

## THE EFFECT OF FRANGIPANI FLOWER (*PLUMERIA ACUMINATA* AIT) EXTRACT WITH GRANULE FORMULATION ON MORTALITY OF *Aedes Aegypti* Linn Instar III Larvae

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### ABSTRACT

Dengue hemorrhagic fever is a disease transmitted through the bite of the *Aedes aegypti* mosquito and is widely found in Indonesia. The main key in dealing with *Aedes aegypti* mosquitoes is to control their spread and eradicate larvae. Vegetable larvicide frangipani flower extract formulated in granule form can be an alternative in vector control. The purpose of this study was to analyze the effect of frangipani flower (*Plumeria acuminata* Ait) extract with granule formulation as vegetable larvicide against *Aedes aegypti* Linn instar III larvae. The study was conducted in the laboratory of B2P2VRP Salatiga in 2015. The research method used was a pure experiment with 6 concentrations of 1.6%; 1.9%; 2.7%; 3.4%; 4.3% and 4.7% were accompanied by 4 repetitions. The total sample of larvae in the study amounted to 800 heads. Observations were made for 2,4,6 and 24 hours. Based on the results of study, the effective concentration of frangipani flower extract with granule formulation against the mortality of *Aedes aegypti* larvae is 4.7% because it is able to kill larvae 100%. The results of probit analysis obtained  $LC_{50}$  values of 1.572% and  $LC_{90}$  3.025%, while the  $LT_{50}$  value at an effective concentration of 4.7% was 2.902 or 3 hours and the  $LT_{90}$  value is 9.421 or 9 hours. The ANOVA test obtained p value = 0.000 ( $p < 0.05$ ). The results showed that there were significant differences in various concentrations of frangipani flower extract granules on the mortality of *Aedes aegypti* larvae. The conclusion was that the 4.7% concentration in the formulation of frangipani flower extract granule (*Plumeria acuminata* Ait) was effective as a vegetable larvicide.

**Keywords** : *Aedes aegypti* larvae, larvicide, frangipani flower, *Plumeria acuminata* Ait, granule formulation

### ABSTRAK

Demam berdarah dengue adalah penyakit yang ditularkan melalui gigitan nyamuk *Aedes aegypti* dan banyak ditemukan di Indonesia. Kunci utama dalam menangani nyamuk *Aedes aegypti* adalah mengendalikan penyebarannya dan membasmi larva. Ekstrak bunga kamboja larvasida nabati yang diformulasikan dalam bentuk granul dapat menjadi alternatif dalam pengendalian vektor. Tujuan penelitian ini adalah untuk menganalisis pengaruh ekstrak bunga kamboja (*Plumeria acuminata* Ait) dengan formulasi granul sebagai larvasida nabati terhadap larva *Aedes aegypti* Linn instar III. Penelitian dilakukan di laboratorium B2P2VRP Salatiga pada tahun 2015. Metode penelitian yang digunakan adalah eksperimen murni dengan 6 konsentrasi 1.6%; 1.9%; 2.7%; 3.4%; 4.3% dan 4.7% disertai dengan 4 pengulangan. Total sampel larva dalam penelitian ini berjumlah 800 ekor. Pengamatan dilakukan selama 2,4,6 dan 24 jam. Berdasarkan hasil penelitian, konsentrasi efektif ekstrak bunga kamboja dengan formulasi granul terhadap kematian larva *Aedes aegypti* adalah 4.7% karena mampu membunuh larva 100%. Hasil analisis probit diperoleh nilai  $LC_{50}$  sebesar 1.572% dan  $LC_{90}$  3.025%, sedangkan nilai  $LT_{50}$  pada konsentrasi efektif sebesar 4.7% sebesar 2.902 atau 3 jam dan nilai  $LT_{90}$  sebesar 9.421 atau 9 jam. Uji ANOVA diperoleh p value = 0.000 ( $p < 0.05$ ). Hasil penelitian menunjukkan bahwa terdapat perbedaan yang signifikan dalam berbagai konsentrasi butiran ekstrak bunga kamboja terhadap mortalitas larva *Aedes aegypti*. Kesimpulannya adalah konsentrasi 4.7% dalam formulasi granul ekstrak bunga kamboja (*Plumeria acuminata* Ait) efektif sebagai larvasida nabati.

