 bites, in the eighth minutes 11 bites, in the ninth minutes 12 bites, in the tenth minutes 11 bites, in the eleventh minutes 6 bites, in the twelfth minutes 8 bites, in the thirteenth minutes 6 bites, in the fourteenth minutes 10 bites, and in the fifteenth minutes 13 bites, the number mosquitoes that bite the arm at a concentration of 20% are 195 bites.

At a concentration of 30% in the first minute 6 bite, in the second minutes 7 bites, in the third minutes 14 bites, in the fourth minutes 13 bites, in the fifth minutes 13 bites, in the sixth minutes 18 bites, in the seventh minutes 16 bites, in the eighth minutes 14 bites, in the ninth minutes 14 bites, in the tenth minutes 6 bites, in the eleventh minutes 7 bites, in the twelfth minutes 8 bites, in the thirteenth minutes 8 bites, in the fourteenth minutes 7 bites, and in the last fifteenth minutes 7 bites. The number of mosquitoes that bite the arm are 164 bites.

At a concentration of 40 % in the first minute 2 bites, in the second minutes 6 bites, in the third minutes 7 bites, in the fourth minutes 11 bites, in the fifth minutes 11 bites, in the sixth minutes 13 bites, in the seventh minutes 11 bites, in the eighth minutes 16

bites, in the ninth minutes 19 bites, in the tenth minutes 14 bites, in the eleventh minutes 6 bites, in the twelfth minutes 4 bites, in the thirteenth minutes 4 bites, in the fourteenth minutes 8 bites, and in the fifteenth minutes 7 bites. The number of mosquitoes that bite the arm that are 138 bites.

At a concentration of 50% in the first minute 3 bites, in the second minutes 9 bites, in the third minutes 9 bites, in the fourth minutes 10 bites, in the fifth minutes 9 bites, in the sixth minutes 9 bites, in the seventh minutes 8 bites, in the eighth minutes 7 bites, in the ninth minutes 14 bites, in the tenth minutes 14 bites, in the eleventh minutes 7 bites, in the twelfth minutes 9 bites, in the thirteenth minutes 7 bites, in the fourteenth minutes 9 bites, in the fifteenth minutes 4 bites. The number of mosquitoes that bite the arm are 129 bites.

At a concentration of 60% in the first minutes 3 bites, in the second minutes 3 bites, in the third minutes 3 bites, in the fourth minutes 4 bites, in the fifth minutes 4 bites, in the sixth minutes 7 bites, in the seventh minutes 3 bites, in the eighth minutes 8 bites, in the ninth minutes 3 bites, in the tenth minutes 9 bites, in the eleventh minutes 9 bites, in the twelfth minutes 9 bites, in the thirteenth minutes 9 bites, in the fourteenth minutes 8 bites, and in the fifteenth minutes 6 bites. The number of mosquitoes that bite the arm are 88 bites.

Based on the result obtained that there are 70% of mosquito that bite the arm at concentration of 10%; there are 72,14% of mosquito that bite the arm at concentration of 20%; there are 76,57% of mosquito that bite the arm at concentration of 30%; there are 80,28% of mosquito that bite the arm at concentration of 40%; there are 81,57% of mosquito that bite the arm at concentration of 50%; and there are 87,42% of mosquito that bite the arm at concentration of 60%.

Table 4. Descriptive Data in Each Reject Power Test Concentration of Neem Leaf Extracts Against Aedes Aegypti

Anova: Single Factor
SUMMARY

Groups	Count	Sum	Average	Variance
0	3	1500	500	46875
0.1	3	420	140	4204
0.2	3	390	130	3721
0.3	3	328	109.3333	2641.333
0.4	3	276	92	1731
0.5	3	258	86	1519
0.6	3	176	58.66667	814.3333

Table 5. Results of Data Analysis of Variance Test of The Reject Power Test Concentration of Neem Leaf Extracts Against Aedes Aegypti

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	419499.81	6	69916.63	7.957258	0.000713	2.847726
Within Groups	123011.333	14	8786.524			
Total	542511.143	20				

