

The thesis is completed at the
NATIONAL INSTITUTE OF MALARIOLOGY,
PARASITOLOGY AND ENTOMOLOGY

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The thesis will be defended in front of the Institutional Defense Committee at the National Institute of Malaria, Parasitology, Entomology

At....., date.... month...year.....

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LIST OF AUTHOR'S PUBLICATION RELATED TO THE THESIS

- 1 Dao Minh Trang, Vu Duc Chinh (2018), "Assessment of killing efficacy of transfluthrin insecticandles to some mosquito laboratory strains", *Journal of Malaria and Parasite diseases control*, 4(106), pp. 64 – 70.
- 2 Dao Minh Trang, Vu Duc Chinh, Bui Le Duy (2018), "Assessment of efficacy of transfluthrin insecticandles against mosquitoes for farm hut sleeping in Khanh Hoa" *Journal of Malaria and Parasite diseases control*, 5(107), pp. 51 – 56.
- 3 Dao Minh Trang, Vu Duc Chinh, Truong Van Hanh, Bui Le Duy, Nguyen Van Dung (2022), "Distribution of Anopheline species according to habitat diversity and biting activities of *Anopheles dirus* at night in Son Thai commune, Khanh Vinh district, Khanh Hoa province". *Journal of Malaria and Parasite diseases control*, 1(127), pp. 13 – 21.

INTRODUCTION

Malaria vector control in the Greater Mekong Subregion (GMS) is almost entirely based on the provision of long-lasting nets (LLINs), which have been shown to be remarkably effective against malaria transmission. However, the malaria still persist, even when widespread coverage of insecticide-treated bed nets and other interventions in large scale (e.g. indoor residual spraying IRS); This transmission is called residual malaria transmission (RMT). Residual malaria transmission may be the result of vector behavior and/or human habits and practices, which increase the malaria exposure and reduce the effectiveness of other vector prevention methods.

Determining the factors of residual malaria transmission so that effective vector control measures can be taken while people are still active in forests and fields is essential to contribute to reducing the burden of malaria. Therefore, I carry out the project: "**Study on residual malaria transmission related to malaria-transmitting mosquitoes and efficacy of mosquito repellent candles contain transfluthrin in Khanh Vinh district, Khanh Hoa province (2016 - 2019)**". The objectives of the study are:

- 1. Description of residual malaria transmission factors associated with malaria-transmitting mosquitoes in Son Thai commune, Khanh Vinh district, Khanh Hoa province.*
- 2. Evaluation of the efficacy and side effects of mosquito repellent candles contain transfluthrin in the laboratory and in the field*

NOVELTY, SCIENTIFIC AND PRACTICAL SIGNIFICANCE OF THE THESIS

1. This is the first study conducted in the area of Son Thai commune, Khanh Vinh district, Khanh Hoa province to analyze and describe the factors of residual malaria transmission that are: the source of malaria parasites that frequently exist. in the community; The circulation of malaria vectors in forest and upland habitats; The important transmission role of *An. dirus* combined with people's living habits easily exposed mosquitoes; Measures to control malaria vectors have not reached coverage in forested areas.

2. Detecting the early starting time of biting (17 hours) is a new and significant record, creating a basis for further research on the influence of

ecological conditions on the vector's biting behavior. In addition, data on the difference in the rate of biting indoors and out in the farm house of *An. dirus* at different times (beginning, end and middle) during the rainy season and specific data were obtained on tracking the movement of people who regularly go to the forest, on a satellite map through the use of navigation equipment. GPS locations are valuable new contributions, serving as the basis for the addition of malaria vector control measures.

3. For the first time studying the efficacy of mosquito repellent candles containing transfluthrin and the efficacy of individual protection and community acceptance of mosquito repellent candles in malaria vector control for forest goer and farm goer who work and sleep in remote forest and farm locations in Vietnam.

THESIS STRUCTURE

The thesis consists of 115 pages divided into the following parts: Introduction 2 pages; literature review 32 pages; Study methods: 20 pages; Study results 34 pages; Discussions: 24 pages; Conclusion 2 pages; Recommendation 1 page. There are 10 figures, 28 tables of data and 163 references.

Chapter 1:

LITERATURE REVIEW

1.1. Malaria situation in the world and in Vietnam

1.1.1. Malaria situation in the world

Malaria is one of the leading deadly diseases for humans, caused by several species of Plasmodium parasites. There are about 350-500 million cases of malaria each year and at least one million of them die all around the world. Of these, 90% are in sub-Saharan Africa, in remote areas with underdeveloped health services. However, other regions such as Asia, Latin America, and the Middle East are still being affected [9].