**Kata kunci** : larva *Aedes aegypti*, larvasida, bunga kamboja, *Plumeria acuminata* Ait, formulasi granul

## INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is one of the infectious diseases characterized by sudden high heat for no apparent reason accompanied by red spots on the skin. DHF is caused by the dengue virus which is transmitted to humans through the bite of the *Aedes aegypti* mosquito (Kementerian Kesehatan RI, 2011). DHF cases have increased drastically throughout the world, it is estimated that 50-100 million people worldwide are infected with dengue hemorrhagic fever every year (Kementerian Kesehatan RI, 2014). The number of dengue cases in Indonesia in 2013 was reported as many as 112511 cases with the number of deaths of 871 people (Incidence Rate = 45.85 per 100000 population and CFR / death rate = 0.77%) there was an increase in the number of cases in 2013 compared to 2012 which amounted to 90245 cases with IR 37.27 (Dinas Kesehatan Provinsi Jawa Tengah, 2013).

In 2013 the number of dengue cases in Semarang City was 2364 cases, up 89.11% from 1250 cases in 2012. The number of deaths in 2013 was 27 cases, up 22.73% from 2012 which amounted to 22 cases. The Incidence Rate (IR) of dengue fever in Semarang City from 2006 to 2013 has always been much higher than the IR of Central Java DHF and the IR of National DHF. In 2013 the IR of dengue in Semarang City was twice as high as the IR of dengue in Central Java (Dinas Kesehatan Kota Semarang, 2013).

Based on the report of the Semarang City Health Office in 2022, the situation of dengue fever cases for the last five years (2017–2022) had decreased in 2018 and then increased. The number of cases in 2022 was 865 cases with 33 deaths so that the IR value was 51.7 per 100000 population and CFR 3.82% (Dinas Kesehatan Kota Semarang, 2022). This shows that dengue cases since 10 years ago until now are still concerning and require handling efforts in eradicating *Aedes aegypti* mosquitoes.

Larval eradication is a key strategy of vector control programs worldwide (Daniel, 2008). Accumulative use of chemicals can have a negative impact on humans and the environment (Husna, Syarifah, Bambang P., 2014). The use of temephos insecticide within 25 years as a control of the spread of *Aedes aegypti* mosquitoes, is known to be able to cause resistance (Soebaktiningsih, Roekistiningsih, 2014). The results of research by Oroh, M. Y., Pinontoan, O. R., & Tuda, 2020 stated that the incidence of dengue fever is related to the presence of physical environmental factors, biological, human, health service, so that in eradicating mosquito nests, efforts need to be made to modify the environment and community behavior. One plant that can be modified into plant-based insecticides is the Cambodian plant. Cambodian plants belong to the family Apocynaceae (Heyne, 1987). Based on the results of research by Devprakash, Rohan T, Suhas, Senthil, and Tamzih M, 2012 explained that the preliminary phytochemical screening of frangipani flower extract found the presence of several compounds such as flavonoids, fulvoplumierin, saponins, terpenoids, tannins, glycosides, steroids. In the research of Farooque, A. M. D, Mazunder, A., Shambhawe, S., and Mazunder, 2012 also stated that the active substances in frangipani flowers are flavonoid compounds, tannins, saponins and terpenoids. In addition, frangipani flowers also contain essential oils that have pharmacological activity as antimicrobials, these essential compounds can also provide a relaxing effect. In frangipani flowers, several essential compounds are found that are the main cause of the flower smelling good (Zaheer, Z., Konale, A. G., Patel, K.A., Subur, K.W., and Farooqui, 2010). The results of research by Fikayuniar, L, Abhimanyu, A. P, Maria, A. D, Sathi'ah, F. A, Yuliani, N. D, & Mahfud, 2023 stated that the oil contained in frangipani flowers (*Plumeria acuminata* Ait) can repel *Aedes aegypti* mosquitoes.

