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Toxicity of *Tangiayu Incense* Smoke as an Insecticide Against *Aedes aegypti* Mosquito Mortality

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ABSTRACT

Background: Natural substances such as *beluntas* leaves, lemongrass leaves, and wood powder packaged in *Tangiayu incense* can be used to control vectors.
Objective: This study was to determine the toxicity of *Tangiayu* smoke insecticides on *Aedes aegypti* mosquito mortality.
Methods: Experimentation with a completely randomized design was applied to eight hundred seventy-five mature *Aedes aegypti* mosquitoes, divided into seven treatment groups with five replications, each unit consisting of 25 mosquito tests (n = 25). The data was collected from *Aedes aegypti* mosquito mortality following exposure to *Tangiayu incense* smoke for 5, 10, 15, 20, and 25 minutes. The ANOVA, LSD, and Probit tests were used to examine mortality data.
Results: According to the research, there was an increase in *Aedes aegypti* mosquito mortality following the exposure smoke *Tangayu incense*. The exposure duration that resulted in 50% mortality was 18 min 30 s (LT_{50}=5.9011), which is classified as extremely hazardous. The probit test resulted in equation 5= 0.0582x + 5.627. The coefficient of determination (R^2) is 0.9718 (97.18%) and the coefficient correlation (r) is 0.9858 (98.58%).
Conclusion: Exposure to *Tangiayu incense* smoke on *Aedes aegypti* mosquitoes mortality is extremely hazardous and effectively used as insecticides.

Keywords: *Aedes aegypti, Insecticide, Smoke, Tangiayu Incense,Toxicity.*

INTRODUCTION

Every year, infections caused by the *Aedes aegypti* mosquito vector experience a significant increase, one of which is dengue hemorrhagic fever (DHF).[1] It is known that this virus belongs to the genus flavivirus subgenus Stegomyia which has four serotypes with different signs and symptoms.[1] At its most dominant level, clinical manifesting is the DEN-3 serotype and has implications for infection of DHF cases with moderate to severe categories and the appearance of visible symptoms. [2,3]

Dengue Hemorrhagic Fever is included in *Extraordinary events* in various parts of Indonesia, one of which is in Bali Province.[4] Based on the Ministry of Health of the Republic of Indonesia in 2020, the prevalence of DHF in Bali was 12,173 cases, the *incidence rate* was 280.7 per hundred thousand population, and the *Case Fatality Rate* was 0.4%. The IR figure exceeds the limit set by the Ministry of Health, which is <49 per 100,000 population, and currently, Bali Province occupies the second position with the highest incidence of dengue cases in Indonesia.[5]

Several factors contribute to the high incidence of dengue cases in Bali Province, including the ineffective implementation of the Mosquito Nest Eradication Movement (GPSN), the low demand for the 4M-Plus Program, and the significant increase in mosquito populations.[6] This resulted in people switching to synthetic insecticides to suppress the *Aedes aegypti* mosquito population. However, excessive use of synthetic insecticides causes mosquitoes to resist certain active compounds [7], increases environmental pollution because they cannot be degraded quickly in the environment, and poses a threat to human health if inhaled for an extended period and in high concentrations.[8]

Furthermore, the smoking of incense containing synthetic colors and scents that can induce cancer in the respiratory tract [9], chronic obstructive pulmonary disease (COPD)[10], and other disorders has resulted in a degradation in the quality of the environment and human health. he resulting smoke contains compounds of carbon monoxide, sulfur dioxide, nitrogen dioxide, and hydrogen sulfide, which are toxic.[10,11]

In order to minimize the impact resulting from exposure to synthetic insecticides (Mosquito Coils) and commercial incense, strategic efforts are needed to control dengue disease vectors that are safe for health, cheap, easy to apply, and biodegradable. The efforts are to use plant parts as raw materials for natural insecticides such as *Beluntas* leaves (*Pluce indica* Less), lemongrass leaves (*Andropogon nardus*), and wood powder packaged in *Tangiayu Incense* preparations.