1.1.2. Malaria situation in Vietnam

In general, the malaria situation nationwide tends to decrease year by year. However, the malaria situation in some localities is still complicated, mainly in the Central-Central Highlands-Eastern provinces. In the South, the risk of malaria returning and the risk of malaria outbreak is still high in many localities. Especially

now, the status of drug-resistant parasites and insecticide-resistant mosquitoes appear and are at risk of spreading in many localities.

1.1.3. Malaria situation in Khanh Hoa

The trend of malaria in Khanh Hoa is similar to some provinces in the Central-Central Highlands. Although malaria cases have decreased compared to before, the number of malaria cases in Khanh Hoa is still high compared to other provinces in the region and is one of the 10 provinces with the highest average annual number of malaria parasites in the country in the period 2016-2020 [19]. However, if calculating the rate of parasites/population in malaria-endemic areas, it is very high and mainly concentrated in two mountainous districts, Khanh Vinh and Khanh Son in Khanh Vinh district, both of which are highly endemic (table 1.2.), in which Son Thai commune is one of the communes with the highest rates of malaria and malaria in the district (table 1.3.). There are circulation of main vector of malaria *An. dirus* and *An. minimus*, in addition to some secondary vector of malaria such as *An. maculatus*, *An. jeyporiensis*, *An. aconitus*. Although the locality has complied with the application of malaria control measures under the direction of the National malaria Control Program, especially the prevention of malaria, malaria still residual, especially for the group of people that work and sleep in remote forest and farm locations.

1.2. Study on distribution of Anopheles mosquitoes in the world and Vietnam

1.2.1. Study on the distribution of Anopheles mosquitoes in the world

Sinka M. et al (2012) synthesized 465 species of Anopheles in the world, of which about 70 species are capable of transmitting human malaria and 41 species are major malaria vectors[22].

Based on the research results of many authors around the world, it has been determined that in each geographical area, there are Anopheles mosquito species with the ability to transmit malaria at different levels, which are divided into main vectors and vectors. Secondary vectors depending on their ability to transmit disease in each region. Main or secondary roles can change over time, geographically. The presence of main and secondary vectors is a determinant of malaria transmission.

1.2.2. Study on distribution of Anopheles mosquitoes in Vietnam

In 2008, the National Institute of Malaria, Parasitology, Entomology published a list of Anophelinae mosquitoes in Vietnam including 63 Anopheles species. [33]. Of which, 10 species were identified as the main and secondary vectors of malaria. The distribution of these vectors can be summarized as follows [34]:

+ **Main vector:**

- National mountainous region: *An. minimus*
- Mountains and forests from 20 degrees north latitude to the south: *An. minimus*, *An. dirus*
- Southern brackish water coastal area: *An. epiroticus*

+ **Secondary vectors:**

- National mountainous region: *An. aconitus*, *An. jeyporiensis*, *An. maculatus*.
- Northern coastal region: *An. subpictus*, *An. sinensis*, *An. vagus*
- Southern Coast: *An. sinensis*, *An. subpictus* và *An. campestris*.

1.3. Study on the behavior of malaria vectors

1.3.1. Host preference behavior

An. dirus is a species that prefers to feed on human blood than cattle. The ratio of human to animal bites varies from place to place, but mosquitoes are more likely to bite humans than animals.

An. minimus has a affinity for human blood, the density of mosquito bites is usually higher than that of cattle. However, this density also varies in different regions.

An. maculatus is a species that prefers biting cattle than biting humans, but there are a few that biting both indoors and outdoors.

1.3.2. Blood biting activity behavior

The time of biting activities of *An. dirus* throughout the night, biting people both inside and outside the house, the density of mosquito bites is higher in the first half of the night [38]. Predation outdoors tends to be higher than indoors, and activity peaks vary by region and by season.

Many studies indicate that *An. minimus* prefers to biting outdoors than indoors. The biting activity of *An. minimus* throughout the night, and the peak of the stinger varies in space and time [64].

An. maculatus biting both indoors and outdoors. The density of biting outside the house is usually higher than indoors.

1.4. The role of malaria transmission of Anopheles

1.4.1. The role of malaria transmission of *An. dirus*

An.dirus mosquito plays an important role in malaria transmission in the forest area.

1.4.2. The role of malaria transmission of *An. minimus*

An. minimus is the main vector of malaria in all regions where they are present.

1.4.3. The role of malaria transmission of *An. maculatus*

An. maculatus is the main malaria vector in Malaysia, Thailand and Laos, but is considered as a secondary vector of malaria in Vietnam.

