

**MINISTRY OF EDUCATION AND TRAINING- MINISTRY OF HEALTH
NATIONAL INSTITUTE OF MALARIOLOGY, PARASITOLOGY
AND ENTOMOLOGY**

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**THE SITUATION OF DENGUE VECTORS AND
EFFECTIVENESS OF SOME INSECTICIDES
AGAINST AEDES MOSQUITOES IN
DIEN KHANH DISTRICT, KHANH HOA PROVINCE
PERIOD 2015-2019**

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is a communicable disease of group B caused by the virus of the Flaviviridae family, transmitted by the female mosquito *Aedes* [1]. Among the 5 Southeast Asian countries with the highest dengue burden [5], Vietnam has implemented epidemiological surveillance measures for dengue and proactively prevented dengue transmitting mosquitoes to cut off the source of dengue virus transmission. Mosquitoes suck the blood of sick people and pass them to healthy people when they bite blood. Two popular measures to prevent dengue transmission mosquitoes that are recommended by WHO in the world and the Ministry of Health of Vietnam instructed in the national Dengue control program are the use of ULV spray to against adult mosquitoes and larvicide [6], [7]. Among the provinces with high prevalence of dengue, Khanh Hoa in the Central region has the highest number of dengue per 100,000 population in many years [13].

To answer the question about the current status of the vector of dengue transmission in some districts with high risk of dengue fever such as Dien Khanh district, Khanh Hoa province, there are changes in some biological characteristics such as species composition, behavior, price can stay, reproductive nests; Does sensitivity to insecticides reduce the effectiveness of currently commonly used insecticides when spraying, killing larvae, mosquitoes transmitting dengue?

At the same time, the study also evaluated some new insecticides for the method of spraying ULV and killing mosquito larvae *Ae.aegypti* recommended by WHO for the first time field trial in Vietnam, which is the basis for proposing measures and The insecticide is suitable for areas with high prevalence of SXHD and a high risk of mosquitoes *Ae.aegypti* has been resistant to the Pyrethroid group insecticide. Therefore, we conduct the project "Assessing the vector status of dengue fever and the effectiveness of some *Aedes* mosquito control measures in Dien Khanh district, Khanh Hoa province, the period 2015 - 2019" with 2 goals. as follows:

1. To assess the vector status of Dengue in Dien Khanh district, Khanh Hoa province in the period of 2015-2017.
2. To evaluate the effectiveness of some *Aedes* mosquito control measures in Dien Khanh district, Khanh Hoa province in the period of 2018-2019.

NOVELTY, SCIENTIFIC AND PRACTICAL SIGNIFICANCE OF THE THESIS

1. For the first time, a new insecticide, fludora co-max, which combines two active ingredients in two different groups, is recommended by WHO for the first time testing in Vietnam for a highly effective method of spraying ULV with *Ae.aegypti* population resistance to pyrethroid insecticides.
2. For the first time, a new formulation of sumilarv 2MR in Vietnam recommended by WHO to have a long-lasting effect, easy to use for larviciding against *Ae.aegypti* was resistant to insecticides in Vietnam.
3. Up to date new data on the ecological characteristics of Aedes mosquitoes (the behaviours of resting, seeking, larvae breeding site...) and the resistance level of *Ae.aegypti* to insecticides Pyrethroid group at Dien Khanh district, Khanh Hoa province.

THESIS STRUCTURE

The thesis consists of 120 pages divided into the following sections: Introduction 2 pages; Literatures review 30 pages; Research method: 20 pages; Research results: 37 pages; Discussion: 25 pages; Conclusion: 2 pages; Recommendation: 1 page. The thesis has 29 tables, 22 figures and 150 references.

CHAPTER 1

LITERATURE REVIEW

1.1.Dengue situation in the World and Vietnam

According to Wilder-Smith (2019) study over 13 years (2000-2013) showed that the number of dengue cases increased by 400% globally. Author Lee (2017) stated that Vietnam is one of the countries with the highest prevalence of dengue in the region. dengue has direct effects on the health, economy and society, especially countries in the dengue regions endemic as Vietnam [21].

The research of Do T.Thanh Toan (2015) stated that dengue is common throughout the country, the North mainly develops in the summer, autumn, the South and the Central is hot all year round, so dengue circulates all year. [23]. SXHD gradually increased and spread to 63 provinces and cities, from populous cities to rural towns, the time gap between services was also closer [23], [26]. Therefore, it is important to characterize the vector of disease transmission, and then apply some

appropriate *Aedes* mosquito control measures, especially in some provinces with highly endemic dengue [26].

Khanh Hoa is the province with the highest prevalence of dengue per 100,000 population in the Central region as well as the highest rate in the country, especially in the period 2008 to 2015. Among the districts and towns of Khanh Hoa province, Dien Khanh, Ninh Hoa and Cam Lam has a high number of cases of dengue per 100,000 population compared to other districts [30]. In Dien Khanh district in the period 2011–2014, the number of dengue cases was highest concentrated in Dien Khanh town, followed by Dien Phu and Dien Dien communes, which were bordered by Nha Trang city and Ninh Hoa district, where there are the highest prevalence in Khanh Hoa province [30].

1.3. Vector indicators for Dengue vector surveillance

Dengue vector transmission studies have identified *Aedes aegypti* as the main vector of disease transmission. Survey results of Phan Thi Kim Lien (2015) in Hanoi showed that 64.8 - 83% of the caught mosquitoes were *Aedes aegypti* [69]. According to WHO (2017), indicators used to monitor *Ae. aegypti* and *Ae. albopictus* mosquitoes include density index, house mosquito index [71].

1.4. Insecticide applied strategies

According to WHO guidelines (2016, 2018) on a global assessment of insecticide resistance against malaria vectors in the 2010-2016 period, to control insecticide resistant mosquitoes, the strategy for insecticide resistance is needed. comply with 3 specific methods [89]: alternating use of insecticides, alternating use of insecticides and coordination of many insecticide groups.

1.5. Some methods to against *Aedes* mosquitoes

Based on some international research, in order to prevent *Aedes* mosquitoes transmitting dengue to prevent and cut the transmission of dengue virus in epidemic outbreaks, the interventions focus on the adult stage and kill larvae. In it, spray space to kill adult mosquitoes and insecticides to kill - larvae inhibitors.

