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The Egg Hatchability and The Development of *Aedes aegypti* Mosquitoes in Ethanol Extracts of The Leaves of Bitter Melon (*Momordica charantia L.*) and Basil (*Ocimum basilicum L.*)

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Abstract. The botanical insecticides acquired from the ethanol extracts of the leaves of Bitter Melon (*Momordica charantia L.*) and Basil (*Ocimum basilicum L.*) has been conducted on dengue fever mosquitoes' vector of *Aedes aegypti* by observing the parameters; hatchability of eggs-larvae, the development of larvae-pupae, and pupae-adult using a complete randomized design (CRD) factorial, 5 iterations, with 6 concentration treatments (0, 200, 400, 600, 800, 1000 ppm) on 25 eggs of *A. aegypti*, and parameters of secondary metabolite contents with quantitative screening methods. The results showed that the hatchability of eggs-larvae and the development of *A. aegypti* larvae were compared with control level of 0% in all concentration treatments of the ethanol extracts of bitter melon and basil are decreasing, namely; hatchability rate of eggs-larva decreases to 24% - 64% and 32% - 68% in the ethanol extracts of Bitter Melon and Basil respectively; the development of larvae-pupae declines to 32% - 80% in Bitter Melon and to 40% - 84% in Basil's ethanol extract. However, there is no downturn in the development of pupae-adult (100%) with effective concentration at 800 ppm. The results of phytochemical screening showed that there were 5 secondary metabolites (alkaloids, terpenoids, steroids, tannins, saponins) in the ethanol extract of Bitter Melon, and two metabolites (Flavonoids, Tanin) in Basil.

1. Introduction

Indonesia is a country with the increasing cases of dengue fever every year. One of them happened in North Sumatra Province and has caused death victims namely: in 2015, 44 died from 5,688 cases, 48 died from 7,777 cases in 2016. Medan ranked first in dengue fever cases, with seven victims died from 1470 cases in 2015, and ten died from 1540 cases in 2016 [1]. The high number of dengue cases in Medan is due to the city being the main hope to have a better living for the job seekers as a result of the high population growth rate. However, the residential development for the society in environmental health has not been well managed; consequently the environment quality has declined.

The government of Medan city has attempted to prevent dengue fever, including controlling the development of mosquito larvae using larvicidal abate synthetic insecticides. However, its use is thought to have caused resistance to *A. aegypti* mosquitoes. This is due to uncontrolled usage and tends to increase both in quality and quantity. It is reported that the resistance of *A. aegypti* has occurred in several cities, including; Jakarta, Indonesia [2]. Tamil District Nadu, India [3], Mansehra District, Pakistan [4], Brazil [5].



Several studies; [6], [7], [8], [9] use plants as alternative insecticides to overcome these issues. This is because the secondary metabolites contained in the plants have the quality to be insecticides, often called botanical insecticides with specific work characteristics and different from one another, namely; toxic, and inhibits appetite and growth. This kind of insecticides minimizes the possibility of resistance. Moreover, Botanical insecticides are easily decomposed in nature, and environmental damage can be avoided [10]. The leaves of Bitter Melon (*M. charantia*), and Basil (*O. basilicum*) have potential as insecticides [11].

Based on the descriptions, authors conducted research on how the hatchability of eggs and the development of *A. aegypti* mosquitoes when treated with ethanol extracts of the leaves of Bitter Melon (*M. charantia*), and Basil (*O. basilicum*) with the aim of analyzing the secondary metabolites of both plants and their effectiveness as botanical insecticides against the eggs hatchability rate and the development of *A. aegypti* mosquitoes.

2. Methodology

2.1. Experimental animals

This study used *A. aegypti* eggs obtained from the Medan Center for Environmental Health Engineering and Control of Communicable Diseases.

2.2. Research architecture

The tests of the eggs hatchability and the development of *A. aegypti* implemented the Complete Randomized Design (CRD) factorial; three iterations; two types of plants, Bitter Melon (*M. charantia*), and Basil (*O. basilicum*); and five extract concentrations (0, 200,400, 600,800, 1000 ppm.).

2.3. Procurement of ethanol extracts of Bitter Melon leaves (*M. charantia*) and Basil leaves (*O. basilicum*) [12].

The leaf powder of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*) were macerated with 96% ethanol for 3 x 24 hours until the macerate has a transparent color. A rotary evaporator evaporated the obtained macerate (at a temperature of 40° C), then obtained the concentrated ethanol extracts of Bitter Melon leaves (*M. charantia*) and Basil leaves (*O. basilicum*).

Observation of testing parameter

2.3.1. The test on the secondary metabolite content of the ethanol extracts of Bitter Melon (*M. charantia*), and Basil (*O. basilicum*) [12].