1.5. Insecticide resistance of malaria vectors

1.5.2. Study on resistance to insecticides of malaria vectors in the world and in Vietnam

The development of insecticide resistance of malaria vectors makes reduce the effectiveness of malaria vector control. Therefore, regularly investigating vector density and assessing the level of resistance to insecticides will help entomologists and epidemiologists to choose chemicals to proactively respond with risk of malaria epidemic.

1.6. Study on malaria vector control measures

1.6.3. Personal measures

In addition to products such as repellent cream, incense, and mosquito repellent candles have been researched and tested in the prevention of disease-transmitting insects. Mosquito repellent candles containing transfluthrin, a chemical of the pyrethroid group, have been tested to be effective in some countries such as Malaysia, Australia... in the prevention of disease-transmitting insects.

Chapter 2: STUDY METHODS

2.1. Objective 1: Description of residual malaria transmission factors associated with malaria-transmitting mosquitoes in Son Thai commune, Khanh Vinh district, Khanh Hoa province.

2.1.1. Subjects, time and place of the study

2.1.1.1. Research subjects

Anopheles mosquitoes and other malaria parasites infecting mosquitoes in the study area.

Species of malaria parasites infecting humans in the study area

People live in Son Thai commune, Khanh Vinh, Khanh Hoa.

2.1.1.2. Research time

Duration: From January to December 2016.

2.1.1.3. Research location

Studied site: Son Thai commune, Khanh Vinh district, Khanh Hoa province.

2.1.2. Methods

2.1.2.1. Study design

- Cross-sectional descriptive study
- Experimental research at the laboratory.

2.1.2.2. Sample size

- The sample size to determine the malaria infection rate in Son Thai was calculated by the formula:

$$n = Z_{(1-\alpha/2)}^2 \frac{pq}{d^2}$$

- Sample size of mosquito survey: Collect all samples of *Anopheles* mosquitoes in village, farms and forests in 3 surveys, July, October and December.

- Survey sample size observing indoor activity and net use status of people and house structure:

+ In the village: 35 households in the residential area for 1 survey, a total of 3 times is 105 households,

+ In the farm: 9 field houses have people living around the area to catch mosquitoes.

- Sample size of survey to observe people's outdoor activities: each survey 5 routes in village and farms, 1 route per night in 3 surveys of July, October and December.

- Tracking people's movements with GPS devices: 80 turns of people often go to the forest to sleep in the farms.

2.1.3. Study content

- Assess the situation of malaria parasite infection in Son Thai commune

- Investigation of species composition and density of *Anopheles* mosquitoes

- Investigate indoor activity and nets usage status

- Investigate and observe various types of outdoor activities

- GPS tracking for individuals that work and sleep in the forest and farms

2.1.5. Indicators

- The rate of malaria parasite infection in humans through blood smear and PCR

- Density of *Anopheles* mosquito species collected by human landing catch method and cattle sheds catch method

- Rate of parasite infection in mosquitoes (%):

- Percentage of households sleeping under nets (%)
- Insect transmission index year (Annual Entomological Inoculation Rate – AEIR) [120] $AEIR = ma \times s \times 365$

2.2. Objective 2: Evaluation of the efficacy and side effects of mosquito repellent candles contain transfluthrin in the laboratory and in the field

2.2.1. Subjects, time and place of the study

2.2.1.1. Study subjects

- Mosquito repellent candle cup contains the active ingredient transfluthrin ($C_{15}H_{12}Cl_2F_4O_2$)
 - *An. dirus*; *An. minimus* strains raising in the laboratory.
 - *Anopheles* mosquito species at the study site
 - Participant in testing candles in the laboratory.
 - People work and sleep in the forest and in the farms, people take part in the experiment

2.2.1.2. Study duration

- From January to December 2018.

2.2.1.3. Study sites

- Laboratory of Entomology Department of National Institute of Malaria, Parasitology and Entomology
- Son Thai commune, Khanh Vinh district, Khanh Hoa province

2.2.2. Methods

2.2.2.1. Study design

- Experimental research in the laboratory
- Trial with control in the field.

2.2.2.2. Sample size

- Candle sample size: at least 10 candles/each concentration with 4 candle concentrations

- Test sample size to evaluate the efficacy of candles in the laboratory: the tests were repeated 3 times with 2 tested mosquito species, *An. dirus*, *An. minimus*.

- Sample size to evaluate the protective effect of mosquito repellent candles: tested at 8 farming houses for 8 nights in which 4 experimental houses, 4 control houses, each house has 1 person to catch mosquitoes inside or outside the house [128].

- Sample size to evaluate side effects: 6 participants tested candles in the laboratory, 8 participants tested candles in the field, 22 people lived in the farmhouse area of Son Thai commune.