1.4.1. Ultra low volume spraying (ULV)

According to WHO guidelines, the ultra-small particle volume (ULV) space spray method is the first common priority measure to intervene with an outbreak with fast time and low cost. Insecticides are sprayed in the form of a mist with a tiny volume of insecticide particles <50µm into the space so that

the insecticide particles are suspended in the air for a period of time and stick to mosquitoes when flying or sitting. Fludora co-max is the first spatial spray product consisting of a mixture of two active ingredients of two different groups: flupyradifurone (butenolide group) and transfluthrin (pyrethroid group) with two other action mechanisms. This helps to increase effectiveness in killing mosquitoes, slow the development of insecticide resistance in mosquitoes. Developed by the Bayer CropScience company, fludora co-max is used in indoor and outdoor mosquito spraying [101].

1.4.2. Larviciding

Temephos belongs to the group of organophosphates used effectively in the dengue control program for the prevention of temebate Aedes aegypti larvae developed by Imaspro Resources Sdn Bhd., Malaysia. Pyriproxyfen is an insect growth regulator that inhibits the larvae stage and cannot hatch into a mosquito. Sumilarv 2MR (Pyriproxyfen 2% w / w) is a new preparation published by Sumitomo Insecticide in 2014 with a group of U- no acute toxicity (classified by WHO) used to inhibit the preterm birth hormone of larvae. do not allow to develop into cast and adult mosquitoes

CHAPTER 2

STUDY SUBJECTS AND METHODS

2.1. Study subjects

- *Ae.aegypti* and *Ae. albopictus* in Dien Khanh district, Khanh Hoa province.
- Fludora co-max: a combination of 5.0% of the pyrethroid and 2.5% flupyradifurone of the 2.5% pyrethroid and flupyradifurone group of the butenoline group in the form of an oil-in-water emulsion (EW) produced by Bayer Corp in Germany (2019) [120]
- K-othrine 2EW: component deltamethrin 2% single substance pyrethroid group in the form of oil in water emulsion (EW) produced by Bayer Corp in France (2019) [121]
- Sumilarv 2MR: pyriproxyfen active ingredient 2% (20 g ai / kg \pm 25% w / w) larvae growth inhibition group, long-lasting matrix plastic (Matrix release-MR) Sumitomo Corp made in Japan (2018) [123]
- Temebate: active ingredient Temephos 1% w / w granular OP group (Granule-G) produced by Imaspro Company in Malaysia in 2019.

2.2. Research time and place

- Research and implementation from January 2015 to December 2019.

- Criteria for selection of district site: Purposely selecting Dien Khanh district, where the highest prevalence of dengue per 100,000 population in Khanh Hoa province, bordering Nha Trang city and Ninh Hoa district with the highest number of dengue cases in the province . The district conducts key surveillance activities of the annual national dengue prevention program.
- Criteria for selection of research communes of Dien Khanh district:
 - Choose on targeted 02 communes: Dien Phu (intervention) and Dien Dien (control).
 - Two communes with the highest number of dengue cases compared to the rest of the district (2011-2014). Being a key monitoring commune for Dengue prevention.
 - The two communes have similar habitat and geographic characteristics and are bordering Nha Trang city and Ninh Hoa district.

2.3. Research Methods

-Cross-sectional descriptive studies incorporating analysis. Observational research in the laboratory. Small scale field experimental studies to evaluate the effectiveness of some insecticides. Study of community intervention with case control study, comparison before after intervention.

2.4. Sample and sampling method

The sample size was selected according to the vector surveillance regulations of the National Dengue Control Program in Decision 3711/2014 / QD-BYT issued by the Ministry of Health and WHO's guidelines for mosquito investigation. 2016 [6], [114] with 100 investigators at each study site for each survey:

- 100 houses in Dien Phu commune and 100 houses in Dien Dien commune were selected for the investigation and monitoring of mosquitoes and larvae.
- The first household is randomly selected from the list provided by the commune People's Committee, the following households are in the order of the following list up to 100 households.

2.5. Research activities

- The number, density and composition of mosquitoes, larvae, and Aedes throws, the habitats of Aedes mosquitoes. Monthly mosquito indicators, *Ae.aegypti* larvae index.

- Determine the sensitivity and resistance of *Ae.aegypti* mosquito to insecticides.

Correlation of vector indicators with dengue patients

- Correlation of the density index of mosquitoes, houses with mosquitoes, the Breteau index, the index of houses with larvae, the index of IUDs with rodents with dengue patients.

Evaluate the potency of ULV sprayers indoors

- Killing effect of insecticides
- Selecting insecticides with best killing effect for narrow field intervention.
- Assessment of mosquito density index, houses with mosquitoes before and after the intervention.

- Document undesirable responses and community approvals.

Evaluate the effectiveness of insecticides to kill larvae

2.6. Indicators of larvicidal and inhibitory efficacy

- According to WHO guidelines (2005.13) [116], to conduct parasitic surveillance at both trial and control sites, 1 day before and 7 days, 14 days, 30 days, 60 days after intervention and 90 days.

2.7. Methods of analysis and data processing: Data were analyzed and processed by Epi Info software.

2.8. Research ethics: The research has complied with the requirements and regulations on biomedical research ethics of the Institute of Malariology, Parasitology and Entomology.

CHAPTER 3 STUDY RESULTS

3.1. Current situation of the vector of dengue transmission in Dien Khanh in the period 2015-2017

3.1.1. The composition of Aedes mosquitoes:

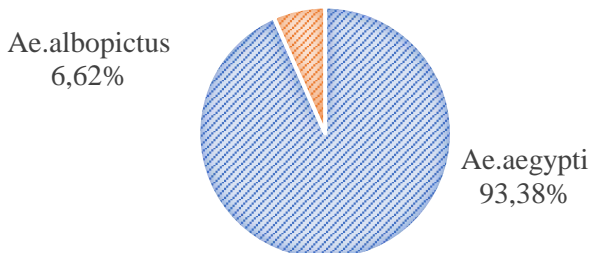


Figure 1: Composition of Aedes mosquitoes at the study site

Figure 1 was shows only 2 species of Aedes mosquitoes, of which *Ae.aegypti* was majority with 93.38% of the 921 Aedes mosquitoes caught. *Ae.albopictus* only 6.62%, the % difference between 2 species of Aedes mosquito was statistically significant ($P = 0.03 < 0.05$).

