ORIGINAL ARTICLE

EFFECT OF CITRONELLA (*Cymbopogon nardus*) EXTRACT ON KNOCKDOWN TIME AND *AEDES AEGYPTI* MORTALITY BY THERMAL FOGGING METHOD

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ABSTRACT

Various methods have been carried out either naturally or chemically to reduce the mosquito population, to prevent and eradicate mosquito-borne diseases or disorders caused by mosquitoes. However, efforts to control mosquitoes should be made so as not to cause harm humans and the environment. Controlling mosquitoes by using chemicals can be done, among others, by using the anti-mosquito spray and anti-mosquito lotion that have been circulating on the market. Meanwhile, other ways are more 'environmentally friendly' to overcome mosquito-borne disorders, by utilizing anti-mosquito plants, such as citronella. This study aims to compare the knockdown time and Aedes aegypti mosquito mortality in the thermal fogging system between the use of citronella (Cymbopogon nardus) extract. This research was an experimental study. The sample size was 30 mosquitoes for each treatment group; 5 treatment groups with citronella extract doses of 100, 200, 300, 400, and 500 ml/ha. Data were analyzed with an ANOVA test. The highest knockdown time of Aedes aegypti mosquitoes was at a dose of 500 ml/ha with 20 mosquitoes in 8 hours. The lowest knockdown time was at a dose of 100ml/ha and 200 ml/ha with 0.25 mosquitoes in 5 min. There was a positive effect. The higher the concentration of citronella (Cymbopogon nardus) extract, the higher the number of knockdown time of Aedes aegypti (p-value = 0.004). In conclusion, citronella extract (Cymbopogon nardus) can be used as an alternative Aedes aegypti mosquito killer to replace malathion.

Keywords: *Aedes aegypti, citronella extract (Cymbopogon nardus), mosquito repellent, anti-mosquito plant.*

INTRODUCTION

Aedes aegypti mosquito raises fears of sickness and spread of Dengue Hemorrhagic Fever (DHF). World Health Organization (WHO) reports that dengue is the fastest-growing mosquito-borne diseases. Around 1 million cases are reported to the World Health Organization every year, with 20 thousand deaths annually[1]. From 2019 until February, there were 15,132 DHF sufferers recorded in 34 provinces in Indonesia. As many as 145 of them died. DHF cases in 23 districts/cities in Aceh in January 2019 reached 174 cases, including two deaths (in Banda Aceh and Nagan Raya)[1].

Humans use various methods to kill mosquitoes by using chemical-based poisons, including by using the anti-mosquito spray and anti-mosquito lotion that has been circulating on the market[2]. The government carried out the DHF vector control program was adult mosquitoes control through fogging using malathion[3]. Chemical thermal fogging is known as fogging.

Malathion short-term impacts can cause respiratory problems and damage the nervous system. If it exceeds the observed effect limit (OEL), it can cause death. Also, continuous exposure for a long time may lead to sensitivity to the skin. Cholinesterase inhibition can cause cumulative effects[3].

The use of chemical insecticides is now beginning to turn to the utilization of plant secondary metabolites as plant-based insecticides. Plant-based insecticides are safer for human health, leaving no residue in nature, thereby reducing pollution. The content of chemical compounds in some plants can be used as mosquito repellents, for example, in the form of anti-mosquito lotions such as lavender, eucalyptus, citronella, vetiver, cloves, and mimba[4].

Citronella extract contains natural chemical compounds that can be used to control and eradicate disease vectors, including essential oils, saponins, steroids, and tannins, which have the nature of insecticides. These chemical compounds' structure produces the ability as a plant-based insecticide consisting of carbon, oxygen, nitrogen, and hydrogen. Plant-based insecticides in nature will be easily degraded. Some studies mentioned that citronella extract concentration of at least 1.56% could kill mosquito larvae by 68% and LC_{90} by 4.898%, LC_{50} by 1.068% (dominant chemical content of vetivetate acid)[5]. The use of plants of the genus Cymbopogon as a mosquito repellent, namely the pure oil from the citronella plant, protected 2 h from insect bites and found that citronella with a concentration of 0.05-25% was able to protect from *Aedes aegypti* bites for 20 min[5]. Thorsell *et al*. reported that 10% citronella concentration in ethanol 70%, 8 hours after application, gave a 31.9% repellent effect to *Aedes aegypti* in the laboratory[6]. A study that used the extract from the soaking of citronella stems or citronella solution compared to fragrant pandan leaves showed that citronella solution used as a poison in fogging could kill 90% of *Aedes aegypti* mosquitoes[7].