2.2.3. Study content

- Evaluation of the killing effect of candles containing transfluthrin according to 4 concentrations of 0.02%, 0.03%, 0.04%, 0.05 with 2 species of *An. dirus*, *An. minimus* laboratory strain in a glass chamber measuring 70 cm x 70 cm x 70 cm [122] and a Peet Grady chamber measuring 180 cm x 180 cm x 180 cm according to WHO guidelines [123]

- Evaluation the efficacy of repellent mosquito candles containing transfluthrin at candle burning intervals of 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 24 hours.

- Evaluation of side effects on participants in the laboratory

- Evaluate the efficacy of repellent mosquito candles in the field

- Evaluation of side effects and the community's acceptance of using candles.

2.2.5. Indicators

- Rate of mosquitoes knockdown (%)

- Ratio of mosquitoes mortality after 24 hours(%)

-The density of Anopheles mosquito species collected by human landing catch method was calculated according to the method of the National Institute of Malaria-Parasites-Entomology [5].

- KT_{50} , KT_{95}

- Rate of side effects of participant in testing candles

- Efficacy of repellent mosquito candles in the field

2.4. Data processing and analysis methods

2.4.1. Data processing

- Data was entered into Microsoft Excel software

- Processed by SPSS software to determine KT_{50} , KT_{95} values.

- GPS data is acquired by i-gotU gt-120 GPS logger, downloaded into the corresponding software and exported to Google Map..

2.4.2. Data analysis

- Calculate frequency, percentage of variables.

- Calculate the density of Anopheles mosquito species.

- Compare the KT_{50} , KT_{95} values

- Comparison of malaria vector density

2.5. Ethical clearance

The study was approved by the Ethics Committee of the National Institute of Malariology, Parasitology and Entomology..

Chaper 3: RESULTS

3.1. Malaria epidemiology and residual malaria transmission factors related to malaria-transmitting mosquitoes in Son Thai commune, Khanh Vinh, Khanh Hoa

3.1.1. Malaria epidemiological situation in Son Thai commune

3.1.1.4. Results of longitudinal monitoring of malaria parasite status in Son Thai in 2016

Bảng 3.3. Number of malaria cases by parasite species in 2016

Species of parasites	Number of malaria cases by 12 months												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
<i>P. falciparum</i>	4	2	1	2	2	0	29	4	5	1	1	8	59
<i>P. vivax</i>	0	2	0	1	0	1	2	0	1	0	0	0	7
Total	4	4	1	3	2	1	31	4	6	1	1	8	66

With the data obtained on malaria cases from Son Thai commune health station, Khanh Vinh district health center and longitudinal follow-up investigation by the research team, it shows that the situation of malaria infection in Son Thai commune occurs all year round. The composition of malaria parasite species is mainly *P. falciparum*, *P. vivax* accounts for a low proportion.

3.1.1.5. Analysis of malaria parasite situation through cross-sectional survey

Bảng 3.4. Results of detecting malaria parasites by testing methods in Son Thai commune in July 2016

Test method for parasites	The rate of malaria infection (%)		
	Bo Lang Village	Giang Bien Village	Total
Total number	357	343	700
1. Blood smear Test			
The rate of malaria infection	1,12 (4/357)	2,04 (7/343)	1,57 (11/700)
Malaria parasites			
<i>P. falciparum</i>	18,18 (2/11)	54,55 (6/11)	72,73 (8/11)
<i>P. vivax</i>	0	9,09 (1/11)	9,09 (1/11)
Gametocyte infected	18,18 (2/11)	0	18,18 (2/11)
2. PCR Test			
The rate of malaria infection	1,40 (5/357)	2,04 (7/343)	1,71 (12/700)
Malaria parasites			

<i>P. falciparum</i>	41,67 (5/12)	50,00 (6/12)	91,67 (11/12)
<i>P. vivax</i>	0,0 (0/12)	8,33 (1/12)	8,33 (1/12)
Malaria infection rate without symptoms (%)	80,0 (4/5)	42,86 (3/7)	58,33(7/12)

The total number of malaria parasites detected through 2 methods is 12 parasites. The composition of malaria parasite species through cross-sectional survey (table 3.4.) was mainly *P. falciparum*, while *P. vivax* accounted for a low proportion. A total of 12 parasites were detected, accounting for 1.71% with 11 cases of *P. falciparum* infection and 1 case of *P. vivax* infection, no other parasite species were detected. Through scintigraphy, 2 cases of gametocyte formation were detected. The rate of malaria infection without symptoms is high, accounting for 58.33% of the total number of detected infections.