The reason why the author chose frangipani flowers is because frangipani flowers are easy to find and rarely used by the public. In addition, frangipani flowers are one of the plants that are thought to have potential as vegetable insecticides because the content contained in frangipani flowers contains flavonoids, tannins, saponins, terpenoids and essential oils so that

they can kill *Aedes aegypti* larvae. In this study, researchers chose extraction using the maceration method because the content of frangipani flowers which functions as an insecticide is soluble in oil (ethanol) then formulated into granule form to be more applicable in its use. Granules are more durable in storage and do not cause resistance. The advantage of granules is that they can protect active compounds from confounding factors, and other factors that may not be controlled (Dwi W, Joko W, 2015).

## METHOD

The type of research used is true experimental design with a post-test only control group design research design. The study was conducted in Salatiga B2P2VRP in 2015. The purpose of this study was to determine the effectiveness of frangipani flower extract (*Plumeria Acuminata* Ait) with granule form formulation against mortality of *Aedes aegypti* larvae. The population used was *Aedes aegypti* instar III larvae from Salatiga B2P2VRP Laboratory. Based on WHO references in 2005, the number of experimental animals for larvicide testing is 25 mosquito larvae per 100-200 ml (World Health Organization, 2005). Based on these data, the number of larvae needed is 175 *Aedes aegypti* larvae as many as 7 containers including control for preliminary tests without repeating while for further tests 625 *Aedes aegypti* larvae are needed as many as 7 containers including control with four repeats.

Making a test solution in the form of this extract using frangipani flowers and the solvent in making this test solution in the form of 96% ethanol, extraction by maceration. Frangipani flower extract granules were dissolved using aquades to obtain a concentration of 1.6%; 1.9%; 2.7%; 3.4%; 4.3%; 4.7% and also control up to a volume of 100 ml. Observations of larval mortality were carried out at contact times of 2 hours, 4 hours, 6 hours, and 24 hours. To calculate the data obtained from this study using One Way Anova analysis, if meaningful results are obtained in the test, LSD (Least Significance Difference) analysis of the influence of various concentrations and influences will be carried out to find out which groups are meaningful while for  $LC_{50}$ ,  $LC_{90}$ ,  $LT_{50}$  and  $LT_{90}$  analysis using the probit test.

## RESULT

### Research Environmental Conditions

The pH in the container in this study was determined by measuring using a pH stick on a solution containing frangipani flower extract granules. The pH value in the control is 7. Meanwhile, the pH at the lowest concentration of 1.6% is 5 and the highest concentration of 4.7% is 4. Average room temperature  $26^{\circ}C$  and average humidity 77%. In the results of measuring environmental conditions both water pH, room temperature and humidity do not affect the mortality of larvae. Larvae can live in the pH range between 4-11 and will die at  $pH \leq 3$  and  $\geq 12$  (Clark, T. M., Flis, B. J., & Remold, S. K., 2004). The average temperature of the research room was in the optimum range of larval growth, which was  $25-30^{\circ}C$  (Kusnindar, 1990) while for the average air humidity of the research site also did not affect because it was in the range of larval growth air humidity which was 70-89% (Jumar, 2000).

### Mortality of *Aedes aegypti* Larvae

The results showed that the lowest mortality occurred in the 2nd hour at a concentration of 1.6% as much as 7%. The lowest mortality that occurred at the 4th, 6th and 24th hours also occurred at a concentration of 1.6% as much as 12%; 17% and 56%. The highest mortality at 2nd, 4th, 6th, and 24th hours occurred at the highest concentration of 4.7% as much as 36%; 65%; 74%; and 100% while in the control group there were no deaths in larvae. Mortality in *Aedes aegypti* larvae increases according to increasing concentration.