Based on the results if the data presented in Table 4.4 shows that the value of $F_{\text{count}} = 7.957258$ and $F_{\text{criteria}} = 2.847726$, and the P - value = 0.000713. Therefore $F_{\text{hitung}} > F_{\text{criteria}}$ or P - value < 0.005 , this means that H_0 is rejected. Conclusions based on the results of data processing are there are significant differences reject power test of neem leaf extract with a concentration of 10%, 20%, 30%, 40%, 50%, 60% against the bite activities of *Aedes aegypti*. In other words, the leaf extract of neem (*Azadirachta indica* Juss) is effective in reducing (rejected) the bite activities of *Aedes aegypti*.

Conclusions

1. At a concentration of 10% the bite on the arm are 210 bites within 15 minutes.
2. At a concentration of 20% the bite on the arm are 195 bites within 15 minutes.
3. At a concentration of 30 % the bite on the arm is slightly reduced to 164 bites within 15 minutes.
4. At a concentration of 40 % the bite on the arm is reduced to 138 bites within 15 minutes.

5. At a concentration of 50% the bite on the arm reduced to 129 bites within 15 minutes.
6. At a concentration of 60% the bite on the arm reduced again to 88 bites in 15 minutes.
7. The higher concentration of the extract, the fewer number of bites on the arm at the same time. The entire test results indicate neem leaf extract as repelan which have protective power of less than 90% for 2 hours against *Aedes aegypti* contact. Neem leaf extract can serve as repelan, because it has the contents of ecdyson blockers and salanin.

Recommendations

1. Informed public to use natural insecticides, one of it is an extract of neem leaves to replace the anti-mosquito lotion made from chemicals.
2. It is need to repeated the research by using other mosquito species with different methods in order to know whether the neem leaf extract affected broadly to all types of mosquitoes.

Reference

1. Aradilla , U.S. 2009. Effectiveness Test larvicides Ethanol Extract Neem leaves (*Azadirachta indica*) against larvae of *Aedes aegypti* . Diponegoro University , Semarang .
2. Faculty of Medicine , 2004. Dengue Script Complete Training for Trainers pediatrician and Specialist Doctors in Case Management of Dengue . Jakarta : Hall Publisher University of Indonesia .
3. Hariana , A. 2008. Medicinal Plants and Usefulness . Series 2. New York: Organization .
4. Kardinan , A. 2007. Plant Repellent and Mosquito Repellent . Jakarta : Agromedia Library
5. Shinta.2012 . Media Health Research Volume 22. Potential Leaf Essential Oil Patchouli (*Pogostemon cablin* B.) , leaves Badotan (*Ageratum conyzoides* L) , Flower Boxwood (*Cananga odorata* hook F & Thoms) and leaves of rosemary (*Rosmarinus officinalis* L) For Against *Aedes aegypti* repelan , [online] available at < <http://www.medialitbang.com/html> > [Accessed 13 march 2014] .
6. Soegijanto , S. 2006. The set of papers and Infectious Tropical Diseases in Indonesia . Volume 5. New York: Airlangga University Press .
7. Soegijanto , S. 2004. Dengue Fever . Review and New Findings in 2003. Surabaya Era : Airlangga University Press .
8. Soegijanto , S. 2006. Dengue Fever . Issue 2. Surabaya : Airlangga University Press .
9. Susana, D & Light Uli J Sembiring . 2011. Health Entomology . Jakarta : Indonesian universities .
10. Poster , 2010. Indonesian Herbal Nutritious Scientific Evidence and How Racik . Volume 8. Depok : Poster Organization .
11. Warsidi , E. 2009. Hazard and Prevention of DHF . Bekasi : Key Partners .
12. WHO , 200. Prevention and Pengendahalian Dengue and Dengue . Jakarta : Medical EGC .
13. Widoyono . 2011. Tropical Diseases Epidemiology , Transmission , Prevention , and eradication . Issue 2. Jakarta : Erland .
14. Yuniarsih , E.2010 . Test Effectiveness repelan Lotion Neem Oil (*Azadirachta indica* Juss) against *Aedes aegypti* . State Islamic University , Jakarta