3.1.2. Habits of Aedes mosquitoes:

Bảng 1: Nơi trú đậu của muỗi Aedes tại điểm nghiên cứu

Resting place	<i>Ae.aegypti</i>		<i>Ae.albopictus</i>	
	No.	%	No.	%
I. Indoor	793		3	
- Bed room	399	50,32	0	-
- Kitchen	189	23,83	0	-
- Living room	136	17,15	0	-
- Others (store, toilet..)	69	8,70	3	100
II. Outdoor	67		58	
- Hidden place	41	61,19	25	43,10
- Tree hood, waster containers	15	22,39	24	41,38
- Larvae breeding site	3	4,48	3	5,17
- Others	8	11,94	6	10,34

The data in Table 1 was shows that *Ae.aegypti* mosquitoes are the most resting in the bedroom with the rate of 50.32% ($p < 0.05$) compared with 23.83% and 17.15% respectively. in the kitchen and living room. Only 3 mosquitoes *Ae.albopictus* scanned and caught in the bathroom account for a very small proportion. *Ae.albopictus* mosquitoes preferred to rested in dark holes outside the home and stumps of discarded plants outside the house with 43.10% and 41.38% respectively, the difference was not statistically significant ($p = 0.78 > 0.05$). Investigating the substrates and settling altitude of Aedes mosquitoes, the results are shown in Table 2:

Table 2: The substrate and settling elevation of Aedes mosquitoes at the study site

Resting place	<i>Ae.aegypti</i>		<i>Ae.albopictus</i>	
	No.	%	No.	%
Clothes hanged on the wall	279	35,18	3	100
Dark wall	186	23,46	0	-
Dark place behind furnitures	92	11,60	0	-
Clothes hanged on clothes line	89	11,22	0	

Resting place	<i>Ae.aegypti</i>		<i>Ae.albopictus</i>	
	No.	%	No.	%
Curtain	44	5,55	0	-
Bed nets	36	4,54	0	-
Under beds, chairs, wardrobes, tables	26	3,28	0	-
In the vase	26	3,28	0	-
Indoor drying line	11	1,39	0	-
Bookshelves, shelves	4	0,50	0	-
Resting altitude				
Altitude > 1.5m	116	14,63		
Altitude 0.5-1.5m	552	69,61	3	100
Altitude <0.5m	125	15,76		

Table 2 shows that *Ae.aegypti* mainly resting on wall clothes, accounting for 35.18%, with 23.46% of *Ae.aegypti* mosquitoes on the surface of walls in dark indoor areas. The rate of *Ae.aegypti* staying on surfaces such as curtains, clothes squeezed on the clothesline, and the dark place behind household utensils was not statistically significant. There are 69.61% of *Ae.aegypti* (552) that prefer to rested at a height of 0.5-1.5m on substrates such as wall clothing and wall surfaces in dark areas. However, there was no difference between the rate of *Ae.aegypti* perched above 1.5m and below 0.5m. For *Ae.albopictus*, the preferred perching height is from 0.5-1.5 m.

3.1.2. The characteristics of Aedes larvae:

The cross sectional study was indicated the larvae and the source larvae of Aedes mosquitoes at the study site.

Table 3: Ratio of Aedes larvae in water containers in the study site.

Water containers	<i>Ae.aegypti</i>		<i>Ae.albopictus</i>	
	No.	%	No.	%
Flower vase	420	66,88	0	
Aquariums	130	20,70	0	
Waste bottles	68	10,83	12	100
Big tank, jar	7	1,11	0	
Drums	2	0,32	0	
Buckets, barrels	1	0,16	0	
Total number of larvae	628	100	12	100

The results in Table 3 show that *Ae.aegypti* larvae are present in popular water containers at study site, concentrated mainly in flower vase at the rate of 66.88%, in front porch aquariums, accounting for 20.70%. and 10.83% of the *Ae.aegypti* larvae in waste bottles have water on the porch. *Ae.albopictus* is concentrated only in waste things outside the houses. Results of the survey on the rate of *Ae.aegypti* larvae in the water containers have positive for larvae at the study site are shown in Table 4:

Table 4: Ratio of *Ae.aegypti* larvae in water containers with larvae in the study site

No	Water containers	No. water containers	Infested water containers	% each infested/ each water containers	% each infested/ each water containers
1	Flower vase	280	250	89,29	89,93
2	Aquariums	75	17	22,67	6,12
3	Waste	122	7	5,74	2,52
4	Big tank, jar	51	2	3,92	0,72
5	Drums	3	1	33,33	0,36
6	Buckets, barrels	38	1	2,63	0,36
total		569	278		

With 6 types of water containers with larvae, 89.95% of 278 containers have *Ae.aegypti* larvae, the difference is statistically significant ($p < 0.05$) compared to the rate of containers also concentrating bugs. Aedes sticks with a low rate are aquariums, wastes, jars, jars, drums with the rates of 6.12%, 2.52%, 0.72% and 0.36% of the containers having larvae.

3.1.3. The susceptibility of *Ae.aegypti*

Table 5. Result of susceptibility against *Ae.aegypti* with insecticide impregnated test paper

No	Insecticide name, concentration %	mortality rate %)	Evaluation
Pyrethroid			
1	Deltamethrin 0.05%	27	Resistance
2	Permethrin 0.75%	57	Resistance
3	Lambda-cyhalothrin 0.05%	31	Resistance

No	Insecticide name, concentration %	mortality rate %)	Evaluation
4	Alpha-cypermethrin 30mg/m ²	42	Resistance
5	Cyfluthrin 15%	78	Resistance
6	Pyrethroid -Control	100	Susceptibility
Organophosphates			
7	Pirimiphos-methyl 0.25%	98	Susceptibility
8	Malathion 5%	100	Susceptibility
9	Organophosphate -Control	100	Susceptibility
Carbamate			
10	Propoxur 0.1%	98	Susceptibility
11	Carbamate -Control	100	Susceptibility
Organochlorine			
12	DDT 4%	99	Susceptibility
13	Clo- control	100	Susceptibility

The results in Table 5 was presented *Ae.aegypti* collected in Dien Khanh district, Khanh Hoa province was resistant to 5 common insecticides of pyrethroid group (alphacypermethrin, deltamethrin, lambda cyhalothrin, permethrin and cyfluthrin) with the rate of mosquitoes. death is only 27- 78%.

