Effectiveness of Red Lime with the Local Herbal to Eliminate *Aedes spp.* Larvae in the Semi-Urban Community of Thailand: A Quasi-Experimental Study

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Abstract— Dengue virus (DENV) has been the most important arboviral disease in the world, responsible for significant morbidity and mortality, especially in tropical countries. The aim of this study was to examine the effectiveness of the red lime with the local herbal to eliminate the Aedes Aegypti larvae in the semi-urban community of Thailand before and after 2-weeks intervention program. This is a one-group design of Quasi-experimental study. The 45 samples who were lived in the semi-urban community of Thailand and recruited by using the purposive random sampling. The main outcome consisted of the house index (HI) and Container Index (CI) of Aedes spp. larvae and the incidence rate of dengue fever. The secondary outcomes were assessed the knowledge about dengue fever and awareness on dengue fever. The Shapiro–Wilk test was used to test of normality in all of variables and the paired-sample t-test was used for comparing before and after 2-weeks intervention program within the group. The results showed that the baseline compared with 2-weeks intervention, there was at 11.9% of HI and decreased to be 1.74% of HI after 2-weeks measured. There was at 9.97% of CI and decreased to be 1.34% of CI after 2-weeks measured (p<0.001). The incidence rate of dengue fever was at 622.17 per 100,000 population in the year 2020 and decreased to be 1.56 per 100,000 population. Moreover, the secondary outcomes showed that the knowledge and awareness about dengue fever were increased significantly after 2-weeks intervention program (p<0.001). The finding suggests that the healthcare staff should apply the intervention program with the usual care to eliminate the Aedes Aegypti larvae and prevent and control the dengue fever problem in the future.

Keywords— Red Lime, Intervention, Dengue Fever, Local Herbal.

I. INTRODUCTION

Dengue fever is an urgent public health problem that needs to be prevented and constantly revise combined disease control methods must be used [1]. By the way, dengue fever (DF) is a viral infection carried by Aedes Aegypti and constitutes a national health problem [2]. The spread is attributed to population congestion and improvements in the national transportation system which has allowed the vector to migrate. Dengue fever has four stereotypes (DENV-1 to DENV-4) and is transmitted through the female mosquito known as Aedes Aegypti. Severe complications of dengue fever include Dengue hemorrhagic fever and Dengue Shock Syndrome [3]. Dengue hemorrhagic fever has symptoms of high temperature, bleeding, low platelet counts and plasma leakage due to low concentration of proteins and albumins in blood [4]. Dengue shock syndrome can occur after 2-7 days of dengue hemorrhagic fever along with symptoms of low blood pressure and pulse [5].

KlongSi Subdistrict, KlongLuang District, Pathum Thani Province is a semi-urban community of Thailand which having many characteristics of the population with the high incidence rate of dengue fever [6]. The results showed that KlongSi Sub-district is the high incidence rate of dengue fever and increased in every year, the incidence rate per 100,000 population was at 113.12 in the years 2016, 169.68 in the year 2017, 226.22 in the year 2018, 233.33 in the year 2019, and 622.17 in the year 2020. So, the dengue fever in the area is crucial public health problem which should prevent and control. When considering the characteristics of the area, it was found that there is slum area. Moreover, from the pilot study, the result showed that the population were lacked of the knowledge on dengue fever and awareness on dengue fever and its effect to the incidence rate of dengue fever in the community.

Betel nut chalk or Red lime is often used to eliminate Aedes spp. Larvae. The previous study found that the betel nut chalk could be used to eliminate Aedes spp. Larvae. The Betel nut chalk with concentration as 1,682 mg/L or above was 100% effectiveness to eliminate Aedes spp. Larvae. Moreover, the concentration as 4,485 mg/L or above also caused 100% effectiveness to eliminate larva [7]. This intervention was developed the red lime with the local herbal including Thai basil or Cymbopogon citratus, Lemon Grass or Ocimum basilicum Kaffir lime rind. It is effective to eliminate the Aedes spp. Larvae [8]. The red lime with the local herbal is a concept from local wisdom by bringing herbs that can be easily to find in their community. The 3 herbs have the effect to help for eliminating the Aedes aegypti larvae [9]. In addition to this innovation is useful to the people in the

community and can also be distributed and further developed to society. Therefore, this intervention program was based on the epidemiology triangle of the diseases. The triad consists of an external agent, a susceptible host, and an environment that brings the host and agent together. The developing of the appropriate, practical, and effective public health measures to control or prevent disease usually requires assessment of three components [10]. This intervention program was separated into 3 factors to solve the dengue fever in the community. The objective of this study was to examine the effectiveness of red lime with the local herbal to eliminate *Aedes spp.* larvae in the semi-urban community of Thailand. Moreover, the specific objective of this study, there were to compare the house index (HI) and Container Index (CI) of larvae indicators and the incidence rate of dengue fever. The secondary outcomes were measured the knowledge about dengue fever and awareness on dengue fever.

