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EFFECT OF BIOINSECTICIDE GRANULE MIXTURE OF SUGAR APPLE SEED (*Annonae squamosa*) AND BETEL LEAF (*Piper betle*) ON MORPHOLOGY OF EGGS *Aedes aegypti*

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ABSTRACT

Introduction: Dengue hemorrhagic fever (DHF) is an important public health problem in Indonesia because its morbidity and mortality are quite high. Prevention by vector control methods with chemical insecticides adversely affects the environment. Research on granular bioinsectide mixture of betel leaf extract and sugar apple seeds against *Aedes aegypti* is expected to be used to break the chain of *Aedes aegypti* mosquitoes starting from the juvenile stage (eggs) as an effort to control the vector population naturally. **Objective**: This study aims to analyze the effect of granule bioinsecticide mixture of sugar apple seed extract and betel leaf on changes in the morphology of *Aedes aegypti* eggs. **Methods**: This study is a true-experimental study with a Post test Only Control Group Design design. The treatment group will be given a granular bioinsecticide, a mixture of sugar apple seed extract and betel leaf, positive control will be given abate while negative control will be given aquades. Each group uses 25 eggs with 3 repetitions. On day 3, all eggs will be observed using a stereo microscope to determine morphological changes. The data obtained will be analyzed using ordinal regression test. **Results**: the ordinal regression test got the value of ρ in the Wald test between (0.012 - 0.955)> α = 0.01. **Conclusion**: there is no effect of mixed granules of betel leaf and sugar apple seeds on the mortality of *Aedes aegypti* eggs. Further research is needed on new formulations using other natural ingredients to increase the effect of ovicides on Aedes aegypti.

ABSTRAK

***Latar Belakang**: Penyakit demam berdarah dengue merupakan masalah kesehatan masyarakat penting di Indonesia karena morbiditasnya dan mortalitasnya cukup tinggi. Upaya pencegahan dengan metode pengendalian vektor (Aedes aegypti) dengan insektisida kimia dapat berdampak buruk terhadap lingkungan. Pengujian bioinsektida granula campuran ekstrak daun sirih dan biji srikaya terhadap telur Aedes aegypti diharapkan dapat digunakan untuk memutus rantai perkembangbiakan nyamuk Aedes aegypti mulai dari stadium terkecil (telur) sebagai upaya pengendalian populasi vektor secara alami. **Tujuan**: penelitian ini bertujuan menganalisis pengaruh bioinsektisida granula campuran ekstrak biji srikaya dan daun sirih terhadap perubahan morfologi telur Aedes aegypti. **Metode**: Penelitian ini merupakan penelitian true-experimental dengan desain Post test Only Control Group Design. Kelompok perlakuan akan diberi bioinsektisida granula campuran ekstrak biji srikaya dan daun sirih, kontrol positif akan diberi abate sedangkan kontrol negarif diberi aquades. Masing-masing kelompok menggunakan 25 butir telur dengan 3 kali pengulangan. Pada hari ke 3 semua telur akan diamati menggunakan microscope stereo untuk mengtahui perubahan morfologi. Data yang didapatkan akan dianalisis menggunakan uji regresi ordinal. **Hasil**: berdasarkan hasil uji regresi ordinal di dapatkan nilai ρ pada uji wald antara (0,012 – 0,955) > α = 0,01. **Kesimpulan**: tidak ada pengaruh granula campuran ekstrak daun sirih dan biji srikaya terhadap kematian telur Aedes aegypti. Perlu adanya penelitian lebih lanjut tentang formulasi baru menggunakan bahan alami lain untuk meningkatkan efek ovisida pada Aedes aegypti.*

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***Kata Kunci :** daun sirih, biji srikaya, Aedes aegypti, bioinsektisida*

Keywords : betel leaf, sugar apple seeds, *Aedes aegypti,* bioinsectide

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Introduction:

Dengue hemorrhagic fever (DHF) is an important public health problem in Indonesia.

DHF vector control which is mostly carried out by the community and government, namely The chemical method uses a factory-made insecticide that causes it resistance to mosquitoes, besides the chemicals contained in insecticides can cause environmental damage (Iftita, 2016). The World Health Organization (WHO) has since 1985 advocated seeking new breakthroughs, namely plant-based insecticides (Wahyuni, 2016). Plants that can be used as natural insecticides are betel leaves and sugar apple seeds. Granules of toxic compounds mixed with betel leaf extract and sugar apple seeds are proven to kill 95% of *Aedes aegypti* larvae with a dose of 1 g / 10 L of water in 105 minutes under LC50 (Wahyuni, 2016).