However, *Ae.aegypti* is still susceptibility to pirimiphos-methyl and malathion of organophosphates group, and to propoxur of carbamate group and DDT insecticide of organochlorine group with mortality rate from 98 % - 100%.

3.1.4. Mosquito indicators *Ae.aegypti* for the period 2015-2017

3.1.4.1. Average density index and house of *Ae.aegypti*

Based on the monthly vector surveillance according to the guideline of the Ministry of Health in the study site of Dien Khanh district, the average monthly average *Ae.aegypti* mosquito index in the period 2015-2017 is shown in figure 2

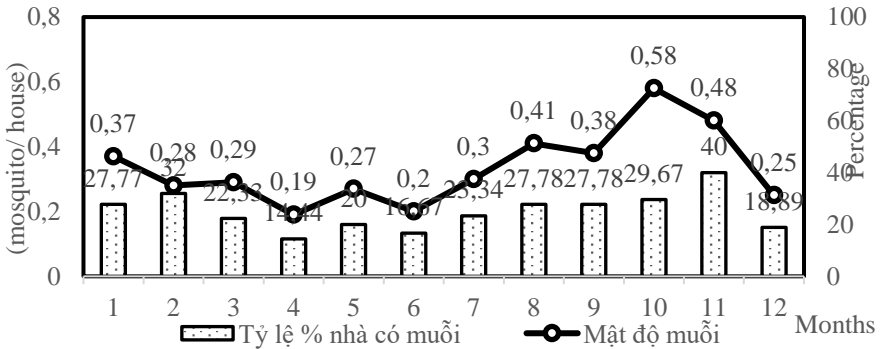


Figure 2: Average density index of houses with *Ae.aegypti* mosquitoes in the period 2015-2017

During the period 2015-2017, the results in Figure 21 show that the *Ae.aegypti* appeared in all months, of which 2 pick seasons were January and the beginning of the year with an average of 0.37 mos/ house and gradually decreasing to June. The density of *Ae.aegypti* increased again from July and highest in October with an average of 0.58 mos/ house. With the mosquito house index, the percentage of houses with mosquitoes *Ae.aegypti* in Dien Khanh has 2 peaks increasing in February with an average rate of 32% of houses with mosquitoes and the second peak in November with the average rate of 40% of investigators have mosquito *Ae.aegypti*.

3.1.3.2. Average *Ae.aegypti* larvae index

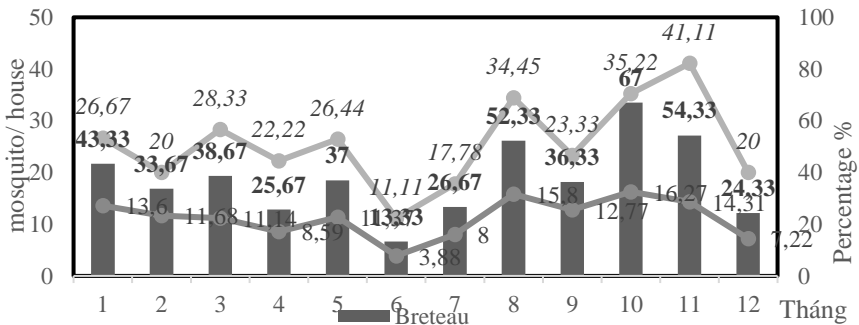


Figure 3: The average of Breteau index, the percentage of water container d and house infested with *Ae.aegypti* period 2015-2017

The results of the Breteau Index survey in Figure 3 show that the Breteau Index averaged high in January with 43.33 and the lowest drop in June with Breteau was 13.33, then peaked in October with only the average Breteau number is 67.00. The average annual rate of water containers with larvae usually increases in January 2 with an average rate of 13.60% with larvae and in October with an average rate of 16.27%. The average annual index of houses with larvae usually increases in March 2, with an average of 28.33% of houses having larvae and in November with an average rate of 41.11% of investigators. sticks *Ae.aegypti*.

1.1.2. The correlation between vector indicators and Dengue case

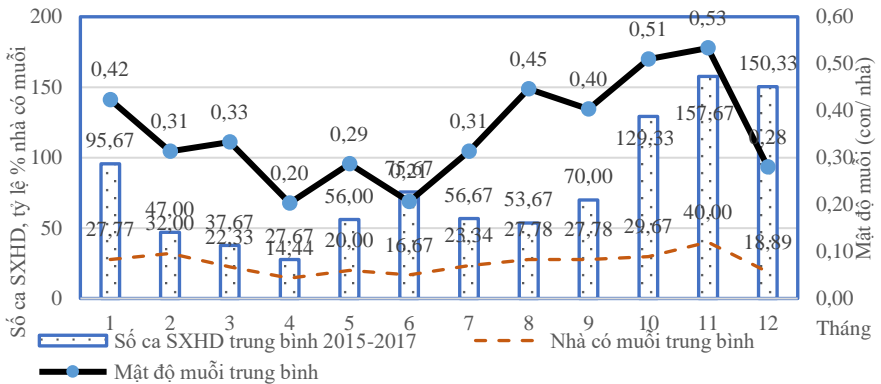


Figure 4. Number of dengue cases, vector index (2015-2017)

There is no correlation between the vector index and the average dengue cases in the period 2015-2017 in Dien Khanh district.

3.2 Evaluation of some Aedes mosquito control measures

3.2.1. Evaluation of ULV spray measure

To compare the potency of two insecticides k-othrine 2EW and fludora co-max with the indoor ULV spray method, the results are shown as follows:

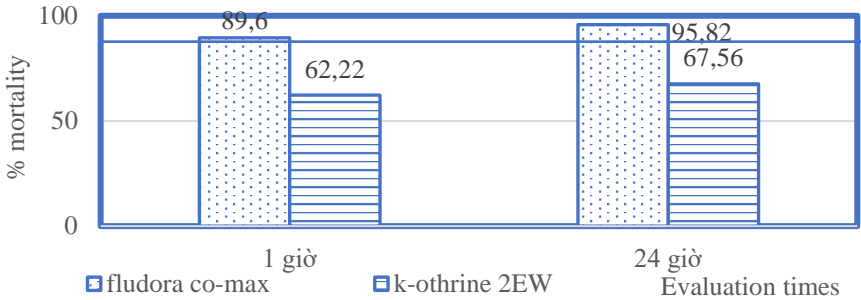


Figure 5. Insecticide potency comparison fludora co-max and k-othrine 2EW

Figure 5 was showed the fast-acting fludora co-max insecticide caused 89.60% of the tested *Ae. aegypti* knock down and good effectiveness mosquito killing effect with 95.82% of the mortality rate of *Ae. aegypti* after spraying 24 hours according to regulations of the Ministry of Health and WHO. Insecticide k-othrine 2EW was unsatisfactory with 62.22% of the collapsed *Ae. aegypti* mosquitoes and 67.56% of *Ae. aegypti* dead mosquitoes lower than the killing effect of fludora co-max. Fludora Co-max was selected to evaluate small scale trial at research sites in Dien Khanh district.

