International Journal of Public Health Science (IJPHS)
Vol. 12, No. 3, September 2023, pp. 1112~1118
ISSN: 2252-8806, DOI: 10.11591/ijphs.v12i3.22811

Container characteristics and density of *Aedes aegypti* larvae: a field trial of *Ziziphus mauritiana* leaf extract

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Article Info

Article history:

Received Dec 5, 2022
Revised May 20, 2023
Accepted Jun 13, 2023

Keywords:

Aedes aegypti
Container index
Larvae
Ziziphus mauritiana

ABSTRACT

The application of larvicide from botanical origin was extensively studied as an essential part of mosquito prevention. This study aimed to assess the difference in container index (CI) based on the characteristics of water containers using *Ziziphus mauritiana* leaf extract solution. A quasi-experiment research was done among 300 selected containers with temephos as a control and 9% of *Ziziphus mauritiana* leaf extract solution as a treatment. The types of containers observed in this study were the type of water, container materials, location, container lids, and community behavior regarding immature *Aedes aegypti* breeding eradication. Water containers without lids and outside the house had a significantly higher CI among treatment (OR=26; 3.69–18.34; p=0.001 and OR=20; 2.04–19.64; p=0.003) and control group (OR= 10.83; 95% CI=1.96–59.83; p=0.005 and OR=6.43; 95% CI=1.02–40.26; p=0.04). Negative community behavior regarding the prevention and eradication of *Aedes* immatures was significantly associated with greater odds of high CI among treatment and control, with OR=37.5; 3.64–38.65; p=0.001 and OR=16.88; 2.56-11.4; p=0.002, respectively. Furthermore, containers with rainwater out of the treatment group had a higher presence of *Aedes* larvae (OR=11.25; 1.15–11.05; p=0.03).

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1. INTRODUCTION

Dengue fever is a severe vector-borne disease around the world. It is transmitted by the *Aedes aegypti* or *Aedes albopictus mosquito* vector and is caused by the dengue virus with symptoms of fever, headache, skin redness, muscle, and joint pain and can even cause bleeding, leakage of blood vessels, and low blood platelets [1]. WHO released that the incidence of dengue has grown in recent decades. The cases increased over eightfold over the last two decades, from 505,430 cases in 2000 to over 2.4 million in 2010 and 5.2 million in 2019 [2]. In Indonesia, one of the dengue endemic regions, 202 thousand cases, and 1,593 deaths were reported in 2017 [3].

The Regency of Kubu Raya is one of the dengue endemic areas with the highest incidence rate in West Kalimantan. It has been reported that 16.5% of total cases are attributed to this area [4]. With a population totaling around 615 thousand individuals, it is the second-largest area in West Kalimantan in population size [5]. The high population density may contribute to dengue transmission due to its potential impact on humans and *Aedes aegypti* contact, both within a person's home and when visiting others.

Assessing the density of *Aedes aegypti* mosquitoes can be used to determine the risk of a dengue epidemic in certain areas [6]. Several studies propose methods and indices to estimate the density of *Aedes* *aegypti*, such as the house index (HI), breteau index (BI), and container index (CI) [7]. An area determined as a high risk of DHF transmission is CI ≥5%, HI ≥10%, and BI ≥50% [7], [8]. These results have allowed the estimation of dengue"s critical thresholds to understand its transmission dynamics better.

The container index (CI), one of the indicators of the density of *Aedes mosquitoes* in households, is suitable for assessing the density of larvae in settlements with a lot of water storage [9]. This method is consistent with the culture of people in Kalimantan who are used to collecting rainwater as an alternative source of clean water. Consequently, containers often become breeding places due to the community's lack of awareness of dengue fever prevention. A previous study also showed that the cause of mosquito breeding was the density of larvae in water reservoirs and the increased density and mobility of the population [10]. Climatic variables, such as rainfall, high humidity, and temperature, also affected the mosquito larvae population, particularly in some areas [11].

In addition, chemical larvicide mainly used to control mosquitoes in households has led to the development of resistance by *Aedes aegypti*, adaptive vector behavior, and environmental health concerns [12]–[14]. These problems have necessitated the need to explore alternative strategies using eco-friendly products. Numerous studies have investigated the larvicidal activity of different plant extracts against *Aedes aegypti*, including *Ziziphus mauritiana* [15], [16].