II.   METHOD

The program was a one-group of the Quasi-experimental study to compare the house index (HI) and container index (CI) of larvae indicators and the incidence rate of dengue fever. The secondary outcomes were measured the knowledge about dengue fever and awareness on dengue fever before and after 2-weeks intervention program and this study did not blind. This program was recruited the participants from the semi-urban community of Thailand and founded the mosquito larva in house. The study area is KlongSi Subdistrict, KlongLuang District, Pathum Thani which is high incidence rate of dengue fever and increased in very years [6]. This program was assigned into one group design. The participants were received the red lime with the local herbal to eliminate *Aedes spp.* Larvae and improve the knowledge of dengue fever and raise awareness to protect themselves from dengue fever. The intervention was conducted between January 2021 to March 2021 through the observational structural-interview and assessment forms. This was designed to 2-weeks intervention program.

A.   Participants

The participants were recruited the population from KlongSi Sub-district, KlongLuang District, PathumThani Province of Thailand. This is a semi-urban community which located in the central part of country. The participants who were founded the mosquito larva in their house and participated by voluntary. Moreover, this program was excluded the participant who were allergized with the red lime from the study and excluded the participants who did not able to survey the container inside in house and cannot participate throughout the project. The purposive random sampling was used to select the participants into the study. As the sample size of this study was calculated by the G-power program which using the confidence interval was 95% study, allowable error was 5% and effect size was 0.33 [11]. After calculation the sample size by the G-Power version program, the total sample size was used 45 participants to select with the study.

B.   Material and Procedure

This research was conducted to use the red lime with the local herbal to eliminate *Aedes spp.* Larvae. This is one group design to measure the pre-post of outcome before and after 2-week trial. The study area was implemented at KhlongSi Sub-district, Khlong Luang District, Pathumthani Province. This intervention was separated into 2 stage includes; the experiment stage, this stage was designed to the experimental study for exploring the effective of the optimal formula of red lime with the local herbal before implementation, and the implemental stage; this stage was designed to actualize in the study area. This intervention program was developed base on the epidemiology triangle of the diseases [10]. The step of the intervention program can describe as follows:

Figure 1. Thai basil

Figure 2. Lemon grass

Figure 3. Kaffir lime rind

Figure 4. Red Lime

Figure 5. The Local Herbal by using Thai basil, Kaffir Lime Rind and Lemon Grass

1.) Experimental stage; this stage was aimed to explore the optimal formula of red lime with the local herbal before implement in the community. This study was chosen the Thai basil, Kaffir red rind and lemon grass to mix with red lime for eliminating *Aedes spp.* larvae. This program was developed with 3 formula of local herbal with the differential ratio of local herbal and controlled with red lime without the local. The conceptual of the experiment can showed as follow the figure 6.

Figure 6. The red lime with the local herbal characteristic

The properties of the ingredients consisted of 1.) Red lime is causing to ceil floating above the water. When the fog covers the surface of the water will make the mosquito larvae unable to breathe. Moreover, the new mosquitoes are unable to lay eggs. 2.) Thai Basil are the main component with eugenol, geraniol, and linalool. These substances have the effect of quickly coiling the mosquito larvae, slow down and eventually to die. 3.) Kaffir lime rind is the main component with Citrus Hystrix Dc. and can act as an oil. The substance can come out of the skin part and causing for Aedes aegypti larvae to die. and 4.) Lemon grass are containing the essential oils of Citronella, Citronellol, and Geraniol. The substances can help to repellent the mosquitoes [12].
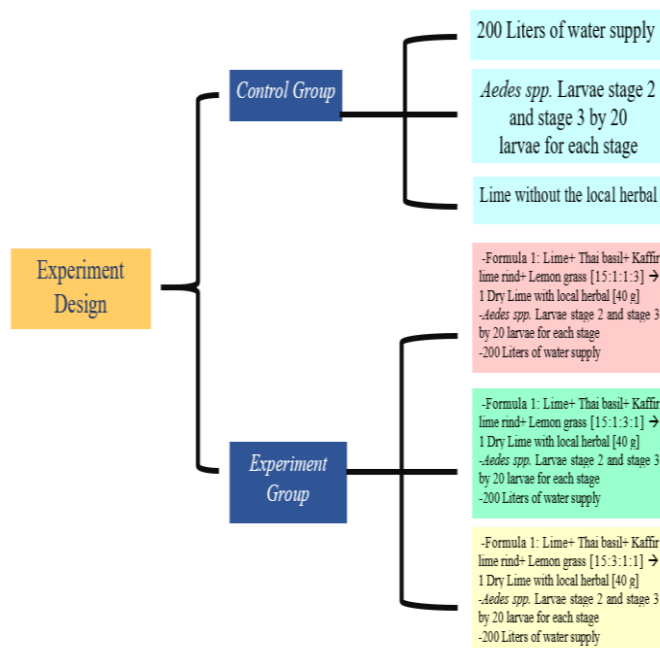