The use of malathion is evidenced to be effective in killing adult Aedes mosquitoes. Still, some studies found that there was a resistance to malathion and deltamethrin in Western Venezuela[8]. *Aedes aegypti* resistance towards malathion (0.8%) and cypermethrin (0.05%) was also reported by Widiarti *et al*. and Zulfikar *et al*. in several regions in Central Java (Indonesia), *i.e.*, Jepara, Blora, Semarang, Salatiga, Surakarta, Tegal, Magelang, and Purwokerto[9,10]. Sayono *et al*. Stated the presence of resistant status in *Aedes aegypti* mosquitoes in Semarang towards cypermethrin insecticide[11]. A study in Cimahi showed that *Aedes aegypti* had been resistant to cypermethrin concentrations of 0.2% and 0.4% or with higher concentrations used in this study[11]. A study in Banjarmasin, South Kalimantan, showed that *Aedes aegypti* samples taken from 5 Community Health Centers were resistant to malathion concentration of 0.8%[12]. In a different condition, a study in Thailand showed that *Aedes aegypti* mosquitoes in some regions were still susceptible to malathion and resistant to permethrin[13]. A study conducted by Ikawati *et al*. showed a resistance *Aedes aegypti* had occurred towards malathion concentration of 0.8% at Semarang, Grobogan, Purbalingga, and Kendal[14].

Citronella leaves and roots contain saponins, flavonoids, and polyphenols, and the leaves also contain essential oils[15]. Several compounds are included in the citronella stems and leaves that can kill mosquitoes, one of which is citronella oil. Citronella oil has poisonous properties that work similarly to contact poisons that can cause death due to continuous fluid loss so that the mosquito's body loses fluids[16]. As a result, mosquitoes become paralyzed or die. Data that describe the use of citronella powder and killing adult mosquitoes compared with malathion was still rare to obtain so far. The previous studies only focused on extract as a mosquito repellent, and there is no utilization of citronella extract as a fogging ingredient as a substitute for malathion. The use of malathion often causes odor, not safe for the residential environment, and a high price. Therefore, the authors are interested in using citronella extract to replace the use of malathion. This study aims to determine the knock down time and death of Aedes aegypti mosquitoes in a thermal fogging system using citronella extract (Cymbopogon nardus).

METHODS

The experiment was a quasi-experimental study with a post-test only with a control group design, with a completely randomized design method (CRD). (**Figure 1**)

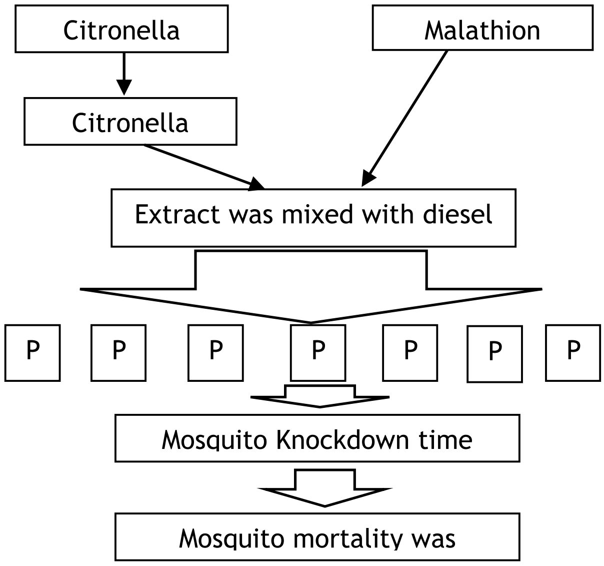

Figure 1. Research design: P- = Negative control (diesel fuel), P+ = Positive control (Malathion), P1 = Treatment 1 (citronella extract dose of 100 ml/h), P2 = Treatment 1 (citronella extract dose of 200 ml/h), P3 = Treatment 1 (citronella extract dose of 300 ml/h), P4 = Treatment 1 (citronella extract dose of 400 ml/h), P5 = Treatment 1 (citronella extract dose of 500 ml/h)

The study was conducted at the Department of Environmental Health of the Health Polytechnic of the Ministry of Health of Aceh. The study object was the citronella (*Cymbopogon nardus*) extract obtained from Aceh Province. The part used was the citronella stem. The study subjects were adult *Aedes aegypti* mosquitoes obtained from the breeding method of eggs obtained from the entomology laboratory of IPB (Institut Pertanian Bogor, Indonesia). The sample size for each treatment was 30 animals, considering the experiment to use 25-30 larvae in each treatment. According to Supranto, the control group and the treatment group respectively received a repetition to produce a more appropriate measure of the effect of treatment on the experiment[17]; the equation used to calculate the number of repetition was:

$(t-1)(r-1) \geq 15$
Information:
t = number of treatment
r = number of repetition

Based on the calculation, the number of repetitions obtained was 4 times for each test. There were 30 mosquitoes in each treatment group. The total samples were 840 *Aedes aegypti* mosquitoes. To get serial concentrations of citronella (*Cymbopogon nardus*) with the doses of 100, 200, 300, 400, and 500 ml/ha, the extract needed for one treatment was 6000 ml/ha (for 4 repetitions).

The tools that used in the study to produce citronella extract were a blender (Waring 8010 Bu+Ss 610, USA), knife (Vicenza V910K, Italy), sieve (Retsch 8 X 2, Germany), tray (Panda Star, Indonesia), spoons (Ikea, Sweden), jars (TP5 Pin Mao, Taiwan), analytical scale (Fujitsu, Japan) funnels (Herma, UK), rotary evaporator (B One RE300, USA), beaker glass (Herma, Uk), and stirrer (Iwaki, Japan). The tools used for the study were 250 ml plastic cups (Iwaki, Japan), 1 ml micropipette (Onemed, Indonesia), sticks, counter, water thermometer (Hanna Italy), pH meter (Hanna, USA), humidity gauge (Hygrometer-Hanna, USA), microscope, mosquito test cage (12 x 12 x 12 cm), Swing fog machine TF50 nozzle 0.8 mm (IGEBA GmbH, Germany), pulverizer/ULV. The ingredients used in this study were citronella (*Cymbopogon nardus*), *Aedes aegypti*, 95% Ethanol, filter paper, rubber bands, and water, malathion, thermo-hygrometer and plastic cups, tweezer, timer, aspirators, cotton wool, wet towels, diesel fuel, malathion, citronella extracts with the concentrations of 100, 200, 300, 400 and 500 ml/ha which were dissolved in 5 L diesel fuel. Malathion 96 EC with a dose of 500 ml/ha was used for comparison.

The efficacy criteria were taken based on 50% knockdown time and 95% of the total number of test mosquitoes calculated from data that have been corrected by mortality and knockdown time of the test mosquitoes in the control group[17]. Percentages of paralyzed/unconscious mosquitoes were calculated at 5, 10, 15, 30, 45, and 60 min; Also, 2, 4, and 8 h after fogging. The percentage of mortality was determined 24 h after maintenance in the laboratory.

Statistical analysis

In data analysis, if the mortality rate in the control group was more than 20%, then the study was considered failed. Furthermore, if the mortality rate in the control group was 5-20%, then it must be corrected with the Abbot formula[18]. Data obtained from the calculation of simplex lattice design (prediction) were compared to the data from the actual test results. Data were analyzed using two-way Analysis of Variant (ANOVA) to find out whether there were significant differences between treatments.

RESULTS

The observation results during the study with 4 repetitions in the treatment groups of *Aedes aegypti* mosquitoes by using citronella extract (dissolved in 5 L of diesel fuel) with concentrations of 100, 200, 300, 400 and 500 ml/ha and 96 ml malathion with a dose of 500 ml/ha for comparison, can be seen in **Table 1.**

Table 1: Percentage of knock down time and death of Aedes aegypti mosquitoes in a thermal fogging system using citronella extract (*Cymbopogon nardus*)

Application of Insecticides (Target Dose)	Mean knock down time in each observation (%)									Mosquito mortality in 24 h
	5 min	10 min	15 min	30 min	45 min	60 min	2 h	4 h	8 h	
Dose I	0.83	2.5	4.167	7.5	9.167	14.17	22.5	37.5	47.5	21.67
Dose II	0.83	6.667	9.167	14.17	12.5	18.33	26.67	36.67	49.17	25.00
Dose III	5	15.83	16.67	24.17	28.33	33.33	47.5	49.17	56.67	28.33
Dose IV	9.17	19.17	20	30	36.67	48.33	52.5	55.83	63.33	38.33
Dose V	10.8	23.33	27.5	39.17	40.83	55.83	59.17	58.33	66.67	48.33
Control (+)	28.3	43.33	65	100	100	100	100	100	100	100.00
Control (-)	0.83	0	0	0	0	0	0	0	0	0.00

Based on the data that obtained in **Table 1**, the highest number of mosquito knockdown time with the highest number of mortality was at the dose of 500 ml/ha (66.67% mosquitos in 8 h). The lowest number was at a dose of 100ml/ha and 200 ml/ha (0.83% mosquitoes in 5 min). The percentage of unconscious mosquitoes are shown in **Figure 2**.