3.3.2. Evaluate the efficiency of indoor ULV spray fludora co-max in Dien Khanh district

The vector surveillance in both Dien Phu commune (intervention commune) and Dien Dien commune (control) 1 day before the intervention. Spraying insecticides fludora co-max in Dien Phu commune. The results are shown in figure 6:

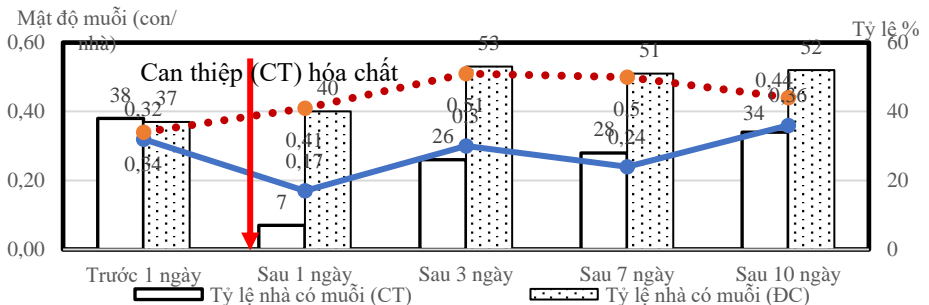


Figure 6. Density and house infested with *Ae. aegypti* in intervention commune (CT) compared with control commune (ĐC)

The results compared with the mosquito density index in the intervention and the control commune before the intervention were similar, corresponding to the density of 0.32 to 0.34 mosquitos / house at the time before 1 day of the survey. not statistically significant ($p = 0.67 > 0.05$). After spraying fludora co-max 1 day, the mosquito density in the intervention commune decreased to 0.17 mosquito/ house compared to 0.41 mosquito / house in the control commune, the difference was statistically significant ($p = 0.02 < 0.05$). After sprayed 7 days, the mosquito density in the intervention commune was 0.24 mosquitos / house compared with 0.5 head / house in the control commune, the results were statistically significant. $p = 0.04 < 0.05$). The mosquito density in the intervention commune increased again and reached 0.36 mosquito / house compared to the control point of 0.44 mosquito / house after 10 days of testing, the difference was not statistically significant ($p = 0.29 > 0.05$). The efficiency of spraying ULV on fludora co-max reached 7 days after spraying to reduce the density of mosquito *Ae. aegypti* resistant strain in Dien Khanh.

Table 6. The acceptant community and side effected for fludora co-max

No.	Questions	Total no. people	Answers	Percentae
1	Participate in trial / exposure	106	106	100
2	Any unpleasant expressions	106	0	0
3	Sneeze	106	2	1,89
4	Dizziness	106	0	0
5	Headache	106	0	0
6	Cough	106	0	0
7	Itching	106	0	0
8	Nausea	106	0	0
9	Itchy eyes	106	0	0
10	Runny nose, stuffy nose	106	0	0
11	Other symptoms	106	0	0
12	The insecticide has an unpleasant odor	106	1	0,94
13	The insecticide is easy to use	6	5	83,33
14	Would like to use insecticides at home	106	102	96,23

For indoor ULV spraying with fludora co-max, the results in table 3.21 show that out of 106 people directly spraying insecticides and representatives of interviewed families participating in insecticide spray testing, only 1.89% of people have sneezing reaction and 0.94% of people complain of unpleasant smell when they smell insecticides in 24 hours of spraying. There are 96.23% of people commenting that insecticides are easy to use and agree to use experimental insecticides with the rate of 96.23% in 106 interviewed people.

3.4. Evaluate larviciding

Comparing the killing, inhibiting and persistence effects of two insecticides sumilarv 2MR and Temebate, the results are shown in the figure below:

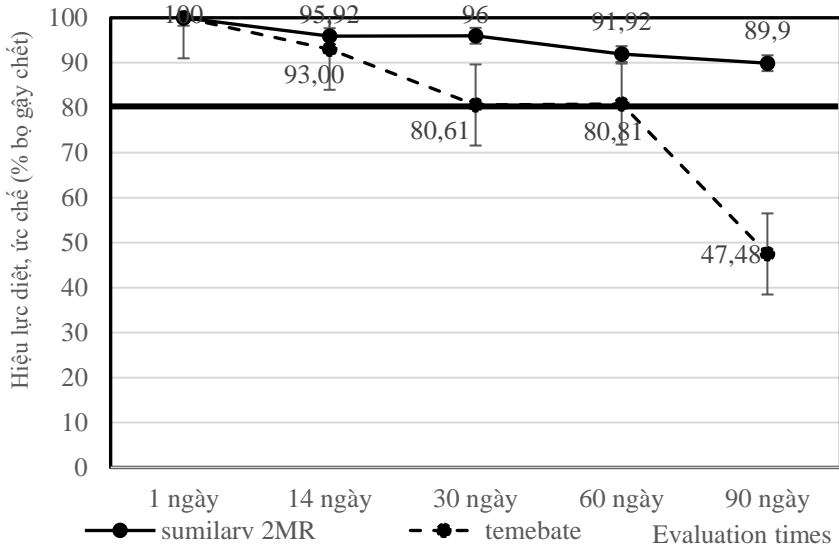


Figure 7. Comparison of killing and inhibiting effect of sumilarv 2MR and temebate under laboratory conditions

According to WHO 2005.13 guideline, if the rate of larvae mortality > 80%, the effect is satisfactory. The comparison results are shown in Figure 3.9, it shows that 2MR sumilarv has good inhibitory effect with 89.80% of *Ae.aegypti* larvae died after 3 months of testing, temebate has a killing effect of 47, 48%. The selected sumilarv 2MR insecticide was tested in the field in Dien Khanh district to evaluate the practical effectiveness in reducing the larvae index.