The raw materials were chosen based on research demonstrating that *Beluntas* and lemongrass leaves have bioinsecticide characteristics. Both plants produce essential oils that mosquitoes dislike.[12] In addition, the lemongrass plant contains a lot of citronella, which is useful as an anti-mosquito.[15] Fitriansyah's research indicated that *Beluntas* and lemongrass leaves include lignan chemicals, saponins, phenyl-propanoids, bensoids, alkaloids, flavonoids, and tannins. Saponin and tannin compounds have activities such as *digestive toxins* and anti-cholinesterases that can inhibit *the enzyme acetylcholinesterase* (AchE)[13].

According to Hasni's research, a synergistic combination of flavonoids, saponins, and tannins can cause the death of *Aedes aegypti* mosquitoes, both adults and larvae, by reducing the surface tension of the mucous membrane and the tractus wall *of the dige*stive tract and disrupting the respiratory system.[14] According to research by Mertha Adnyana, Sudaryati, and Sitepu, the content of flavonoids, citronellal, geraniol, lignan, tannin, cineol, camphene, and pinine inhibits the respiratory *poison* system, reduces the excitatory feeding *of Aedes aegypti* mosquitoes, and inhibits molting and hissing, making it an effective insecticide.[15]

The high potential for developing *Tangiayu Incense* as an insecticide to suppress *the Aedes aegypti* mosquito population in the community, resulting in guarantees of the safety of health products, needs to be improved through pre-clinical trials and clinical trials to determine the resulting impact. The results are used as information related to their efficacy. The objective of this study was to analyze the toxicity of *Tangiayu Incense* smoke as an insecticide against the mortality of *Aedes aegypti mosquitoes*.

MATERIAL AND METHOD

Research Design

Research experimental design follows Completely Randomyzed Design (CRD).[15,16] The study grouped 875 three-week-old adult *Aedes aegypti* mosquitoes into seven treatment groups. Each group consisted of five units (*repetition*), and each unit contained 25 adult *Ae. aegypti* mosquitoes (n = 25). Giving treatment in each unit by exposing *Tangiayu Incense* Smoke at different times, namely P1 (exposure for five minutes); P2 (exposure for ten minutes); P3 (exposure for fifteen minutes); P4 (exposure for twenty minutes); P5 (exposure for twenty-five minutes) and 2 controls, namely K(-) negative control without smoke exposure and K(+) exposure to smoke mosquito coils containing *transfluthrin*®.

The number of treatments and tests is based on *the OECD Guidelines for Chemical Testing, Test Number 403: Acute Inhalation Toxicity,*[17] *World Health Organization Pesticides Evaluation Scheme* (WHOPES),[18] and Indonesian National Standard 06-3566-1994 on the eradication of burn mosquitoes.[19]

Time and Place of Intervention

The research was carried out for three months (January to March 2021) at the Laboratory of Entomology and Parasitology, Department of Environmental Health, Denpasar Health Polytechnic for the enlargement of test mosquitoes and treatment. *Tangiayu Incense* production was carried out at UD. Gandhi Sradha, located in Tusan Village, Banjarangkan District, Klungkung, Bali.

Ethical Eligibility

The implementation of the research has been approved (Laik Etik) by the Health Research Ethics Commission (KEPK) of the Denpasar Health Polytechnic number LB.02.03/EA/KEPK/ 0241/2021.

Instrument and Materials

The instrument used includes Peet Grady Chamber (PGC) size 45×45×45 cm^3, handscone, stopwatch, scissors, hygrometer, measuring flask, digital scales, thermometer, glass aspirator, cotton, tissue, drip pipette, gas lighter, and gadget. At the same time, the materials used include *Aedes aegypti* mosquito eggs two traps, tissue, mosquito repellent containing *transfluthrin*®, *Tangiayu incense*, granulated sugar, and fish pellets.