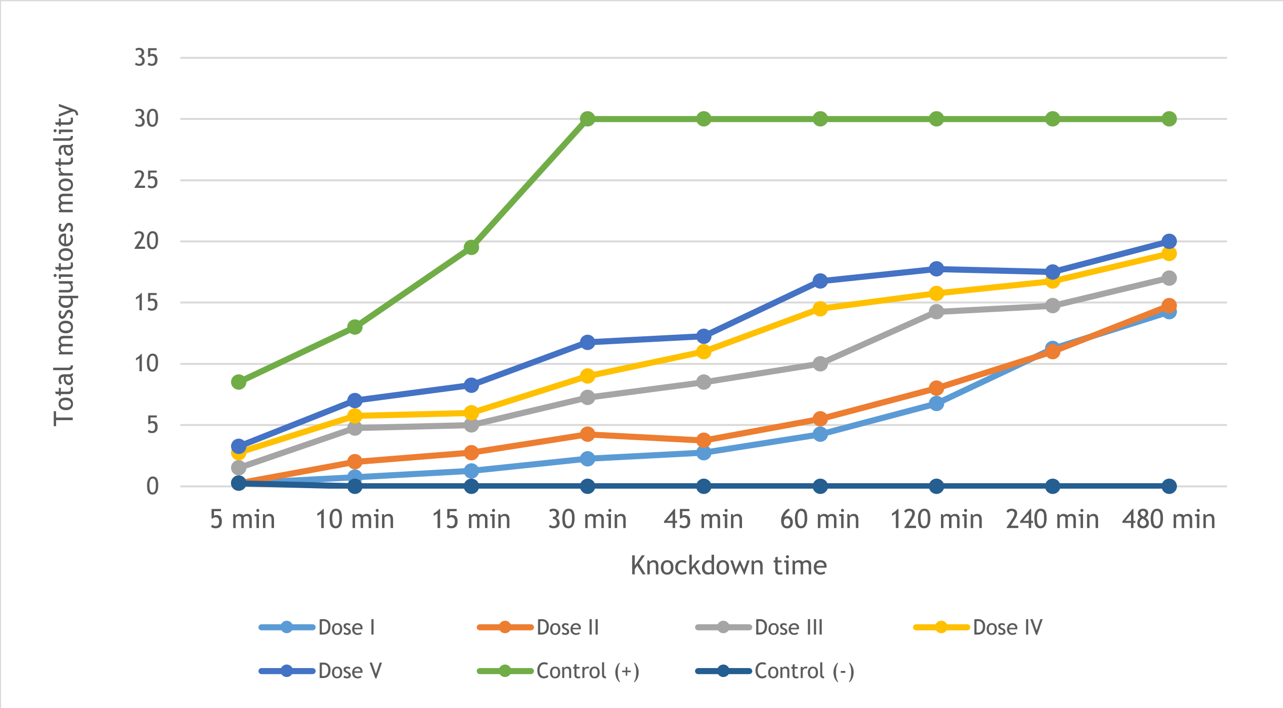

Figure 2. Mosquito knockdown time in each observation.

The knockdown time of *Aedes aegypti* mosquitoes in each observation was then processed using ANOVA to determine whether the treatment with different dosage variations would produce different knockdown time and *Aedes aegypti* mosquito mortality or not. The results of each test are presented in **Table 2.**

Table 2: Results of ANOVA test on mean knockdown time and *Aedes aegypti* mosquito mortality in the thermal fogging system using citronella (*Cymbopogon nardus*) extract.

Variable	Mean	SD	SE	95% CI		P value
				Lower limit	Upper limit	
Dose of 100 ml/Ha	5.02	4.68	1.48	1.67	8.37	
Dose of 200 ml/Ha	5.97	4.41	1.39	2.81	9.13	
Dose of 300 ml/Ha	9.15	4.93	1.56	5.61	12.68	0.004
Dose of 400 ml/ha	11.20	5.33	1.68	7.38	15.01	
Dose of 500 ml/Ha	12.90	5.41	1.71	9.02	16.77	
Total	**8.85**	**5.64**	**0.79**	**7.24**	**10.45**	

Based on **Table 2**, the ANOVA test results obtained p-value = 0.004 ($p < 0.05$), meaning that there was a significant effect of citronella (*Cymbopogon nardus*) extract and malathion on the mean knockdown time and *Aedes aegypti* mosquito mortality in the thermal fogging system. Therefore, an LSD follow-up test was conducted to determine the most effective dose. The LSD test results are presented in **Table 3**.