3.4.1. Assessment of effectiveness of larvicidal in small scale trial

The trial was conducted in Dien Phu commune - similarv 2MR intervention commune at 100 selected houses and in Dien Dien commune - control commune with 100 non-insecticide intervention houses monitored for parasitic ratios. similar to the experimental commune, Dien Phu. The two larvae indicators including Breteau, the index of the percentage of houses with the larvae and the index of the percentage of the IUDs with the larvae were monitored before and after the simultaneous intervention in both communes. Monitoring results of Breteau larvae *Ae.aegypti* index in 2 intervention and control communes are shown in the figure below:

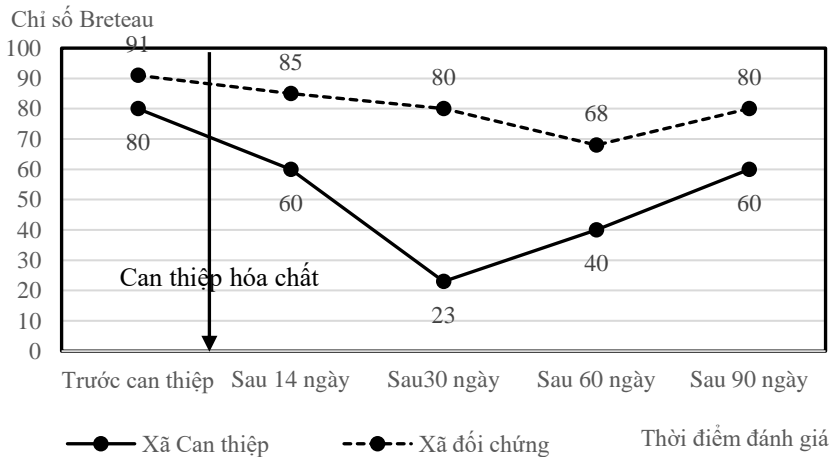


Figure 8. Comparison of Breteau index in similarv 2MR intervention commune with a control commune

Figure 8 shows that similarv 2MR reduces Breteau *Ae.aegypti* larvae index in experimental commune compared to control commune. Before the intervention time, the Breteau index of *Ae.aegypti* larvae in the intervention commune was 80 and the control commune was 91. After 30 days of intervention, the Breteau index at the intervention commune decreased 23 compared to the index. The control commune Breteau was 80.

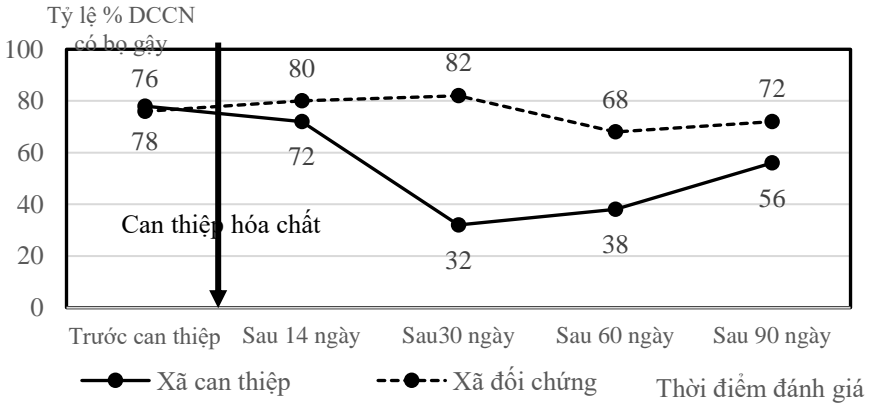


Figure 3.12 Comparison of IUDs with larvae in similar 2MR insecticide-interfered commune with control commune without insecticide intervention

Comparing the percentage of water container infested larvae (WCIL) in 2 communes, 2MR similarv insecticide reduces the percentage of WCIL with larvae *Ae.aegypti* in the narrow field of intervention commune. After 30 days of intervention, similarv 2MR reduced the proportion of houses with *Ae.aegypti* larvae to 32% compared with 82% of WCIL with *Ae.aegypti* larvae in the control commune. Comparing the difference between the larvae indicators: Breteau, percentage of households with larvae and percentage of WCIL with larvae in the 2 intervention and control communes, the results are summarized in Table 3.30:

Table 7. Results of the synthesis of the effects of 2MR similarv against for *Ae.aegypti* larvae in intervention commune compared to the control commune of Dien Khanh district

No	Evaluation times	Prevention commune (CT)			Control commune			p
		Breteau	% house infested larvae	% water container infested larvae	Breteau	% house infested larvae	% water container infested larvae	
1	Before	80	64	78	91	62	76	0,64 > 0,05

No	Evaluation times	Prevention commune (CT)			Control commune			p
		Breteau	% house infested larvae	% water container infested larvae	Breteau	% house infested larvae	% water container infested larvae	
2	After 14 days	60	60	72	85	64	80	0,19 > 0,05
3	After 30 days	23	20	32	80	70	82	0,001 < 0,05
4	After 60 days	40	46	38	68	74	68	0,005 < 0,05
5	After 90 days	60	40	56	80	68	72	0,02 < 0,05
Giá trị p		So sánh (1):(2) 0,18 > 0,05 (1): (3; 4; 5): 0,006; 0,004; 0,002 < 0,05			So sánh (1):(2; 3; 4; 5): 1; 0,88; 0,60; 0,60 > 0,05			

Table 7 shows the non-statistically significant difference between the *Ae. aegypti* larvae indexes: Breteau, percentage of houses with a larva, the percentage of WCIL with a larva in the intervention commune and control commune ($p = 0.64 > 0.05$). For Dien Phu commune-intervention: the Breteau index, the percentage of households with a larva, the percentage of IUDs with a larva in the intervention commune was still lower than before the intervention and lower than in the control commune ($p = 0.006; 0.004; 0.002 < 0.05$). Sumilarv 2MR in a narrow field intervention reduced the larvae indicators: Breteau, percentage of houses with larvae, percentage of IUDs with larvae up to 3 months after release. Interview results showed that of the 6 experimental volunteers and 100 representatives of families participating in the 2MR similarv insecticide test, no one had any adverse reactions within 24 hours of the intervention. Total 100% of people commented that similarv 2MR was easy to use and agreed to use similarv 2MR insecticide in preventing dengue transmission mosquitoes at the rate of 100%.