3.1.2. Residual malaria transmission factors associated with malaria-transmitting mosquitoes in Son Thai commune, Khanh Vinh district, Khanh Hoa province

3.1.2.1. Anopheles species composition in 3 landscapes in Son Thai commune

Table 3.5. Anopheles species composition in 3 landscapes in Son Thai commune, 2016

N ^o	Species	Number of mosquito		
		Village	Field	Forest
1	<i>An. aconitus</i> Doenitz, 1902	36	0	0
2	<i>An. barbirostris</i> Van Der Wulp, 1884	33	0	0
3	<i>An. dirus</i> Peyton & Harrison, 1979	0	206	65
4	<i>An. jamesi</i> Strickland & Chowdhury, 1927	110	0	0
5	<i>An. kawari</i> (James, 1903)	2	0	0
6	<i>An. kochi</i> (Dönitz, 1901)	200	0	0
7	<i>An. maculatus</i> Theobald, 1901	11	10	3
8	<i>An. nivipes</i> (Theobald, 1903)	1	0	0
9	<i>An. peditaeniatus</i> (Leicester, 1908)	0	2	0
10	<i>An. philippinensis</i> Ludlow, 1902	114	0	0
11	<i>An. sinensis</i> Wiedemann, 1828	118	0	0
12	<i>An. splendidus</i> Koidzumi, 1920	48	0	0
13	<i>An. vagus</i> Doenitz, 1902	263	0	0
	Total	936	218	68

In 3 landscapes of Son Thai commune, Khanh Vinh district, Khanh Hoa province, 13 species of Anopheles mosquitoes were collected in all 3

surveys, the species were mainly caught in village. The main malaria vector *An. dirus* cannot be collected in village, only in farm and forested areas. *An. minimus* can't be collected during three surveys.

Bảng 3.7. The density of biting indoor and outdoor of malaria vectors in the farm house through 3 surveys in 2016

N ^o	Species	Density of biting indoor in the farm house (individual/person/night)			Density of biting outdoor in the farm huouse (individual/person/night)		
		July	October	December	July	October	December
1	<i>An. dirus</i>	8,8	3,0	4,29	9,0	0,4	10,00
2	<i>An. maculatus</i>	0	0	0,29	0,14	0	0,86
3	<i>An. peditaeniatus</i>	0	0	0,00	0	0	0,29

By HLC inside and outside the farm house of Son Thai commune, through 3 surveys, 3 species of Anopheles mosquitoes were obtained *A. dirus*, *An. maculatus*, *An. peditaeniatus*. The main malaria vector *An. dirus* collected in 3 surveys with both indoor and outdoor with the highest density in July, October and December, respectively 9.0 (individual/person/night); 3.0 (individual/person/night) and 10.00 (individual/person/night).

Table 3.8. Density of Anopheles mosquitoes in the forest through 3 surveys in 2016

N ^o	Species	Density of malaria vectors biting human in the forest (individual/person/night)		
		July	October	December
1	<i>An. dirus</i>	5,2	0	5,57
2	<i>An. maculatus</i>	0,6	0	0

In 3 surveys, in the forest of Son Thai commune with HLC method, 2 species of Anopheles mosquitoes were obtained, in which the main vector *An. dirus* is the dominant species with densities of 5.2 individuals/person/night (July) and 5.57 individuals/person/night (December), secondary vector *An. maculatus* was only collected in the July survey at a density of 0.6 individuals/person/night (table 3.8.).

3.1.2.3. The biting activity of *An. dirus*

The biting activity of *An. dirus* inside and outside the farm houses

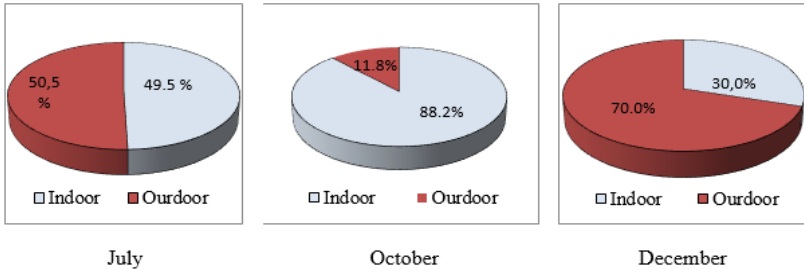


Figure 3.1. Ratio (%) of blood biting inside and outside the farm houses of *An. dirus*

There was a change in the rate of mosquito bites inside and outside farm house of *An. dirus*, in July (the beginning of the rainy season) the rate of human blood biting inside and outside the house was similar (50.5% and 49.5%), but in October (mid the rainy season) the tendency to bite people in houses were higher (88.2% and 11.8%), at the end of the rainy season, the rate of human arson reversed (30% and 70%).