**Table 1. Mortality of *Aedes aegypti* Larvae at Various Concentrations of Frangipani Flower Extract Granules on 2, 4, 6, and 24 Hours**

Concentration (%)	Number of <i>Ae.aegypti</i> larvae (tails) die at the hours				Average mortality of <i>Ae.aegypti</i> larvae (tails) at the hours				Percentage of mortality of <i>Ae.aegypti</i> larvae (%) at the hours			
	2	4	6	24	2	4	6	24	2	4	6	24
Control	0	0	0	0	0	0	0	0	0	0	0	0
1.6	7	12	17	56	1.75	3	4.25	14	7	12	17	56
1.9	9	18	28	63	2.25	4.5	7	15.75	9	18	28	63
2.7	15	29	43	71	3.75	7.25	10.75	17.75	15	29	43	71
3.4	17	39	57	84	4.25	9.75	14.25	21	17	39	57	84
4.3	29	46	65	95	7.25	11.5	16.25	23.75	29	46	65	95
4.7	36	65	74	100	9	16.25	18.5	25	36	65	74	100

This is possible because the toxicity of frangipani flower extract granules has begun to affect *Aedes aegypti* Linn larvae and it is proven that the death of test larvae at various concentrations of frangipani flower extract granules is caused by the active substance content contained in frangipani flowers is not influenced by environmental factors because the control does not kill *Aedes aegypti* larvae at all. The content of chemical compounds from frangipani flowers such as flavonoids, tannin saponins, terpenoids and essential oils (Farooque, A. M. D, Mazunder, A., Shambhawe, S., and Mazumder, 2012). Flavonoids and essential oils can work as powerful respiratory inhibitors or as respiratory toxins. Flavonoids and essential oils have a way of working, namely by entering the body of the larva through the respiratory system which will then cause wilting in the nerves and damage to the respiratory system and cause the larva not to breathe and eventually die (Robinson, 1995).

The essential oil used will evaporate into the air. This odor will be detected by chemical receptors (chemoreceptors) found on mosquito antennae and relayed to nerve impulses. The smell of this essential oil is not liked by mosquitoes. This is then translated into the mosquito's brain so that mosquitoes will express to avoid the source of odor (Shinta, 2012). Tannins have a bitter taste so they can cause a feeding inhibition mechanism in test animals. The possibility of the bitter taste causes test animals not to eat so that test animals will starve and eventually die (Yunita, E. A., Suparpti, N. H., & Hidayat, J. W. 2009). In addition, the compound inhibits taste receptors in the larval mouth area. This results in larvae failing to get a taste stimulus so they are unable to recognize their food so that the larvae starve to death (Febrianti & Rahayu, 2012).

The results of research by Nurseha, Q., & Asngad, 2019 stated that there is an anti-mosquito content in white frangipani flowers and suren leaves that can kill mosquitoes. Anti-mosquito compounds in white frangipani flowers are more influential than compounds in suren leaves. The results of Citra's study, 2023, there is a difference in the death of *Aedes aegypti* instar III larvae with concentrations of 0%, 1.5%, 3% and 6% frangipani flower extract. The effective concentration in killing *Aedes aegypti* larvae is a concentration of 6% because it reaches 100%. The above research is in line with the results of research researchers in 2015 who succeeded in proving the effectiveness of frangipani flower extract able to kill *Aedes aegypti* larvae 100%. Although this research was conducted 8 years ago, it is still relevant to the latest research results in 2023. This shows that frangipani flowers can be used and modified as a way of controlling the spread of *Aedes aegypti* mosquitoes.