Bioactive compounds like essential oils, flavonoids, camphene, pinene, tannins, polyphenols, and saponins are found in *Tangiayu incense*. These compounds come from incense raw materials and are identified by phytochemical tests.

Data Analysis

Data on *Aedes aegypti* mosquito mortality was collected and analyzed statistically using SPSS Inc. version 25.0 using a one-way ANOVA test, which was followed by an LSD test at 99% confidence (p<0.01) to determine the difference in mortality in treatment unit. Toxicity values are calculated using linear regression with the equation y = ax + b to obtain a lethal time value of 50% (LT_{50}).

RESULTS

The study's results (Table 1) show that the exposure of *Tangiayu Incense* smoke to the mortality of adult *Aedes aegypti* mosquitoes at different times has increased significantly. The treatment unit on negative control (K-) found no mortality in test mosquitoes. The mean mortality rate was higher in the P3, P4, and P5 treatment groups compared to those exposed to mosquito coils containing *transflutrines*® (K+). In contrast, the mean of P1 and P2 was less than that of the positive control (K+). The mortality data of the *Aedes aegypt*i mosquitoes were processed using statistical parametric assays and through stages of analysis. The normality test results using *Shapiro Wilk* obtained probability values (p>0.01); thus, the data distribution between treatment groups was normally distributed. The results of the test homogeneity using *the Levene test* obtained a value of F_{count} of 7,718 with a probability value (p>0.01); thus, the mortality of *Ae. aegypti* mosquitoes in each group has the same variance (homogeneous). The results of the *one-way* ANOVA obtained a value F_{count} of 198.955 with probability 0.000 less than the value p<0.01 thus, the *mean* mortality of the *Ae. aegypti* mosquito between different treatment groups is very real with a confidence level of 99%.

Table 1. Mortality of *Aedes aegypti* mosquitoes exposed to smoke from *Tangiayu incense*

Treatment Unit	Mean ± SE	*Shapiro Wilk*	*Levene Test*	*p-value*
K-	0.000 ± 0.000	0.000		
K+	22.20 ± 0.374	0.314		
P1	20.20 ± 1.158	0.501		
P2	22.00 ± 0.894	0.440	0.439	0.000**
P3	23.80 ± 0.374	0.314		
P4	24.00 ± 0.316	0.325		
P5	24.40 ± 1.402	0.314		

Source: Data processed, 2021
Description: ** = Very real different (p<0.01).

Based on the results of further testing using the LSD test (Table 2) showed that the treatment with exposure to mosquito coils contained *transflutrines*® compared to treatment P1, P2, P3, P4, and P5 probability values (*p*> 0.01), treatment at P1 was compared with P2 probability value (*p*>0.01) and treatment on P2 compared with P3, P4, and P5 probability values (*p*>0.01). Thus, the treatment differs markedly but insignificantly.

Table 2. LSD Test Results Mortality of *Aedes aegypti* mosquitoes between treatment groups

	K-	K+	P1	P2	P3	P4	P5
K-	-	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
K+		-	0.03*	0.82*	0.07*	0.04*	0.01*
P1			-	0.04*	0.00**	0.00**	0.00**
P2				-	0.04*	0.03*	0.01*
P3					-	0.82*	0.65*
P4						-	0.65*
P5							-

Source: Data processed, 2021
Description: * = Different real (*p*>0.01); ** = Different very significant (*p*<0.01).

The results of the probit test (*unit probability*) exposure to *Tangiayu Incense* smoke on the mortality of adult *Aedes aegypti* mosquitoes with different exposure times (Table 3) obtained *a p-value* of 0.002 less than the probability value (*p*<0.01). The lower limit value is 7,102 (20 min 26 s), and the upper limit value is 26,302 (34 min and 5 s), with a 99% confidence level. The results of the one-way ANOVA test obtained $a_{n\ F\ count}$ > F value of 103,311 > 0.002; thus, there is an influence of the length of exposure time of *Tangiayu Incense* (X) smoke on the mortality of *Aedes aegypti* (Y) mosquitoes.