Aedes aegypti has a perfect metamorphose cycle. The metamorphosis phase consists of eggs, larvae, cocoons (pupae) and adult mosquitoes (imago) (Hestiningsih, Widjanarko, & Purwantisari, 2019). Stadium eggs are a stage that is very susceptible to insecticides, so that when eradicated at the egg stage it will be more profitable (Maretta, Kuswanto, & Septikayani, 2019). Bioactive compounds that play a role in the inhibition process of turning eggs into larvae are flavonoids, alkaloids, triterpenoids, saponins, and essential oils (Hidana et al., 2017). Sugar apple seeds and betel leaves also contain ovicidal properties such as flavonoids, saponins, essential oils, and alkaloids (Wahyuni, 2016). Research using a granular bioinsecticide mixture of sugar apple seed extract (*Annonae squamosal*) with betel leaf (*Piper betle*) is expected to break the chain of *Aedes aegypti* mosquitoes starting from the smallest stage (eggs) as an effort to control the vector population of mosquitoes that cause DHF with natural ingredients. This study aims to analyze the effect of granule bioinsecticide mixture of sugar apple seed extract and betel leaf on changes in the morphology of *Aedes aegypti* eggs.

Methods:

This research is a true-experimental research with design (Posttest Only Control Group Design). In this design there are two groups, each randomly selected. The treatment group will be given a granular bioinsecticide, a mixture of sugar apple seed extract and betel leaf, positive control will be given abate while negative control will be given aquades. The research was conducted at the Laboratory of Tropical Diseases Center, Airlangga University, Surabaya.

Selection of eggs in the study using a stereo surgical microscope Olympus CSWH10X/22 with 4 times magnification. The eggs used have a characteristic black color, the egg length is 1 mm with an oval or elongated round shape (Madona, 2020). The treatment group will be given a bioinsecticide granule mixture of Sugar Apple seed extract and Betel leaf with concentrations of 1000 ppm, 2500 ppm, 5000 ppm, 7500 ppm, and 10000 ppm. The positive control group will be given abate 1000 ppm and 10000 ppm while the negative control will be given aquades. Each group used 25 eggs with 3 times repetitions (WHO, 2005). On the 3rd day after treatment, all eggs were observed using a stereo surgical microscope Olympus CSWH10X/22 with 4 times magnification to check the shape and general morphology (surface) of the eggs. The characteristics of the egg that died after 3 days were marked by the opening of the egg shell. Another characteristic that the mosquito eggs die is the shape of the mosquito eggs becoming flattened and deflated (Yulidar 2014). The data obtained will be analyzed using ordinal regression test. This study has obtained ethical eligibility from the Health Research Ethics Commission of the Faculty of Dentistry, University of Jember with number. No.396 / UN25.8 / KEPK / DL / 2019.

Results:

The test results on changes in the morphology of *Aedes aegypti* eggs can be seen through the ordinal regression test at table 1.2.

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Table 4.7 Wald Test Results

Material	Concentration Group (PPM)	*Estimate*	*Std. Error*	*Wald*	*df*	*Sig.*	*99% Confidence Interval*	
							Lower Bound	*Upper Bound*
Abate	1000	-.247	.154	2.566	1	0.109	-1.317	-.451
	10000	.342	.155	4.870	1	0.012	-.644	.150
Aquades	0	1.000	.172	33.604	1	0.955	.555	1.444
Mixed Betel Leaf Granules and Sugar apple Seeds	1000	1.966	.233	71.208	1	0.955	1.366	2.566
	2500	1.967	.244	71.288	1	0.955	1.366	2.566
	5000	1.966	.233	71.208	1	0.955	.555	1.444
	7500	23.328	.539	1.876.4	1	0.955	21.940	24.715
	10000	-1.765	.216	66.474	1	0.955	-2.322	-1.207

The results of Wald parameter testing are in table 1.2. above explains that all concentration groups have no effect on changes in the morphology of *Aedes aegypti* eggs with a significance of ρ (0.012 - 0.955)> (α = 0.01) or in other words Ho is accepted.

Differences in the morphological conditions of eggs exposed to abate, mixed granules of sugar apple seed extract and betel leaf and the control group can be seen in the figure 1.1.