Figure 7. The Trail design of red lime with the local herbal

The researcher was conducted the experiment to test the effective of red lime with the local herbal in the laboratory room at primary health care unit. This experiment was separated into 2 group; the experiment group was proposed to use the red lime with the local herbal. These experiments have 3 formula to eliminate the *Aedes spp.* Larvae by using the difference ratio of local herbal followed by figure 7. Each Red lime with the local herbal has 40 g. per 200 L. of water supply. The results of each formula can show as the result part.

2.) Implementation stage; this stage was aimed to appraise the effectiveness of the intervention program before and after 2-week. This study was measured on 2-weeks before implementation which based on the latent period of dengue fever (5-8 days) [13]. The stakeholders were selected to join with the community forum for criticizing of the appropriately of the intervention program. After the researcher were received the community need and choose the optimal formula of red lime with local herbal found that this intervention was used the ratio of red lime with local herbal as follow; lime: Thai basil: Kaffir lime rind**:** Lemon grass with 15:3:1:1 g. The intervention program can separate into 3 *Sessions* and described as follows;

Session I; Be aware of the breeding grounds of Aedes aegypti larvae activity.

Session II; Organize a campaign to eliminate Aedes aegypti larvae.

Session III; Promote to use red lime with the local herbal to eliminate *Aedes spp.* Larvae with 1 lime, accounting for 40 g. per 200 L. of the water supply.

For data collection, this study was ordered the process of data collection as follows; 1.) Survey community profile of Moo16 communities, KlongSi sub-district, KlongLuang district, PathumThani Province, Thailand 2.) Analyze the data for exploring the community characteristics. 3.) Developing the community forum for sharing and discussing with the implementation for prevention and control the dengue fever in the community. 4.) After the researcher was get the data from the community need, the researchers were brought to develop the red lime with the local herbal and other intervention process 5.)The researchers were used the pilot study to assess the effect of red lime with the local herbal in the laboratory room of Klongsi primary Health Care Unit and consulting with the traditional medicine and reform the proportion of the ingredient to make the red lime with local herbal 6.)the researcher was proposed the intervention protocol to the head of the community to understand the concept of the intervention program and allowed to conduct the intervention program 7.) After the study protocol was approached by head of the community, the researchers were implemented the intervention program on 2-weeks of intervention and 8.) When the intervention finished, the researchers were measured the outcome followed by data collection planning, analyzing and interpreting the effectiveness of the red lime with the local herbal to eliminate *Aedes spp.* larvae in the semi-urban community of Thailand.

C. Instruments

This program was used the observational structural form and assessment form to measure the outcomes. The social distancing, mask cover and washing hand were considered to prevent the Coronavirus-19. The program was measured the

validity by peer from 3 experts on health behavior and reported the IOC value. The IOC value was ranged between 0.5-1.0. The reliability was tested in 30 participants in the same district with the study area whose characteristics were similar to those of the participants. The reliability of Cronbach's alpha coefficients was more than 0.95, it was acceptable of tool. The detail of the instrument consisted of 4 part and can be described as follows:

Part I: The baseline characteristics questionnaire and medical record form: The total of this part was 8 questions. This part was to record the general characteristics, health data and economic data such as gender, age, household number, marital status, educational level, occupational, monthly income, health problem. The medical record form was used to record the incidence rate of dengue fever at the baseline and after 2-week of intervention program.

Part II: The knowledge of dengue fever assessment form; the total of this part was 10 questions. Each question has 4 choices. The score ranged from 0-10 points. The high total scores indicated high level of knowledge about dengue fever.