The content of secondary metabolites (Saponins, Tannins, Alkaloids, Terpenoids, and Flavonoids) from the ethanol extracts of the leaves of Bitter Melon (*M. charantia*), and Basil (*O. basilicum*) were observed qualitatively.

2.3.2. Saponin

The ethanol extracts of the leaves of Bitter Melon (*M. charantia*), and Basil (*O. basilicum*) were diluted with hot water up to 10 times, then filtered and shaken for five minutes. Let the mixture stand to see the presence or absence of foam formation. If the foam is formed, then it may contain Saponin. The test was carried out by adding a few drops of concentrated sulfuric acid to the foam formed. Qualitatively, saponin compounds can be seen if the foam is formed stable after dripping the concentrated sulfuric acid.

2.3.3. Tannin

The ethanol extracts of the leaves of Bitter Melon (*M. charantia*), and Basil (*O. basilicum*) were diluted to 10 times with hot water, then filtered. Gelatin was added to the filtrate. Qualitatively, tannin compounds can be known if white precipitates are formed after the addition of gelatin drops.

2.3.4. Alkaloid

Four drops of ethanol extracts of the leaves of Bitter Melon (*M. charantia*), and Basil (*O. basilicum*) were inserted into two plates (each plate contains two drops of ethanol extracts). The first plate, contained two drops of ethanol extracts of Bitter Melon (*M. charantia*), and Basil (*O. basilicum*), formed a deposition of orange to red-brown, while the second plate containing two drops of ethanol extracts of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*) with addition of Mayer reagents formed a white precipitate.

2.3.5. Terpenoid

Two drops of ethanol extracts of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*) with extra 2-3 drops of acetic anhydride (AC₂O) were added in one hole, and 1-2 drops of concentrated H₂SO₄ 1-2 were put in another hole as a comparison. The portion added with AC₂O was stirred slowly for a while until dry, then inserted 1-2 drops of concentrated H₂SO₄. Green or bluish-green coloration indicates the terpenoid.

2.3.6. Flavonoid

Two drops of ethanol extracts of the leaves of Bitter Melon (*M. charantia*), and (*O. basilicum*) were put into a plate with two drops of HCl and Magnesium powder. Formation of orange color indicates the presence of flavonoids.

2.4. The test on the ethanol extract of the leaves of Bitter Melon (*M. charantia*), and Basil (*O. basilicum*) against the eggs hatchability rate and the development of *A. aegypti*

The egg hatchability test was carried out by setting six concentrations of ethanolic extracts of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*), below the concentration of LC₅₀ and one control, each with three iterations. In a glass, 100 ml of the solution was poured in each concentration of ethanol extracts of the leaves of Bitter Melon (*M. charantia*), and Basil (*O. basilicum*), then 25 test eggs were placed in the solution and were examined 24 hours for 15 days. Afterward, the number of eggs, larvae, pupa-adult were observed.

2.5. Data analysis

Each data from the observation parameters were analyzed by variance, if there were significant differences followed by the Tukey test at the 5% confidence level (SPSS release 18), so at the end of the study, it can be seen which concentrations of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*) have the most effective impact on the eggs hatchability and the development of *A. aegypti*.

3. Results and Discussions

The results of the study on the hatchability rate of eggs-larvae and the development of larvae-pupae, pupae-adult of *A. aegypti* mosquitoes in the ethanol extract of the leaves of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*) were obtained as shown in the following figures and table:

From Figure 1, it can be seen that the hatchability of eggs-larvae in all treatment concentrations compared to the control (0%) has decreased and was directly proportional to the concentration increment in both types of plants; Bitter Melon (*M. charantia*) and Basil (*O. basilicum*). The hatchability of eggs-larvae with an amount of 9-19 larvae samples was at 36% -76%, then it decreased to 24% - 64% and the hatchability rate of 8-17 larvae in the ethanol extracts of Basil has also decreased to 32% - 68%.