Table 3 showed that there were significant differences only at a dose of 100 ml/ha compared to 400 ml/ha and 500 ml/ha and also at a dose of 200 ml/ha compared to 400 ml/ha and 500 ml/ha with a value of p = <0.05. The other doses showed no significance. The phytochemical content test result showed that citronella (*Cymbopogon nardus*) contained secondary metabolite contents, namely flavonoids, tannins, phenols, triterpenoids, alkaloids, and saponins. These contents are shown completely in **Table 4**.

DISCUSSION

The results of the study showed that the higher the dose of citronella (*Cymbopogon nardus*) extract and the longer the time, the higher the number of knockdown time and also the higher the *Aedes aegypti* mosquito mortality rate. Simultaneously, the thermal fogging system with malathion reached 100% mortality rate at 24 h of observation. The highest knockdown time of *Aedes aegypti* mosquitoes was at a dose of 500 ml/ha with 20 mosquitoes in 8 h. The lowest knockdown time was at a dose of 100 ml/ha and 200 ml/ha with 0.25 mosquitoes in 5 min. Regarding the results of the percentage of unconscious mosquitoes using citronella extract with concentrations of 100, 200, 300, 400, and 500 ml/ha, which were dissolved in 5 L of diesel fuel and by using 96 EC malathion with a dose of 500 ml/ha for comparison, it was shown that the longer the observation time, the greater potency as an insecticide. This was supported by the ANOVA test results, which obtained a p-value = 0.004. There was a significant difference between mosquitoes' knockdown time with citronella (*Cymbopogon nardus*) extract and malathion as the comparator.

Table 3: LSD test results on knockdown time and *Aedes aegypti* mosquito mortality in the thermal fogging system using citronella (*Cymbopogon nardus*) extract and malathion

Citronella Extract Dose		Difference in mean (I-J)	P-value	95% Confidence Interval	
				Lower limit	Upper limit
Dose of 100 ml/Ha	Dose of 200 ml/Ha	-0.95	0.67	-5.42	3.52
	Dose of 300 ml/Ha	-4.12	0.07	-8.60	0.35
	Dose of 400 ml/ha	-6.17*	0.01	-1065	-1.69
	Dose of 500 ml/Ha	-7.87*	0.01	-12.35	-3.39
Dose of 200 ml/Ha	Dose of 300 ml/Ha	-3.17	0.16	-7.65	1.30
	Dose of 400 ml/ha	-5.22*	0.02	-9.70	-0.74
	Dose of 500 ml/Ha	-6.92*	0.00	-11.40	-2.44
Dose of 300 ml/Ha	Dose of 400 ml/ha	-2.05	0.36	-6.52	2.42
	Dose of 500 ml/Ha	-3.75	0.09	-8.22	0.72
Dose of 400 ml/Ha	Dose of 500 ml/Ha	-1.7	0.44	-6.17	2.77

** The mean difference was significant at the 0.05 level*

Table 4: Phytochemical content of citronella (*Cymbopogon nardus*)

Extract Dose	Flavonoid	Tannin	Phenol	Poly-phenol	Steroid	Triter-penoid	Alkaloid	Saponin
Dose of 100 ml/ha	√	√	√	√	-	√	√	√
Dose of 200 ml/ha	√	√	√	√	-	√	√	√
Dose of 300 ml/ha	√	√	√	√	-	√	√	√
Dose of 400 ml/ha	√	√	√	√	-	√	√	√
Dose of 500 ml/ha	√	√	√	√	-	√	√	√

√ = *present*

During the spraying process of citronella (*Cymbopogon nardus*) extract with different concentrations, it was found that the conditions of *Aedes aegypti* mosquitoes flew more quickly, partially fell in a standing position but did not fly anymore. After a while, the *Aedes aegypti* mosquito fell then died. The content of flavonoids may cause this result. As a bioactive compound in citronella (Cymbopogon nardus) extract, mosquitoes swallowed the content through the mouth and the respiratory tract or spiracles on the skin's surface, which caused nerve disorders. So that, the wings wilted, stiffed, and the mosquitoes were not able to fly anymore. Gautam *et al.* explained that the flavonoids in the citronella extract could destroy (disintegrate) the integument (wrapper), which is associated with loss of the chitin layer and abnormal stretching of the mosquito's body. This is thought to be due to the neurotoxic effect of flavonoids[19].