Ziziphus mauritiana (locally called: *bidara*) has long been used as traditional medicine in Indonesia. This plant has been tested in a laboratory and has been shown to provide antiplasmodial and antibacterial effects due to the presence of alkaloids, flavonoids, triterpenoids, saponins, and tannins [17]–[19]. A previous laboratory-scale study showed that 9% *Ziziphus mauritiana* leaf extract effectively killed mosquito larvae [20]. Furthermore, the greater solution of bidara leaf extracts impacts the higher the mortality rate of *Aedes aegypti* larvae [21]. Little study has investigated *Ziziphus mauritiana* leaf as a larvicide against *Aedes aegypti* and none of the studies were applied in field-scale studies to assess better effects. In this study, we assess its effect on *Aedes aegypti* in the field by counting the larvae density according to the water containers' different characteristics.

2. METHOD

This study was a quasi-experiment design. Two groups were used: 45 houses as control given abate, and 45 homes intervened by giving 9% *Ziziphus mauritiana* extract solution. A total of 300 containers in both groups were surveyed for the presence of *Aedes aegypti* larvae.

The study was conducted in Rasau Jaya District, in the Regency of Kubu Raya, West Kalimantan. The District of Rasau Jaya has a temperature-warm climate with two seasons, the mean temperature of the coldest month (December) is 27.4 °C, and the warmest is 28.4 °C. The study site is located near the geographic center of the district in a crowded neighborhood where dengue transmission usually occurs from September-January. Surveys took place in each settlement over the course of 1–3 days to reduce the effects of seasonal or temporal variation on catch sizes.

Field activities were undertaken from May-July 2020. All containers in which water could be retained (i.e., upright and not broken) were counted. The sampling method was carried out by randomly selecting 90 households with 300 containers and then dividing them into two groups. The treatment group was given 9% of *Ziziphus mauritiana* leaf extract, with a dosage of 10 ml per 100 liters of water [20], and the control used abate (10 gram: 100-liter water). The average container capacity in this study was 500-1,000 liters.

The independent variables are the type of water, container material, container location, covered container, and community behavior. Community behavior is a community action in eradicating mosquito nests. The assessment of eradicating mosquito nests includes burying, draining, and closing containers; if one of the actions is not carried out, then it is included in the category of not eradicating mosquito nests.

Environmental factors, including temperature, pH, and light, were observed daily during the study. An optimal temperature was defined if 26-30 °C and pH 6-7. To assess the lighting, the investigator determined whether the container was shaded or sunlit. The index container is the level of mosquito density in the water reservoir calculated based on the number of positive containers divided by all the containers examined. The number of containers examined for one CI value is five, so 30 CIs are obtained in each group. Index containers are grouped into two criteria, more than or equal to 5% and less than 5% [8]. Bivariate analysis used chi-square to detect the container index difference between different container characteristics types.

This study has approved by Ethics Commission of the Health Polytechnic of the Health Ministry of Pontianak (No. 146/KEPK-PK.PKP/VI/2020). The participants were informed about the study if they wished to participate before signing a consent. A total of 90 households provided informed consent and completed the questionnaire and observations.

3. RESULTS AND DISCUSSION

The observations in Table 1 show that the environmental condition in both groups mainly was in the optimal temperature, pH, and light for larvae. The present study found that most larvae were in containers with 26-30 °C [22], pH 6-7 [23], and sunlit. These results prove that the presence of larvae was affected by environmental factors [24], [25]. In addition, there was no statistically significant difference between the treatment and control groups, therefore, it can be assumed environmental factors homogenous between the two groups (p>0.05 for all factors).

Table 1. Homogeneity test between control and treatment group

Variable	Control (n=150)		Treatment (n=150)		p-value
	n	%	n	%	
Temperature (˚C)					
Optimal (26-30)	145	96.7	146	97.3	0.82
Suboptimal (26 or >30)	5	3.3	4	2.7	
pH					
Optimal (6-7)	135	90	133	88.7	1.00
Suboptimal (<6 or >7)	15	10	17	11.3	
Light					
Shaded	6	4	5	3.3	0.19
Sunlit	144	96	145	96.7	

The presence of larvae between the control and treatment was an insignificant difference according to container characteristics and community behavior (Table 2). Nonetheless, a higher presence of *Aedes aegypti* larvae was reported among containers with rainwater inside, made from cement, located outside the house with open lids in both groups. In addition, respondents who did not eradicate mosquito breeding tended to find more *Aedes aegypti* larvae in their containers.