- The nighttime biting activity of *An. dirus*

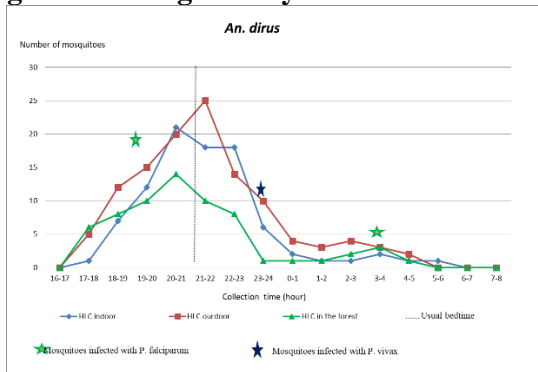


Figure 3.2. The nighttime biting activity of *An. dirus*

An. dirus biting activities throughout the night from 18-6 hours, in which the strongest biting time both indoors and outdoors is from 20-23 hours. In December, the investigation period was extended from 16 hours, and *An. dirus* mosquitoes were collected early from 17 hours.

3.1.2.4. The biting activity of *An. maculatus*

- The nighttime biting activity of *An. maculatus*

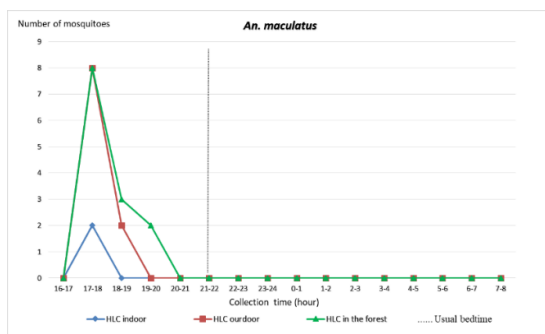


Figure 3.3. The nighttime biting activity of *An. maculatus*

An. maculatus biting activities occur before 9 p.m., including indoors, outdoors, in the forest and farm of Son Thai commune.

3.1.2.5. Role of malaria transmission of *Anopheles* mosquito

Bảng 3.11. Results of detecting parasites in mosquitoes by PCR

N ^o	Species	Number	Number detecting parasites		
			Pf	Pv	Total
1	<i>An. aconitus</i>	32	0	0	0
2	<i>An. barbirostris</i>	13	0	0	0
3	<i>An. dirus</i>	271	2 (0,74%)	1(0,37%)	3 (1,11%)
4	<i>An. jamesi</i>	26	0	0	0
5	<i>An. kawari</i>	2	0	0	0
6	<i>An. kochi</i>	40	0	0	0
7	<i>An. maculatus</i>	24	0	0	0
8	<i>An. nivipes</i>	1	0	0	0
9	<i>An. peditaeniatus</i>	2	0	0	0
10	<i>An. philippinensis</i>	14	0	0	0
11	<i>An. sinensis</i>	7	0	0	0
12	<i>An. splendidus</i>	9	0	0	0
13	<i>An. vagus</i>	114	0	0	0
	Total	555	2	1	3

In 555 mosquito samples analyzed to identify parasites by PCR, 3 samples were positive for parasites. All 3 samples positive for parasites identified as *An.dirus* were collected during the survey in July, in which 1

sample positive for *P. falciparum* (+) was collected outside the farm house from 3- 4 hours, 1 sample positive for *P. vivax* (+) was collected outside the farm house between 23 and 24 hours and 1 sample *P.falciparum* (+) was collected in the forest between 19 and 20 hours hours. The percentage of mosquitoes *An. dirus* infection with parasites is 1.11%.

Bảng 3.12. The Entomological Inoculation Rate index of *An. dirus*

Landscapes	Index		AEIR
Outside farm hut	Density (individuals/person/night)	6,882 (117/1/7)	21,47
	<i>P. falciparum</i> infection rate (%)	0,855 (1/117)	
	Density (individuals/person/night)	6,882 (117/1/7)	21,47
	<i>P. vivax</i> infection rate (%)	0,855 (1/117)	
Forest	Density (individuals/person/night)	4,643 (65/1/14)	26,07
	<i>P. falciparum</i> infection rate (%)	1,538 (1/65)	

The Annual entomological inoculation rate Index for *P. faciparum* and *P. vivax* of *An. dirus* outside the farm house is 21.47; for *P. faciparum* of *An. dirus* in the forest was 26.07.