Other compounds contained in citronella that affects the mortality of *Aedes aegypti* mosquitoes are saponins. Saponin compounds are bitter so that they are suspected to damage cell membranes and disrupt Aedes aegypti mosquitoes' metabolic processes and reduce the surface tension of the mucous membrane of the digestive tract. Thus, the walls of the track become damage, and the mosquitoes died[20]. According to Chaieb, saponin compounds can act as an insecticide by changing mosquito feeding behavior by inhibiting the food uptake in the digestive tract[21]. Saponin can also cause changes in membrane permeability and cause molecular disorganization. Saponin is a strong surfactant; only a low concentration can be toxic to mammals because it causes hemolysis of red blood cells[22].

Jiang *et al.* and Chaieb *et al.* state that saponin compounds have a pungent characteristic, so it is toxic in small animals. Saponin is also an entomotoxicity agent that can cause egg damage and death, reproduction disruption in female insects that cause fertility disorder[21, 23].

Citronella extract contains natural chemical compounds that can be used to control and eradicate disease vectors, including essential oils, saponins, steroids, and tannins, which have the nature of insecticides. These chemical compounds' structure produces the ability as a plant-based insecticide consisting of carbon, oxygen, nitrogen, and hydrogen. Plant-based insecticides in nature will be easily degraded. Some studies mentioned that citronella extract concentration of at least 1.56% could kill mosquito larvae by 68% and LC_{90} by 4.898%, LC_{50} by 1.068% (dominant chemical content of vetivetate acid)[4]. A study that used the extract from the soaking of citronella stems or citronella solution compared to fragrant pandan leaves showed that citronella solution, which was used as a poison in fogging, could kill 90% of *Aedes aegypti* mosquitoes[10], 40% citronella, and 10% cloves protected 7-8 h, they can protect the body from mosquito bites due to their strong aroma[24].

Contact poison will seep into the animal's body through the outer skin, and the animal will die if the skin is touched. As explained by Wudianto, that contact poison will enter the mosquito's body through the cuticle so that if the insecticide contacts directly to the skin, then smoothly, the insecticide molecule will enter the mosquito's body. Over time the accumulation of insecticides in the mosquito's body can cause death[25].

Eugenol (phenol) can cause burns and toxins, and cause allergies if exposed to the skin. This may result in the *Aedes aegypti* mosquito mortality and cause the physical form of dry and brownish color that looks like burning. Eugenol is a phenol compound with alcohol groups to weaken and disrupt the nervous system. It is suspected that this substance causes the body of *Aedes aegypti* mosquitoes to look stiff. Moreover, it can be concluded that the active substances contained in citronella (*Cymbopogon nardus*) extract have the potential as a plant-based insecticide. The more the active substance molecules from citronella (*Cymbopogon nardus*) extract enter the *Aedes aegypti* mosquito's body, the greater the effect. Besides that, the increasing exposure time of citronella extract will increase the number of active substance molecules that enter the *Aedes aegypti* mosquito's body to cause a greater toxic effect[26].

Furthermore, citronella (*Cymbopogon nardus*) is easy to obtain; the residue is easily decomposed; it does not pollute the environment and is safe for other living things. As explained by Utami, plants that have the potential as plant-based insecticides can be widely used since they are easily obtained in nature everywhere. The biomass can be obtained in abundance. They are easily decomposed in nature. They do not pollute the environment and are relatively safe for humans and pets because the residues are easily decomposed[27]. Of course, this is also supported by a variety of other theories and literature that citronella (*Cymbopogon nardus*) is very useful for the community for the next step in the use of alternative insecticides from environmentally friendly plants, especially in controlling *Aedes aegypti* mosquitoes, which is likely to have potential as an alternative to replacing chemical fogging.

CONCLUSION

The highest knockdown time of *Aedes aegypti* mosquitoes was at a dose of 500 ml/ha with 66.67% mosquitoes in 8 h. The lowest knockdown time was at a dose of 100ml/ha and a dose of 200 ml/ha with 0.83% mosquitoes in 5 min. Besides, malathion caused knockdown time for *Aedes aegypti* mosquitoes with a mean mortality rate of 100%. There was a positive effect; the higher the concentration of citronella (*Cymbopogon nardus*) extract, the more *Aedes aegypti* mosquitoes with knockdown time (p-value = 0.004).

Conflict of interest

The authors have no conflict of interest to declare.

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