Table 2. The differences between control and treatment groups based on container characteristics and community behavior in mosquito breeding eradication to the existence of *Aedes aegypti* larvae

Containers characteristics	Presence of *Aedes aegypti* larvae				p-value
	Control (n=150)		Treatment (n=150)		
	n	%	n	%	
Type of water					
Rainwater	35	70.00	28	62.22	0.16
Non-rainwater	15	30.00	17	37.78	
Container materials					
Cement	20	57.14	10	55.56	0.75
Plastics	15	42.86	8	44.44	
Container location					
Outside	23	56.10	12	66.67	0.08
Inside	18	43.90	6	33.33	
Container lids					
No	18	69.23	12	60.00	0.10
Yes	8	30.77	8	40.00	
Community behavior in mosquito breeding eradication					
No	18	64.29	28	73.68	0.10
Yes	10	35.71	10	26.32	

Table 3 shows a Chi-square analysis of predictors of the CI. Among control, containers outside the house (OR=6.43; 95% CI=1.02–40.26; p=0.04), in an open state (OR=10.83; 95% CI=1.96–59.83; p=0.005), and negative community behavior regarding eradicating mosquitoes" nests were significantly associated with greater odds of high CI. Among the Z. mauritiana group, almost all container characteristics investigated in this study increase the likelihood of the CI, except containers' materials (p=0.08).

The type of container materials did not show any differences in the CI, both in the control and intervention groups. The previously mentioned study showed different results where a significant difference in the proportion between *Aedes aegypti* density and types of containers was found (p=0.012<0.05) [26]. Although some previous studies reported that water storage containers, such as earthen jars and drums, were consistently more likely to contain Aedes larvae, [27]–[29] it is noteworthy that the container's location may affect the larvae density.

Table 3. The differences between control and treatment groups in characteristics and container index (CI) for *Aedes aegypti*

Container characteristics	Control CI ≥ 5%	Control CI < 5%	Control p-value	Control OR (95% CI)	Treatment CI ≥ 5%	Treatment CI < 5%	Treatment p- value	Treatment OR (95% CI)
Type of water								
Rainwater	16 (59.3)	11 (40.7)	0.40	2.91 (0.23-36.16)	15 (65.2)	8 (34.8)	0.03*	11.25 (1.15 – 11.05)
Non-rainwater	1 (33.3)	2 (66.7)		Ref.	1 (14.3)	6 (85.7)		Ref.
Containers materials								
Cement	14 (58.3)	10 (41.7)	0.53	1.40 (0.23 - 8.42)	13 (65)	7 (35)	0,08	4.33 (0.85 – 22.23)
Plastics	3 (50)	3 (50)		Ref.	3 (30)	7 (70)		Ref.
Container location								
Outside	15 (68.2)	7 (31.8)	0.04*	6.43 (1.02 - 40.26)	15 (71.4)	6 (28.6)	0.003*	20 (2.04 – 19.64)
Inside	2 (25)	6 (75)		Ref.	1 (11.1)	8 (88.9)		Ref.
Container lids								
No	13 (81.3)	3 (18.8)	0.005*	10.83 (1.96 – 59.83)	13 (86.7)	2 (13.3)	0.001*	26 (3.69 – 18.34)
Yes	4 (28.6)	10 (71.4)		Ref.	3 (20)	12 (80)		Ref.
Community behavior in mosquito breeding eradication								
No	15 (78.9)	4 (21.1)	0.002*	16.88 (2.56 – 11.4)	15 (78.9)	4 (21.1)	0.001*	37.50 (3.64 – 38.65)
Yes	2 (18.2)	9 (81.8)		Ref.	1 (9.1)	10 (90.9)		Ref.

*p-value <0.05

An essential characteristic factor for water containers influencing the presence of *Aedes* immature production was the location of the water container. This study only revealed a significantly different CI in the control (p=0.04) and intervention (p=0.003) with OR=6.43 and 20, respectively, concluding that water containers outside households had a higher presence of *Aedes* immatures than indoor containers. This result may be supported by the fact that water containers outdoors are never emptied as the people allow them to collect rainwater continuously, making them perennial breeding sites for *Aedes mosquitoes* [29]. This is also related to the habit in which the local community is very dependent on rainwater as a source of clean water, so the intensity of emptying the container for cleaning is arduous. In addition, higher temperatures in the container outdoors can increase the development rate of larvae [7], [30], [31]. However, there was no significant difference in the presence of *Aedes aegypti* larvae between the control and treatment groups according to container location (p=0.08). The number of larvae based on the container's location was not affected using *Ziziphus mauritiana* as a larvicide. *Ziziphus mauritiana* and abate showed the same effect that containers outside the home increased the risk of CI but did not differ significantly between the two groups.