#### Analysis Probit and One Way Anova

The  $LC_{50}$  value of frangipani flower extract granules (*Plumeria acuminata* Ait) is 1.572%, this means that to kill 50 % *Aedes aegypti* larvae within 24 hours a concentration of 1.572% is needed while for the  $LC_{90}$  frangipani flower extract granules (*Plumeria acuminata* Ait) of

3.627%, means that to kill *Aedes aegypti* larvae 90% within 24 hours requires a concentration of 3.627%. Larvicide concentration is considered effective if it can cause death of test larvae between 10-95% which will be used to find lethal concentration values (World Health Organization, 2005). The statement confirmed that the concentration of frangipani flower extract granules is effective for killing *Aedes aegypti* larvae because within 24 hours it can kill test larvae 90%.

**Table 2. LC<sub>50</sub> and LC<sub>90</sub> Values of *Aedes aegypti* Larvae at Various Observation Times**

Time (hours)	LC <sub>50</sub> (%)	LC <sub>90</sub> (%)
2	7.399	26.299
4	4.073	11.147
6	3.055	7.778
24	1.572	3.627

**Table 3. LT<sub>50</sub> and LT<sub>90</sub> Values of *Aedes aegypti* Larvae at Various Concentrations**

Concentration (%)	LT <sub>50</sub> value (hours)	LT <sub>90</sub> value (hours)
1.6	20.446	128.878
1.9	14.666	96.847
2.7	9.318	70.802
3.4	5.819	30.382
4.3	4.005	16.851
4.7	2.902	9.421

The LT<sub>50</sub> value for all concentrations of frangipani flower extract granules given is 50% effective in killing *Aedes aegypti* larvae because the time needed to kill *Aedes aegypti* Larvae does not exceed 24 hours and for LT<sub>90</sub> values not all concentrations are effective as Larvicide, but there are two highest concentrations that are effective to kill test larvae, such as: concentration of 4.3% (16 hours) and concentration of 4.7% (9 hours) effective as larvicide because the time needed does not exceed 24 hours to kill 90% of *Aedes aegypti* Linn larvae, then carried out a one-way anova test where the significance value or p-value is 0.000 which means it has a value smaller than 0.05, so the null hypothesis (H<sub>0</sub>) assumption is rejected, this means that there is a significant difference between the average number of deaths of *Aedes aegypti* instar III larvae due to exposure to frangipani flower extract granules at various different concentrations.

**Table 4. LSD Test Based on the Effect of Various Concentrations**

(I) Frangipani Flower Concentration	(J) Frangipani Flower Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.6	1.9	-1.750*	.672	.018	-3.16	-.34
	2.7	-3.750*	.672	.000	-5.16	-2.34
	3.4	-7.000*	.672	.000	-8.41	-5.59
	4.3	-9.750*	.672	.000	-11.16	-8.34
	4.7	-11.000*	.672	.000	-12.41	-9.59
1.9	1.6	1.750*	.672	.018	.34	3.16
	2.7	-2.000*	.672	.008	-3.41	-.59
	3.4	-5.250*	.672	.000	-6.66	-3.84
	4.3	-8.000*	.672	.000	-9.41	-6.59
	4.7	-9.250*	.672	.000	-10.66	-7.84
2.7	1.6	3.750*	.672	.000	2.34	5.16
	1.9	2.000*	.672	.008	.59	3.41

(I) Frangipani Flower Concentration	(J) Frangipani Flower Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	3.4	-3.250*	.672	.000	-4.66	-1.84
	4.3	-6.000*	.672	.000	-7.41	-4.59
	4.7	-7.250*	.672	.000	-8.66	-5.84
3.4	1.6	7.000*	.672	.000	5.59	8.41
	1.9	5.250*	.672	.000	3.84	6.66
	2.7	3.250*	.672	.000	1.84	4.66
	4.3	-2.750*	.672	.001	-4.16	-1.34
	4.7	-4.000*	.672	.000	-5.41	-2.59
4.3	1.6	9.750*	.672	.000	8.34	11.16
	1.9	8.000*	.672	.000	6.59	9.41
	2.7	6.000*	.672	.000	4.59	7.41
	3.4	2.750*	.672	.001	1.34	4.16
	4.7	-1.250	.672	.079	-2.66	.16
4.7	1.6	11.000*	.672	.000	9.59	12.41
	1.9	9.250*	.672	.000	7.84	10.66
	2.7	7.250*	.672	.000	5.84	8.66
	3.4	4.000*	.672	.000	2.59	5.41
	4.3	1.250	.672	.079	-1.16	2.66

\*. The mean difference is significant at the 0.05 level.