Part III: The awareness with the dengue fever; this part was used to assess the awareness with the dengue fever. The total of this part was 5 questions. The score ranged from 5-15 points. The high total scores indicated high level of awareness with the dengue fever.

Part IV: The House index and Container Index of Larvae indicator; the vector surveillance is required to sustain the control measures and detect any increase in vector density. The most used indicators for vector surveillance are the House index (HI): percentage of houses infested with larvae and/or pupae and container index (CI): percentage of water-holding containers infested with larvae or pupae.

D. Ethical Consideration

This study was approved by the ethics review committee for health research. The researcher and co-researchers were told the participants about the study protocol and risk of the program before they signed a consent form.

E. Statistical analysis

The data analysis was performed using SPSS 22.0 for windows. Socio-demographic data were analyzed by descriptive statistics including frequency, percentage, range and standard division. The Shapiro–Wilk test was used to measure the normality test and normal distribution. The results showed that there was the normal distribution in all variable. As the objective of this study was to compare the house index (HI) and container index (CI) of larvae indicators, the incidence rate of dengue fever, the knowledge on dengue fever and awareness on dengue fever. The paired sample t-test was used to compare before and after 2-weeks intervention program within the group. All results with $p<0.05$ was considered statistically significant.

III.   RESULT

The results of effective to eliminate *Aedes spp.* Larvae by using the red lime in the control group and the red lime with the local herbal in the experiment group. This stage was to measure the effective of red lime with the local herbal to die at the starting to die and all of *Aedes spp.* Larvae death. The results showed that the third formula is highest effective by compared with the control group. The third formula was caused the stage 2 of *Aedes spp.* Larvae starting to die within 12 hours and all of them were died within 48 hours. Moreover, the third formula was caused the stage 3 of *Aedes spp.* Larvae starting to die within 36 hours and all of them were died within 84 hours. This formula was consisted of the lime: Thai basil: Kaffir lime rind**:** Lemon grass with the ratio as 15:3:1:1. Each Red lime with the local herbal has 40 gram per 200 L. of water supply.

TABLE 1. The results of the red lime with the local herbal to eliminate the *Aedes spp.* Larvae compared between the experiment group (lime: Thai basil: Kaffir lime rind: Lemon grass) and control group (Red lime)

Group	Stage 2 of *Aedes spp.* Larvae		Stage 3 of *Aedes spp.* Larvae	
	Strat to die	All dead	Strat to die	All dead
Control Group	36 hours	144 hours	72 hours	196 hours
Formula 1 (15:1:1:3)	24 hours	96 hours	48 hours	144 hours
Formula 2 (15:1:3:1)	12 hours	72 hours	36 hours	96 hours
Formula 3 (15:3:1:1)	*12 hours*	*48 hours*	*36 hours*	*84 hours*

A total of 45 participants in the total who were measured before and after 2-weeks of intervention program. The comparison of the primary outcome within the group revealed that there was at 11.9% of HI and decreased to be 1.74% of HI after 2-weeks measured. There was at 9.97% of CI and decreased to be 1.34% of CI after 2-weeks measured ($p<0.001$). The incidence rate of dengue fever at the baseline was at 622.17 per 100,000 population in the year 2020 and decreased to be 1.56 per 100,000 population in the year 2021. Thus, the secondary outcomes showed that the comparison of the parameters at the baseline and 2-weeks showed that the knowledge about dengue fever was at 6.40±1.55 and increased significantly to 8.90±1.05 ($p<0.001$). Moreover, the awareness about dengue fever was at 12.20±2.30 and increased significantly to 13.89±1.87 ($p<0.001$) which showed as the table 2.

TABLE 2. The results of all outcome before and after 2-weeks intervention program (n=45)

Variable	Baseline (Mean± SD.)	2-weeks Intervention Mean± SD.)	p-value
Knowledge of dengue fever	6.40± 1.55	8.90± 1.05	**<0.001***
Awareness of dengue fever	12.20± 2.30	13.89± 1.87	**<0.001***
Incidence rate of dengue fever (100,000 population)	622.17	1.56	-
HI of *Aedes spp.* Larvae	11.9%	1.74%	**<0.001***
CI of *Aedes spp.* Larvae	9.97%	1.34%	**<0.001***

REMARK: Data were analyzed with paired sample t-test within group.
*Statistically significant at the 0.05 level p-value<0.05.