3.1.2.6. *The factors related to ability exposure between people to mosquitoes in Son Thai commune*

The structure of the farm houuse and the situation of using the nets

Table 3.14. Survey results on types and materials of houses farm

Survey content	Number of farm house	Ratio (%)
Number of farm house	9	100
Materials	Wood/bamboo	9
	Brick	0
Farm house types	Open	4
	Partially open	5
	Close	0
Number of Nets	7	
Number of people	33	
Rate (people/net)	4,71	

In the July survey, no people were observed sleeping in the farms, 6 farm houses were observed in the October survey and 3 farm houses in the December survey. All the farm houses were observed in Son Thai is made

of wood/bamboo, the farm house has a simple structure assembled from bamboo, the walls have gaps or lack walls (has 3 walls).

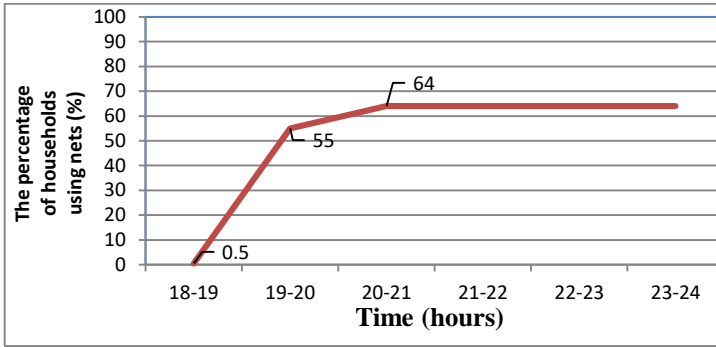


Figure 3.8. Percentage of households using mosquito nets in farm house

The time of using nets of people in the farm houses started at 19 pm. with the rate of 55%, and only reached 64% when the observation time ended at midnight.

3.1.2.7. The sleeping status of the participants in the farm and forest was monitored by GPS locator

GPS tracking data show that the rate of nights sleeping in the farms is 21.4%, the rate of nights sleeping in the forest is 7% (table 3.15), the total rate of nights sleeping in the forest and sleeping in the farm is 28.3%, accounting for nearly One-third of the GPS study participants' total nights sleep.

Bảng 3.15. Percentage of people's nights sleeping in villages, forests and farms via GPS

GPS Tracking	Village		Farm		Forest	
	N ⁰	Ratio (%)	N ⁰	Ratio (%)	N ⁰	Ratio (%)
Number of nights	265	71,6%	79	21,4%	26	7,0%

The status of people going to the forest, sleeping in the farms with complicated travel routes through the locations where malaria is present is a challenge for malaria control for this population when they have not been adequately protected by vector control measures.

3.2. Efficacy of mosquito repellent candles in the laboratory and efficacy of mosquito repellent candles in malaria vector control for farm goers and forest goers

3.2.1. Efficacy of mosquito repellent candles in the laboratory

3.2.1.4. Efficacy of 0.04% transfluthrin candle in Peet Grady chamber

Bảng 3.20. Knockdown and Mortality rate of 0.04% transfluthrin candles in Peet Grady chamber (180cmx180cmx180cm)

Test information	Species					
	<i>An. dirus</i>			<i>An. minimus</i>		
	R1	R2	R3	R1	R2	R3
Number of mosquitoes tested (individuals)	100	100	100	100	100	100
Number of mosquitoes knocked down after 60 minutes (individuals)	100	100	100	100	100	100
Knockdown rate (%)	100			100		
KT ₅₀ (minutes)	4,62±0,15			4,52±0,25		
KT ₉₅ (minutes)	8,75±0,25			9,35±0,29		
Number of deaths after 24 h	300			300		
Mortality rate (%)	100			100		

The mortality rate after 24 hours of 2 mosquito species was 99-100% with 0.04% transfluthrin candle. The knockdown time for mosquitoes KT₅₀ is 4.62±0.15 minutes, 4.52±0.25 minutes, respectively. The knockdown time for mosquitoes KT₉₅ was 8.75±0.25 minutes, respectively; 9.35±0.29 minutes.