Furthermore, an analysis of the LSD (*Least Significance Difference*) test was carried out on the influence of various concentrations. Based on the LSD test, a significant difference was known between the average number of mortality *Aedes aegypti* larvae at various concentrations of frangipani flower extract granules except in pairs of concentrations of 4.3% against 4.7% and vice versa. This is because the concentration of 4.3% and 4.7% of the active substance content in frangipani flower extract granules has the same influence in killing *Aedes aegypti* larvae so that the pair does not have a significant difference.

**Table 5. LSD Test Based on the Effect of Various Observation Times**

(I) Hours	(J) Hours	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
2	4	-4.00000	2.59955	.140	-9.4226	1.4226
	6	-7.08333*	2.59955	.013	-12.5059	-1.6608
	24	-14.83333*	2.59955	.000	-20.2559	-9.4108
4	2	4.00000	2.59955	.140	-1.4226	9.4226
	6	-3.08333	2.59955	.249	-8.5059	2.3392
	24	-10.83333*	2.59955	.000	-16.2559	-5.4108
6	2	7.08333*	2.59955	.013	1.6608	12.5059
	4	3.08333	2.59955	.249	-2.3392	8.5059
	24	-7.75000*	2.59955	.007	-13.1726	-2.3274
24	2	14.83333*	2.59955	.000	9.4108	20.2559
	4	10.83333*	2.59955	.000	5.4108	16.2559
	6	7.75000*	2.59955	.007	2.3274	13.1726

\*. The mean difference is significant at the 0.05 level.

The results of the LSD test analysis of the effect of time on various pairs of average mortality values of *Aedes aegypti* larvae where the pairs of observation time 2 hours to 4 hours, 4 hours to 6 hours did not differ significantly, this is because at the time of observation the

active substance content of frangipani flower extract granules had the same influence in killing test larvae so that it did not have a significant difference. Apart from the observation time above has a significant difference and the most significant observation time is the 24th hour because the active substance from the frangipani flower extract granule has the potential for toxicity levels to increase, so that the mortality of test larvae will increase as the observation time increases. It can be concluded that there is a significant difference in the average mortality of *Aedes aegypti* larvae due to the influence of various levels of observation time.

## CONCLUSION

Granules of frangipani flower extract (*Plumeria acuminata* Ait) have larvicide effectiveness at a concentration of 1.6% capable of killing an average of 14 larvae (56%). A concentration of 1.9% was able to kill an average of 15.75 larvae (63%). A concentration of 2.7% was able to kill an average of 17.75 larvae (71%). A concentration of 3.4% was able to kill an average of 21 larvae (84%). A concentration of 4.3% was able to kill an average of 23.75 larvae (95%). A concentration of 4.7% is capable of killing an average of 25 larvae (100%). The most effective concentration in frangipani flower extract granules against the death of *Aedes aegypti* larvae is 4.7% because it is able to kill *Aedes aegypti* larvae 100%. Lethal Concentration (LC<sub>50</sub>) is 1.572% and Lethal Concentration (LC<sub>90</sub>) is 3.025%. The Lethal Time value (LT<sub>50</sub>) at the most effective concentration of 4.7% is 2.902 hours or 3 hours and the Lethal Time value (LT<sub>90</sub>) is 9.421 hours or 9 hours. This research was conducted 8 years ago in 2015 and is still relevant to research in 2023 so that it can be concluded that the effectiveness of frangipani flower extract which has been formulated in granule form can be used as a reference in controlling *Aedes aegypti*.

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