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IV. DISCUSSION

The study design was pre-post of Quasi-experimental study and designed to measure at the baseline and 2-weeks intervention program. After 2-week intervention program, the results showed that the HI and CI of *Aedes spp.* Larvae was decreased significantly ($p<0.001$). Thus, the incidence rate of dengue fever was decreased significantly when compared at the baseline and 2-weeks intervention program. Thus, the secondary outcomes showed that the comparison of the knowledge and awareness about dengue fever were raised significantly ($p<0.001$). The result is consistent with the study of K. Ratchaneekorn, et al [7] whose study effectiveness of betel nut chalk to eliminate Aedes spp. Larvae, this is a systematic review. The results showed that betel nut chalk could be used to eliminate Aedes spp. Larvae. We found that Betel nut chalk with concentration as 1,682 mg/L or above was 100% effectiveness to eliminate Aedes spp. Moreover, the concentration as 4,485 mg/L or above also caused 100% effectiveness to eliminate larva. Similarly, with K. Pathanunt [14] whose study the assessment of choosing red lime innovation to eliminate Aedes Aegypti larvae. The result showed that the participants were chosen high level of the innovation especially the technology and resource, 74.83 and 71.33%, respectively. Moreover, H. Prapaisee, et al [8] whose study a model development on using red lime to control Aedes larvae. The results showed that after implementing the new model, the HI decreased from 14.29% to 2.08% and the Container Index-CI decreased from 5% to 0.21%. Infection rate of Dengue Hemorrhagic Fever was decreased from 180.83 per 100,000 populations to be none.

The results may be explained that, the situation of dengue fever in the study area was continued to increase in every year. From the condition of the problem was showed that the dengue fever has an outbreak in every year in spite of implement the biological and chemical control to prevent and control as well as developing the capacity of the personnel and selected the high technology to prevent and control, but there was founded the incidence care in every year. In the past, the intervention was measured to eliminate Aedes spp. larvae by focusing on the chemical control [15], but the villager member did not cooperate to use it. The researcher was to study the effectiveness of red lime with the local herbal to eliminate the Aedes spp. larvae. This study was recommended to use the red lime which is highest effective to eliminate the Aedes spp larvae as the third formula (Thai basil: Kaffir lime rind**:** Lemon grass) with the ratio of 15:3:1:1. Each Red lime with the local herbal has 40 gram and recommended to use with 200 L. of water supply. As the result, the effective of the red lime with the local herbal is better more that abet sand and there was no effect after use it. Moreover, using the red lime with the local herbal can eliminate the Aedes spp. larvae which followed the HI and CI were decreased after using the innovation. All sectors, especially the village member by cooperating with the village health volunteers and head of community were able to eliminate the mosquito larvae continually by using such innovation with healthcare provider will coordinate and follow the result after their use the innovation. In addition, the use of this innovation has resulted in the community can reduce the budget cost of abet sand as well as a method that is not difficult and can be done in all areas and most importantly, all sectors are involved especially the population by using the community participation to prevent and control dengue fever in the future. Moreover, the researchers were assessed the satisfaction of the innovation in this study, it was found that they mostly had high level of satisfaction (90.3%) followed by the moderate level of satisfaction (9.7%, respectively)

V. LIMITATION AND RECOMMENDATIONS

There were 3 limitation in this study. Firstly, this study was used one-group designed which there is the internal comparison. The future study should design to 2 group design. Secondly, the intervention was conducted 2-weeks. The study had short duration; the future study should apply this red lime with the local herbal to longer measured to compare the sustain of all outcome. Thirdly, the results of this study cannot generalize to the other area duo to differences in lifestyles, socio-demographic, economic status and other characteristics.

VI. CONCLUSION

This research was to measure the effectiveness of red lime with the local herbal to eliminate the Aedes spp larvae in the semi-urban community of Thailand. The result showed that the red lime with can reduce the incidence rate of dengue fever and HI of larva indicator. Moreover, the third formula was caused the stage 2 of *Aedes spp.* larvae starting to die within 12 hours and this formula was caused the stage 3 of *Aedes spp.* larvae starting to die within 36 hours. Conclusion, the red lime with the local herbal is effective to eliminate the *Aedes spp.* Larvae and the innovation can prevent and control the dengue fever in the community. The next study should demonstrate the effectiveness of red lime with the local herbal to eliminate Aedes spp. in different kinds of water, concentration, and acid-base. Side effects of using water with red lime with the local herbal should also be focused for the next study.

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