CHAPTER 4. DISCUSSIONS

4.1. Dengue vector situation in Dien Khanh district in the period 2015-2017

Results of the survey on the current status of dengue vectors in 2 communes of Dien Khanh district obtained 2 species of *Aedes* mosquitoes, namely *Ae.aegypti* and *Ae.albopictus*, in which *Ae. aegypti* is the dominant species with 93.38% of the *Aedes* mosquitoes collected. *Ae.albopictus* mosquito makes up only a small proportion of 6.62%. It can be seen that in the area bordering Nha Trang city, where there are highly endemic dengue, *Ae.aegypti* mosquitoes are the majority with high densities among a few species of the same species in the study area of Dien Khanh district. The survey results are consistent with research of author Truong Thi Lan Anh (2013) in Khanh Hoa province in which *Ae. aegypti* is the dominant species with 100% of the mosquitoes caught in the study site. The appearance composition of mosquito species *Ae.aegypti* predominates in highly endemic areas not only in Khanh Hoa but also in the North and the South [124].

Research on the *Aedes* mosquito species composition and density is also published by many scientists, author Leta (2018) analyzed the role of *Ae.aegypti* mosquito in the transmission of dengue and competition for environment for reproduction and development [46]. Barrera (2019) also had similar results with species composition and reproductive characteristics, mosquito behavior *Ae.aegypti* [128]

People set up a vase of Phat Loc buds with the desire for a full life. The vase is often placed on the altar and changes water rarely. Washing the flowerpot is not very frequent due to beliefs about restricting touching the altar area. This explains the reason, the density of larvae and mosquitoes *Ae.aegypti* is always in the house all year round and dengue does not decrease. The density of mosquitoes and larvae of *Ae.aegypti* and the typical source of a flowerpot was mentioned by author Nguyen Huu Tai (2019) in the study Situation and some factors related to larvae and mosquito populations. transmission of dengue dengue in Dien Loc commune, Dien Khanh, Khanh Hoa in 2019 [129].

4.2. Vector indicators of dengue transmission in Dien Khanh district, period 2015-2017

The survey results showed that the density of *Ae.aegypti* mosquitoes in 2015 and 2017 increased from January to the beginning of the year due to the impact of the high dengue situation from the end of 2014 and 2018 and the strong development of *Ae.aegypti* mosquito population. Mosquito density is usually high at 2 peaks, the first peak is from January 1-3 with a mosquito density of *Ae.aegypti* > 0.3 birds / house, the second peak is from September to November with a density of > 0.4 head / house. . The index of houses with mosquitoes *Ae.aegypti* in Dien Khanh monitored for 3 years showed that on average > 20% of houses had mosquitoes, of which 2 peaks had a high percentage of houses having mosquitoes from January to March. rate > 30% and the second peak from September to November with the rate of approximately 40% of houses having mosquitoes. The results are consistent with the author Vu Trong Duoc's description (2011) on the mosquito density in the dengue outbreaks during the investigation [131]. Using cross-sectional survey method 2 times in the dry and rainy season, 2011. The results recorded both *Aedes aegypti* and *Aedes albopictus* mosquitoes in Hanoi. Both *Aedes aegypti* and *Aedes albopictus* mosquitoes are found in the inner city and the buffer zone, while only *Aedes albopictus* mosquitoes are found in the suburbs.

Breteau index of *Ae.aegypti* mosquito in Dien Khanh monitored for 3 years showed that Breteau index increased high in January 1-3 and lowest in June, then peaked in October and November. In 2015, the Breteau Index fluctuated abnormally in March to 61 (2015). In 2015, the Breteau Index climbed as high as 90 in October.

4.3. Correlation of vector index with dengue patients in Dien Khanh district 2015-2017 period

There is no correlation between the mosquito density index and the average number of dengue cases in the period 2015-2017 in Dien Khanh district. Other indicators of mosquitoes and larvae did not correlate with the number of dengue cases. The results of correlation analysis in Dien Khanh differ from the study of author Cao Ba Loi (2016) in Tien Giang

on correlation of vector index and SXHD. Number of mosquitoes *Ae. aegypti* caught in an outbreak with the number of dengue patients in outbreaks had no correlation between *Ae. aegypti* with outbreaks of SXHD. This shows that epidemic prevention such as insecticide spraying, mosquito and larvae removal campaign when deployed may not immediately reduce the number of dengue cases but will help control the vector and reduce transmission in the next time [133].

4.4. Evaluate the ULV spray efficiency against *Aedes* mosquitoes

For Pyrethroid insecticides, k-othrine 2EW has low effectiveness with 62.22% of *Ae. aegypti* mosquito falling after 60 minutes of spraying and the killing effect after 24 hours of spraying is only 67.56. %. The results can be seen that if using a insecticide with a single ingredient Deltamethrine 2% in the presence of a population of *Ae. aegypti* mosquitoes that are highly resistant to Pyrethroid insecticides, the insecticide effect will not meet the requirements with over 90% mosquitoes die after 24 hours of spraying.

The results of insecticide tests with Deltamethrin in Dien Khanh differ from some studies with ULV sprayed Deltamethrin and hot blind.

Compared with research by author Nguyen Van Hoang (2015) experimented in Thanh Hoa spraying active insecticides to prevent and control dengue fever epidemic with community participation in two districts Hoang Hoa and Tinh Gia in 2014 for Results of mosquito density and BI index decreased and below the risk level after spraying with insecticide Hantox 200 (Deltamethrin 2%) and Permethrin 50EC compared to before spraying.

4.5. Evaluation of larvicide control method

Intervention in larval stage of mosquito *Ae. aegypti* is a highly effective measure that has been evaluated by many studies. Based on the assessment at the laboratory conditions, the method to kill the larvae with a insecticide inhibiting the hormone similarv 2MR and the insecticide temebate to kill the larvae was evaluated compared. After 3 months of follow-up, temebate decreased in efficacy and *Aedes* larvae eradication

rate <80%. Due to the odor problems of OP insecticides, Temebate is difficult to select for the intervention of larvae in the home.