Table 3. Time of exposure to *Tangiayu Incense* smoke in relationship to *Aedes aegypti* mosquitoes mortality

	Coeff. ± SE	*t*- Stat	*p-value*	Lower 99%	Upper 99%	F	*Sig.*
Intercept	-93.53 ± 10.69	-8.741	0.003**	-156,03	-31.03	103.311	0.002**
Time Log	16.69± 1.642	10.16	0.002**	7.102	26.302		

Source: Data processed, 2021
Description: ** = Different very significant (*p*<0.01).

The results of the acute inhalation toxicity test (Table 4) obtained the equation *y* = *ax* + *b*, namely 5= 0.0582x + 5.627 so that the *Lethal Time* value of 50% (LT_{50}) was 5.9011. Thus, exposure to *Tangiayu Incense* smoke resulted in 50% mortality at 18 min 30 s. The longer the exposure time to *Tangiayu Incense* smoke, the *lethal time* value decreases; on the contrary, the longer the exposure time to *Tangiayu Incense* smoke, the percentage of mortality increases, and the time required to kill the test animal is

faster. The results of the analysis of the relationship between the time of exposure to *Tangiayu Incense* (X) smoke to the mortality of *Aedes aegypti* mosquitoes (Y) (Figure 1) show a value (X) of 0.0582 so that, if there is an additional exposure time of value (X) then the mortality of *Aedes aegypti* mosquitoes (Y) will increase by 0.0582. The coefficient of the positive value indicates that both variables (X) and (Y) have a unidirectional relationship. The coefficient of determination (R^2) of 0.9718 means that 97.18% of the mortality of *Aedes aegypti* mosquitoes is influenced by *Tangiayu Incense* smoke, while other factors influence 2.82. The value of the coefficient correlation (r) of 0.9858 means the relationship between the length of time exposure to *Tangiayu Incense* smoke and the percentage of *mortality of Aedes aegypti* mosquitoes of 98.58% which is included in the robust category (>0.75 – 0.99). A value (r) close to 1 means it is getting stronger.

Table 4. Toxicity of acute inhalation exposure to *Tangiayu Incense* smoke to the mortality of *Aedes aegypti* mosquitoes

Treatment Unit	% Mortality	Time (min.)	Time Log	Probit Value	LT_{50} value	LT_{50} (min)	LT_{50}
P1	81%	5	0.6990	5.88	0,7818	00:18:46	
P2	88%	10	1.0000	6.18	0,6251	00:15:00	x= 5.9011
P3	95%	15	1.1761	6.64	0,5705	00:13:42	(00:18:30)
P4	96%	20	1.3010	6.75	0,5321	00:12:46	
P5	98%	25	1.3979	7.05	0,5046	00:12:07	