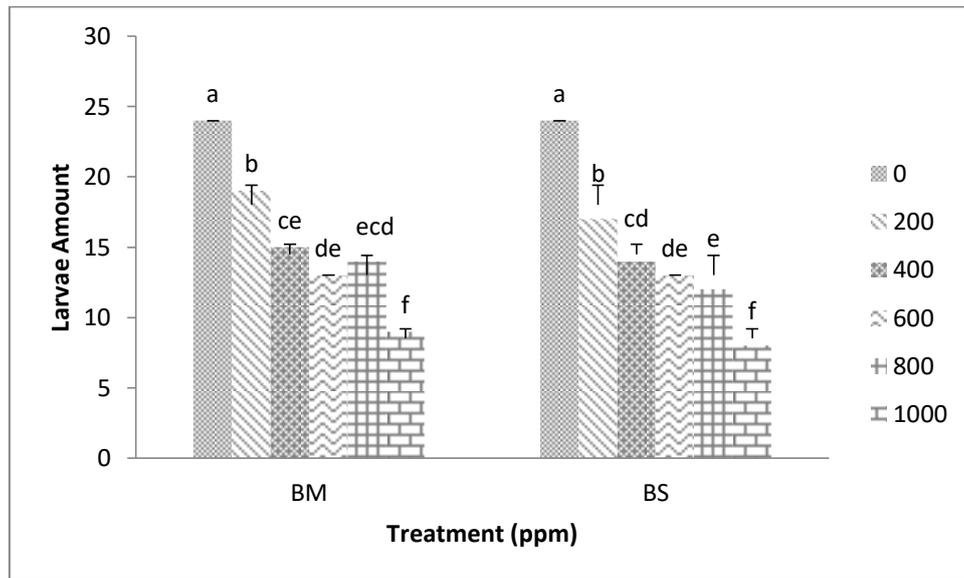


Figure 1. Hatchability rate of *A. aegypti* eggs-larvae in ethanol extracts of the leaves of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*). Details: BM= Bitter Melon, BS= Basil. The graph with the same letter notation in the same group and the different group are significantly different based on the Tukey test at the level of 5%

In Figure 2, it is showed that when compared with the control of 0%, the value of larvae-pupae development with total sample of 5-17 pupae has declined from 20% - 68% to 32% - 80% in the ethanol extract of bitter melon, while in Basil, The development values decrease from 16% -60% to 40% -84% with a sample amount of 4-15 pupae.

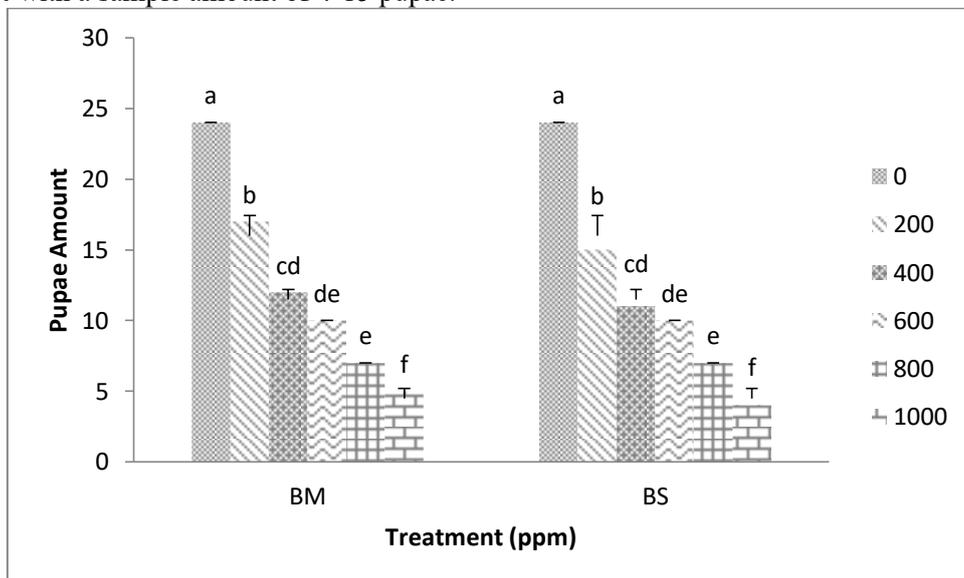


Figure 2. The development of *A. aegypti* larvae-pupae in ethanol extracts of the leaves of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*). Details: BM= Bitter Melon, BS= Basil. The graph with the same letter notation in the same group and the different group are significantly different based on the Tukey test at the level of 5%.

From Figure 3, in general, the development of pupae for all treatment concentrations in the ethanol extract of bitter melon and basil, compared to the control was remained stable, meaning that all pupae develop into adults by 100%