Test results with 2MR sumilarv insecticides in aquariums, front porch waste water tools and Sumilarv tested in vases on the indoor altar for inhibition rate (IE) reached 89.80% up to 3 months under laboratory conditions. Evaluation results are consistent with Mian's research on Sumilarv 2MR products [140].

Results of intervention after 14 days have not been effective due to the action of Sumialrv which is a hormone inhibitor, so its action is slow. Up to 1 month, the slow inhibitory effect of Juvenoid hormone on larvae indicators was effective, causing the larvae index to decrease significantly compared to before the intervention, this result was maintained for up to 3 months of testing. experience. The results showed that the inhibitory effects on Sumilarv's larvae 2MR up to 3 months in field conditions. Compared with the studies of author Hustedt (2017), which tested Sumilarv 2MR and guppies in Cambodia, they showed good effectiveness in killing Aedes larvae in water containers containing larvae [141]. Comparing the inhibitory effect of sumilarv 2MR in Dien Khanh with the results of Min O (2018) when evaluating the larvicidal effect of long-lasting pyriproxyfen resin (SumiLarv@2MR) with the fever transmission vector hemorrhage at school in Hlaing Thar Yar Township, Yangon. The proportion of water containers with larvae fell sharply in schools using larvicide larvicides (OR: 0.24, 95% CI: 0.12–0.48) while a slight decrease in schools not in use (OR : 0.97, 95% CI: 0.55–1.72).

CONCLUSIONS

1. The actual situation of Dengue hemorrhage in Dien Khanh District, Khanh Hoa Province, phase 2015-2017

- At 2 Dien Phu and Dien Dien commune have 2 *Aedes* species, *Ae. aegypti* and *Ae. albopictus*, of which *Ae. aegypti* predominates with a 93.38% ratio.
- No change in behavior of *Aedes* mosquitoes was collected at the study site compared to previous studies, including:
 - + Resting area: *Ae.aegypti* are resting indoors (92.22%), of which 50.32% of *Ae.aegypti* rested in bedroom. *Ae.albopictus* resting outside the house in dark holes, tree stump, waste accounted for 95.08% rate.
 - + Resting position: wall-mounted clothes are where *Ae.aegypti* mosquitoes live the most with 35.18% rate, wall surface in dark areas in the house is 23.46% and some objects in the house with the rate is from 0.5 to 11.60%. There are 69.61% of *Ae.aegypti* mosquitoes and 100% of *Ae.albopictus* preferring to landed at a height of 0.5-1.5 m from the floor.
 - + Larvae main breeding site: *Ae.aegypti* mosquitoes are flower pots in the indoor altar, accounting for 89.92% of the total number of IUDs with larvae. In which 89.29% of the flower pots of buds have *Ae.aegypti* larvae. 100% of *Ae.albopictus* larvae detected in waste equipment outside the home.
- The average vector indicators of dengue transmission in the period 2015-2017:
 - + The *Ae.aegypti* mosquito is present for 12 months with 2 high increasing times in January (0.37 / house) and October (0.58 / house).
 - + *Ae.aegypti* larvae appeared for 12 months and Breteau index and rate of IUDs with larvae increased highest in January (43.33; 13.60%) and October (67.00; 16.27%).
- *Ae.aegypti* mosquito population was resistant to 5 pyrethroid insecticides but still sensitive to organic phosphorus group and carbamate group, organic chlorine group.
- There is no correlation between the indicators of mosquitoes and larvae with the number of dengue cases, postnatal factors.

2. Effective measures against the prevention of the Aedes mosquito in Dien Khanh District, Khanh Hoa Province, period 2018-2019

- Fludora co-max spraying ULV indoors has good killing effect (95.82%) with mosquito Ae.aegypti of Dien Khanh strain. Chemical K-othrine 2EW has a bad killing effect with the death rate of Ae.aegypti mosquito of Dien Khanh strain only 67.56%.
- When spraying ULV in narrow field in Dien Khanh district, fludora co-max was effective in reducing the density of mosquitoes and mosquitoes in the intervention commune after 7 days compared to before the intervention time and with the control commune. . Only 2.83% of 106 people tested undesirable effects (sneezing, unpleasant smell) and 96.23% approved the use of fludora co-max.
- In 3 months of trial, the chemical sumilarv 2MR had good inhibitory effect on Ae.aegypti larvae with the rate of 89.80% of Ae.aegypti larvae of Dien Khanh strain died. Temebate has a bad killing effect with only 47.48% of larvae dying.
- Sumilarv 2MR chemical tested in the narrow field decreased Breteau index, the rate of houses with larvae, the rate of IUDs with larvae up to 3 months after the trial. 100% of test participants had no adverse effects on exposure and approved the use of 2MR sumilarv chemicals.

RECOMMENDATIONS

1. The results of the study are the new evidence for insecticide to the Ministry of Health consider review and additional the list of insecticides for ULV space spraying and larvicide and focused to Aedes mosquito have pyrethroid resistant.
2. In the future, it is necessary to supplement the study of the focus and the relation between the vector indicators and the Dengue cases as the basis for the effective recommendation for prevention of Dengue disease.

LIST OF PUBLICATIONS

1. **Le Trung Kien**, Tran Thanh Duong, (2020). "Some biological characteristics of the Mosquito *Aedes* phase 2015-2017 and the efficacy of Fludora co-max Spray ULV in small scale trial in Dien Khanh District, Khanh Hoa Province", *Journal of Malaria Prevention and parasitic diseases*, No. 4 (118)/2020, Tr55-62.
2. **Le Trung Kien**, Tran Thanh Duong, Ho Dinh Trung and Cs (2019). "Effective evaluation of some insecticide against mosquitoes *Aedes aegypti* have resistant insecticides in Dien Khanh District, Khanh Hoa province". *Journal of malaria prevention and parasitic diseases*, No. 3 (111)/2019, Tr73-81.
3. **Le Trung Kien**, Tran Thanh Duong, Ho Dinh Trung (2019). "An effective review of some insecticides against *Aedes aegypti* insecticide resistance in the laboratory and small scale trial in Dien Khanh, Khanh Hoa province", *Journal of Malaria Prevention and parasitic diseases*, No. 4 (112)/2019, Tr26-34.