Source: Data processed, 2021

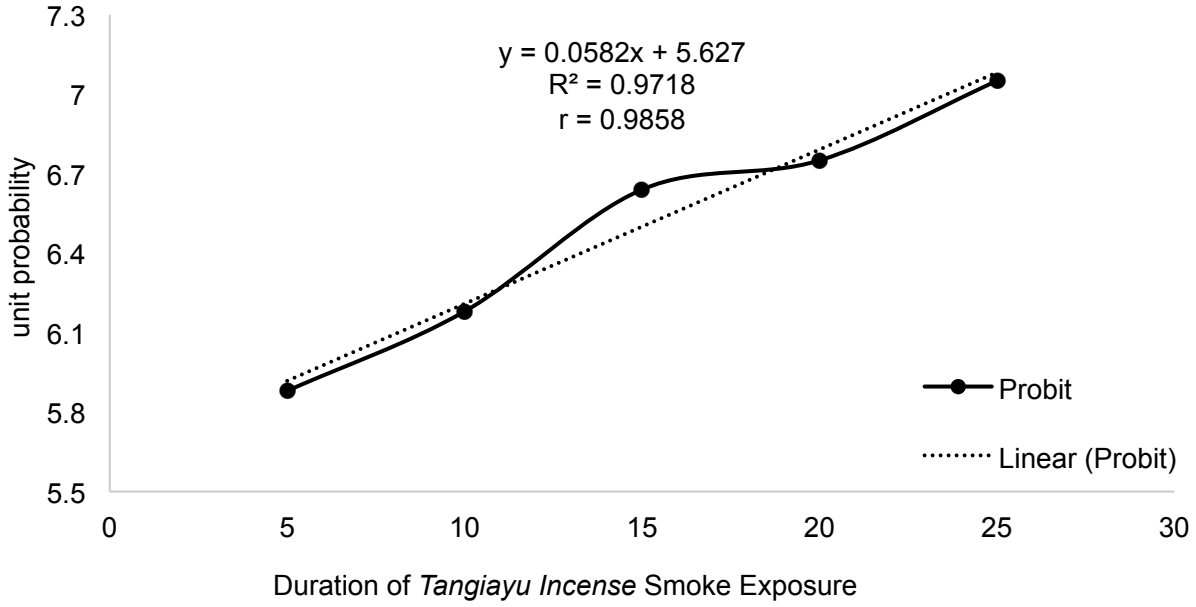

Figure 1. Time-exposure relationship between *Tangiayu Incense* (X) smoke and *Aedes aegypti* (Y) mortality.

DISCUSSION

Control of animal-sourced disease vectors such as the *Aedes aegypti* mosquito can utilize insecticides, most of whose raw materials come from natural materials, one of which is plants. [20] Plants containing essential oils, flavonoids, camphene, pinene, tannins, polyphenols, and saponins are widely used to dispel, nourish, and repellant insects as *Beluntas* leaves and lemongrass leaves. Previous research said mosquitoes did not like the plant's content because of its sharp fragrance, producing toxic effects and causing dehydration. Hence, insects stayed away from plants with these bioactive compounds. [21]

In this study, using *Beluntas* leaves, lemongrass leaves, and wood powder as the primary raw materials for *Tangiayu Incense* is a breakthrough in producing products that are safe for human health, cheap, easy to apply, and environmentally friendly. *Tangiayu Incense* products measure 26 cm with a diameter of 0.1 cm with a weight of 0.3 gr in the shape of a rod. *Tangiayu Incense* products prioritize aspects of product safety, efficacy, and quality based on local wisdom. *Tangiayu incense* is used as a means of the ceremony by *Hindu's* and as an insecticide to suppress the *Ae aegypti* mosquito population.

The high mortality of mosquitoes after exposure to smoke *Tangiayu incense* at varying times indicates that the use of the material has the potential to be developed because it is effective in killing mosquitoes. The study's results in (table 1) show that the mean mortality of *Aedes aegypti* mosquitoes has increased significantly. Influenced by the content of compounds contained in the raw material *of Tangiayu Incense* which can provide a toxic effect on mosquitoes with the mechanism of inhibiting the respiratory tract (proboscis, trachea, spiracles) due to inhalation of incense smoke, inhibiting excitatory eating or *digestive toxin* and resulting in *paralysis*. [9]

Exposure to *Tangiayu* incense smoke with a time of five minutes (P1), ten minutes (P2), fifteen minutes (P3), twenty minutes (P4), and twenty-five minutes (P5) was able to produce mortality of successively 81%; 88%; 95%; 96%; and 98%. These results support the opinion of WHOPES which reveals that insecticides are declared effective and meet the standards if they can have a significant effect in causing mortality in test animals 10-95% for 24 hours. [18] Meanwhile, the Ministry of Health Republic of Indonesia and Minister of Agriculture Regulations Republic of Indonesia, stated that insecticides are effective if they can kill 90-100% of mosquitoes tested for 24 hours. Based on these parameters, P1, P2, and P3 treatments are included in the WHOPES parameters, while the P4 and P5 treatments are included and meet the standards. [18,22,23] Bioactive compounds resulting from the burning of *Tangiayu Incense*, such as flavonoids (apigenin, luteolin, crimineriol), pinene, cineole, alkaloids, camphene,