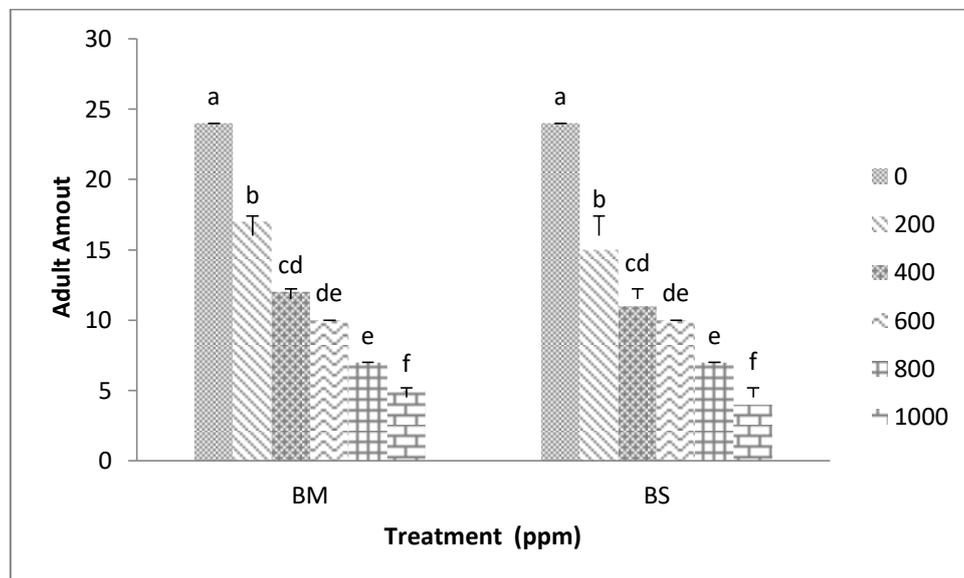


Figure 3. The development of *A. aegypti* larvae-pupae in ethanol extracts of the leaves of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*). Details: BM= Bitter Melon, BS= Basil. The graph with the same letter notation in the same group and the different group are significantly different based on the Tukey test at the level of 5%.

From Figures 1, 2, and 3, it can be seen that there are differences in the inhibition of eggs-larvae hatchability, and the development of larvae-pupae and pupae-adults of *A. aegypti* in ethanol extract of the leaves of Bitter Melon (*M. charantia*), Basil (*O. basilicum*). This happens because the two plants have different contents of secondary metabolites, namely; 5 secondary metabolites (alkaloids, terpenoids, steroids, tannins, saponins) in Bitter Melon and two in basil (flavonoids, tannins) which is shown in Table 1. *Callitris glaucophylla* extract [13], *Moringa oleitera* seeds [14], and *Ipomoea cairica* [15] may also cause *A. Aegyti* developmental mortality and inhibition. The self-defense against insect pest of a plant is determined by secondary metabolites which the plant contains, and its effects on insects will vary, among others, are toxic and inhibiting the development and reproduction of insects [16].

It can be observed in Figure 2, there was an inhibition of the larvae-pupae development in all treatments, but did not affect the adult development (Figure 3). All the adults are 100% developed. This indication shows that when larvae develop into pupae, the larval mortality occurs, but pupa mortality does not happen when developing into adulthood. This is due to apoptosis or cell death at the larval level at all given treatment concentrations, but apoptosis at the pupae stage does not occur. As explained by [17], high concentrations of *Momordica charantia* extract and DMPA can cause cell death (apoptosis). Furthermore, a study explained the inhibition of fecundity (eggs-larvae) and the growth of larvae-pupae and pupae-adults, allegedly because the ethanol extracts of the leaves of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*) can become Insect Growth Regulating hormone, which is Halofenozide molting hormone (RH-0345) [18].

Table 1. Phytochemical Test of Ethanol Extracts of the leaves of Bitter Melon (*Momordica charantia*) and Basil (*Ocimum basilicum*)

Phytochemical Test	<i>Momordica charantia</i>	<i>Ocimum basilicum</i>
Flavonoid	-	+
Alkaloid	+	-
Terpenoid	+	-
Steroid	+	-
Tanin	+	+
Saponin	+	-

Note: + means Positive; - means Negative

From Table 1, the ethanol extracts of the leaves of Bitter Melon and Basil have different secondary metabolites contents, namely; five contents (alkaloid, terpenoid, steroid, tannin, and saponin) in Bitter Melon and two contents (flavonoid and tannin) in Basil. It causes differences in the hatchability rate of eggs-larvae, and the development of larvae-pupae and pupae-adults in the ethanol extracts of Bitter Melon and Basil will be different. The content of different secondary metabolites in a plant will cause a distinctive insecticidal effect on insects [16].

4. Conclusions

Based on the results on the study of the eggs hatchability and the development of *A. aegypti* mosquitoes in the ethanol extract of the leaves of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*), it can be summarized as follows:

- All treatment of ethanol extract of the leaves of Bitter Melon (*M. charantia*) and Basil (*O. basilicum*), when compared with the control, decreased the hatchability rate of eggs-larvae to 24% - 64% and 32% - 68% in Bitter Melon and Basil respectively, while the development of larvae-pupae declined to 32% - 80% in Bitter Melon and 40% - 84% in Basil.
- There's no downturn at the development of pupae-adults in all treatments. All the adults are 100% developed
- The two plants contain different contents of secondary metabolites. Bitter Melon has five contents (alkaloid, terpenoid, steroid, tannin, and saponin) while Basil has two (flavonoid and tannin).

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