The present study also found more *Aedes aegypti* larvae in open-mouthed containers than covered ones. Containers without lids were proven to increase CI 10 (p=0.005) and 26 (p=0.001) times in containers given abate (control) and *Ziziphus mauritiana* extract, respectively. It is well-known that covering water containers with lids has long been promoted to prevent *Aedes aegypti* breeding sites, even though their effectiveness in rigorous studies is only sometimes clear-cut. The literature previously mentioned that this result might be linked to the female mosquito"s habits. They are usually more interested in an open-mouthed container and especially those located in places protected from sunlight to lay their eggs [27], [32]. Furthermore, containers without lids are the most common places for larvae to be found because mosquitoes can easily find water sources that will be used as places to lay their eggs [32]. Therefore, there are differences in the presence of mosquito larvae in tightly closed, semi-open, and fully open container conditions [26].

The types of water collected in this study were categorized as rainwater and non-rainwater. The analysis showed no difference in CI in the control group (p=0.40), while the intervention used 9% *Ziziphus mauritiana* leaf extract showed a significantly different CI (p=0.03). This difference was probably due to the different turbidity levels that inhibit calculating the larvae attached to the wall of the container. A study about the longevity of *Aedes aegypti* larvae in several water sources concluded that rainwater with abate (temephos) resulted in the highest number of pupae [33]. Rainwater is the most preferred medium for hatching eggs of *Aedes aegypti*, with an average of 3-4 heads/day [34].

The most important factor related to CI is public participation in eradicating dengue disease by eliminating the breeding place of *Aedes aegypti*. This variable contributed a higher OR value than other factors (OR=16.87 and 37.5 for the control and treatment groups). Draining water reservoirs is one of the behaviors that aim to prevent the breeding of *Aedes aegypti* mosquitoes in breeding and laying their eggs [35]. However, it seems challenging to be implemented. A more significant effort in collecting water may be the cause. Consequently, uncleaned water reservoirs become breeding places for *Aedes aegypti* mosquitoes [36]. Fostering active community participation is not easy, requiring understanding, awareness, and appreciation by the community of their health problems and efforts to solve them.

The most effective way to control dengue fever is to break the chain of transmission because, until now, the vaccine and medicine have not been found. Control of dengue in tropical countries is mainly achieved through surveillance of *Aedes aegypti* larvae and eliminating larval development habitats by using chemicals such as abate (temephos), which is considered the most effective method [37]. However, challenges were raised regarding the acceptability due to the unpleasant odor and the continuous use of chemical insecticides causing resistance to vector mosquitoes [38]. It is noteworthy to consider herbal larvicides produced from plants, such as *Ziziphus mauritiana*, as environmentally friendly larvicides [39]. Its antiplasmodial and antibacterial effects are due to alkaloids, flavonoids, triterpenoids, and tannins, which are known as components against mosquitos [40], [41].

This review highlights that *Ziziphus mauritiana* is one of the most potentially used interventions against dengue vectors. However, the lack of data linking the toxicological compounds of this plant with entomological indicators on the mortality of *Aedes aegypti* larvae remains a significant knowledge gap in this study. A toxicology laboratory-based study and integrated surveillance with larger samples may need to assess the community effectiveness of *Ziziphus mauritiana*. In addition, lighting was measured inaccurately because it is only based on observation, so we could not convince whether there was an influence on the density of *Aedes aegypti* larvae. Moreover, this study did not assess the density of immature *Aedes aegypti* before and after the treatment; consequently, the results may be potentially biased by this factor.

4. CONCLUSION

The results of this study demonstrate that *Aedes aegypti* larvae present higher CI among containers given abate located outside the house and without lids. Significant factors associated with CI among the *Ziziphus mauritiana* group were containers with rainwater placed outside the house without lids. Individuals' behavior to eradicate mosquito breeding significantly affect the CI for the treatment and control, respectively. It is necessary to continue monitoring the density of *Aedes aegypti* in this bio larvicide to assess the possible confounding in the association between *Ziziphus mauritiana* and CI, including environmental factors. Therefore, future studies need to consider the analysis and measure the CI before and after the treatment to obtain a more precise effect of *Ziziphus mauritiana* compared to abate.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support from the Budget Implementation Statement (DIPA) Health Polytechnic of the Ministry of Health of Pontianak with contract number HK.05.01/I.1/4072.20/2020. The authors are extremely grateful to the study's participants. This study would not have been possible without all the subjects' support and cooperation.

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