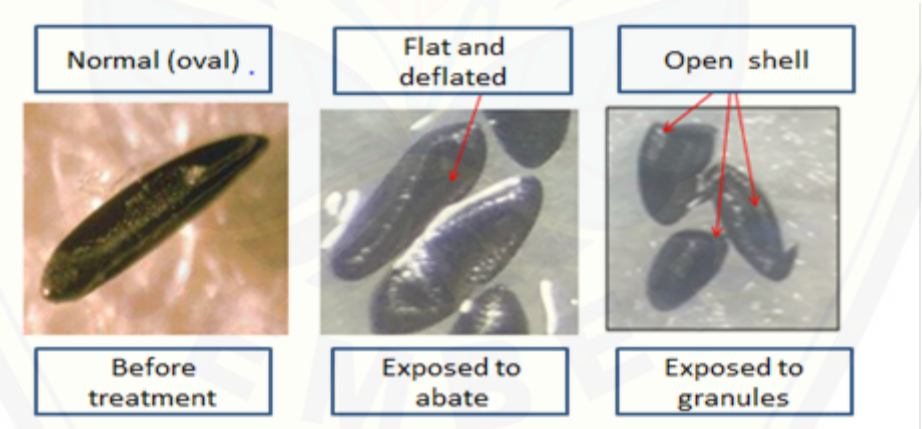

Figure 1.1 Morphological differences of *Aedes aegypti* eggs between the control group and the treatment group

Figure 1.1 shows the differences in egg conditions during the study. Egg condition before treatment looks oval or elongated. Eggs after being exposed to abate for 3 days which are marked with closed shells, the shape of the mosquito eggs becomes flat and deflated. Eggs exposed to the granules are all live eggs which are marked with an open shell.

Discussion:

The penetration of temephos (abate) into the eggs that accumulates in the glycan causes the function of glycans as an

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adhesive and strengthens the egg wall is not optimal and causes the egg wall to become brittle. Damage to the egg wall is also the result of penetration of the triflumurone into their sperm. This triflumuron is absorbed and accumulates into the egg wall, thereby reducing the permeability of the egg wall so that the eggs fail to hatch if exposed to abate. A fragile or damaged shell wall is very easy for the active ingredients to penetrate. The entry of insecticidal active substances into the eggs will enter and interfere with the metabolic process and cause various effects on the embryogenesis process in *Aedes aegypti* eggs (Yulidar, 2014). The egg turns flat when the egg and eggshell is inserted into the abate experiencing endosmosis, in this case when the active compound enters the egg it changes to dryness and is followed by shrinkage and death of the embryo in the eggshell. The endosmosis process can cause reproduction of the embryo in the egg to be inhibited (Subashini, Sivakami, & Jeyasankar, 2017)

The active ingredients contained in sugar apple seeds and betel leaves are thought to be unable or insufficient to damage the shell walls of *Aedes aegypti* eggs so that the eggs remain alive with exposure to the same concentrations as abate. There needs to be an increase in concentration (exceeding the limits of the requirements for larvicide use by WHO) or a new formulation using another part of sugar apple, namely the leaf part to increase the effect of ovicides on eggs. Research conducted by Purwaningsih et.al in 2015 shows that the extract from sugar apple leaves (*Anonna squamosa* L) can inhibit the growth of *Aedes aegypti* eggs into larvae and can even damage the eggs resulting in damage to the egg shells, with a concentration of 100 ppm (Purwaningsih, Kardiwinata, Wayan, & Utami, 2015).

Another reason why the mixed granules of betel leaf and sugar apple seed are unable to inhibit the hatching of aedes eggs is that the combination of sugar apple seed extract and betel leaf is thought to produce new compounds that are protective against *Aedes aegypti* egg shells or cause antagonistic properties. The use of two or more types of pesticides in synergy is said to be compatible with one another. Conversely, if its use decreases its effectiveness, then the pesticide is categorized as antagonistic or incompatible (Andi, 2019). Further research is needed to determine the active ingredient content of the granule mixture of sugar apple seeds and betel leaf to ascertain whether there are new compounds due to the mixture.

Conclusions:

Based on the results of the study, it can be concluded that the granular bioinsecticide mixture of sugar apple seeds and betel leaf cannot cause morphological changes (hatched eggs) in *Aedes aegypti* egg shells. Further research is needed to determine the formulation of another natural ingredient which is *Aedes aegypti* ovicide

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