saponins, tannins, and polyphenols, can inhibit insect feeding excitatory, potent inhibitors of the insect respiratory system, and inhibit hormone molting.[13] Citronellal oleum compounds, geraniol, and pinine have toxic properties by damaging the mosquito cuticle resulting in continuous desiccation [24], and as a contact poison by lowering the elasticity of the mosquito's body surface, as a result of which the body becomes rigid, stiff and ends up rupturing resulting in death.[14]

According Rahmawati et al. the content of polyphenols, flavonoids, tannins, and alkaloids that work synergistically can inhibit insect breathing through blockage of tracheal holes and inhibit the enzyme cholinesterase from causing excitatory impulses in the respiratory system to be disturbed thus, the use of citronella leaves is effective as an anti-mosquito.[25–27] The content of bioactive compounds in *Tangiayu Incense* smoke works with the mechanism of flavonoid compounds to have a respiratory poison effect by clogging spiracles, proboscis, and trachea, which results in inhibition of the nPNH and NADH electron transport processes, which can cause wilting of the nerves, paralysis, decreased excitatory stimulus, and blockage of O_2 transport.[28,29]

Saponin compounds are cytotoxic that inhibit and irritate the mucous membrane of the tractus digestives, as a result of which the pharynx in *Aedes aegypti* mosquitoes experiences drought,[30] inhibiting the formation of new energy, inhibiting uptake take in the digestive tract resulting in changes in membrane permeability and molecular disorganization that result in mosquitoes dying.[20] Polyphenol compounds work as stomach poisons, inhibiting extracellular proteins and solubility, resulting in a decrease in the production of cholinesterase enzymes. These compounds can destroy integuments by shedding the chitin layer with neurotoxic effects. [9]

Based on the exposure to this mechanism, it is corroborated that *Tangiayu Incense* has an effect as an anti-mosquito (Insecticide) by exposing smoke to *Aedes aegypti mosquitoes.* The results of acute inhalation toxicity testing using OECD guideline number 403 revealed that exposure to *Tangiayu Incense* smoke kills the *Aedes aegypti* mosquito vector by 5,9011> 5-50. Therefore, exposure to *Tangiayu Incense* smoke is classified as Extremely Highly Toxic for insects, particularly mosquitoes. The smoke created is non-toxic to humans, is safe for livestock, and is environmentally benign because it is made from natural resources. This research is anticipated to contribute to producing safe, relatively inexpensive, and ecologically friendly insecticides. With the existence of *Tangiayu Incense,* it is envisaged that households will contribute to the suppression and reduction of DHF occurrences in Bali and Indonesia.

CONCLUSION

There was an increase in mosquito mortality *of Aedes aegypti* after exposure to

smoke *Tangiayu incense* at different times. Exposure time resulted in 50% mortality at 18 min 30 s (LT_{50} = 5.9011) with the category extremely hazardous. The results of the Probit test obtained the equation 5=0.0582x + 5.627. The value of the Coefficient of Determination (R^2) of 0.9718 (97.18%) and the coefficient correlation (r) of 0.9858 (98.58%) indicate the relationship of exposure to *Tangiayu Incense* smoke with different times (X) to the mortality of *Aedes aegypti* (Y) mosquitoes are included in the extremely hazardous. The longer the exposure to *Tangiayu Incense* smoke, the mortality of *Aedes aegypti* mosquitoes will increase. Smoke is a respiratory poison and contacts poison. It is hoped that further research will be carried out on the toxic effects produced over a long period due to continuous exposure to *Tangiayu Incense* smoke.

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