Thus, the knockdown and lethal effects of mosquito repellent candles contain 0.04% transfluthrin with *An. dirus*, *An. minimus* is high

3.2.2. Efficacy of mosquito repellent candles contain 0.04% transfluthrin in the field

Bảng 3.23. Protection efficacy against *An. dirus* mosquito of repellent candles contain 0.04% transfluthrin inside farm houses

Time (hour)	Tested (burn candles)		Control (no burn candles)		Protection efficacy (%)
	Number (individuals)	Density (I/H/P)	Number (individuals)	Density (I/H/P)	
18-19	0	0	7	0,4375	100
19-20	7	0,4375	26	1,625	73,08
20-21	4	0,25	27	1,6875	85,19
21-22	0	0	27	1,6875	100
22-23	1	0,0625	13	0,8125	92,31
23-24	1	0,0625	4	0,25	75
24-01	0	0	5	0,3125	100
01-02	0	0	2	0,125	100

Time (hour)	Tested (burn candles)		Control (no burn candles)		Protection efficacy (%)
	Number (individuals)	Density (I/H/P)	Number (individuals)	Density (I/H/P)	
02-03	0	0	2	0,125	100
03-04	0	0	0	0	-
04-05	0	0	1	0,0625	100
05-06	0	0	0	0	-
Total	13	0,8125	114	7,125	88,59
p	p<0,05				

Note:: I/H/P: individuals /hour/person

Mosquito repellent candles contain 0.04% transfluthrin was effective against mosquito bites throughout the night with a protective effect from 73.08% to 100%. The average efficacy of preventing mosquito bites inside the farm house all night (12 hours) is 88.59%.

3.2.2.2. Effect of personal protection against mosquito bites outside the farm house of candles.

Bảng 3.25. Protection efficacy against An. dirus mosquito of repellent candles contain 0.04% transfluthrin outside farm houses

Time (hour)	Tested (burn candles)		Control (no burn candles)		Protection efficacy (%)
	Number (individuals)	Density (I/H/P)	Number (individuals)	Density (I/H/P)	
18-19	1	0,0625	9	0,5625	88,89
19-20	2	0,125	24	1,5	91,67
20-21	6	0,375	21	1,3125	71,43
21-22	4	0,25	22	1,375	81,82
22-23	2	0,125	14	0,875	85,71
23-24	0	0	6	0,375	100
24-01	0	0	3	0,1875	100
01-02	0	0	0	0	-
02-03	0	0	1	0,0625	100
03-04	0	0	1	0,0625	100
04-05	0	0	0	0	-
05-06	0	0	0	0	-
Total	15	0,938	101	6,313	85,15
p	p<0,05				

Note:: I/H/P: individuals /hour/person

Mosquito repellent candles contain 0.04% transfluthrin was effective against mosquito bites throughout the night with a protective effect from 71.43% to 100%. The average efficacy of preventing mosquito bites outside the farm house all night (12 hours) is 85.15%.

3.2.3. Side effects and community acceptance of mosquito repellent candles contain 0.04% transfluthrin

3.2.3.1. Side effects of candles on laboratory for test participants

Table 3.26. Side effects of candles for test participants

N ^o	Interview content	Number of people interviewed	Answer		Percentage answered yes
			Yes	No	
1	Does the test sample have an unpleasant odor?	6	0	6	0
2	Did the following occur during the test?				
2.1	Sneezing	6	0	6	0
2.2	Itchy	6	0	6	0
2.3	Cough	6	0	6	0
2.4	Snivel	6	0	6	0
2.5	Dizzy	6	1	6	17
2.6	Nausea	6	0	6	0
2.7	Eye irritation	6	0	6	0
2.8	Stuffy nose	6	0	6	0
2.9	Headache	6	0	6	0
2.10	Other expression	6	0	6	0

All the test participants did not feel an unpleasant odor when testing the candles and most of the testers did not experience unwanted symptoms.

3.2.3.2. Side effects of candles and community acceptance in the field

Bảng 3.27. Side effects of candles for test participants and users

N ^o	Interview content	Number of people interviewed	Answer		Percentage (%) answered yes
			Yes	No	
1	Does the test sample have an unpleasant odor?	30	0	30	0
2	Did the following occur during the test?				
2.1	Sneezing	30	0	30	0
2.2	Itchy	30	0	30	0
2.3	Cough	30	0	30	0

N ⁰	Interview content	Number of people interviewed	Answer		Percentage (%) answered yes
			Yes	No	
2.4	Snivel	30	0	30	0
2.5	Dizzy	30	1	29	3,3
2.6	Nause	30	0	30	0
2.7	Eye irritation	30	0	30	0
2.8	Stuffy nose	30	0	30	0
2.9	Headache	30	0	30	0
2.10	Other expression	30	0	30	0
3	Does lighting candles affect ormal activities?	22	0	22	0

Almost the test participants and users have no unwanted effects. There are only 01/30 cases of feeling dizzy (3.3%). Using candles does not affect other activities of people in the farm house.

Chapter 4. DISCUSSIONS

4.1. Malaria epidemiology and residual malaria transmission factors related to malaria-transmitting mosquitoes in Son Thai commune, Khanh Vinh district, Khanh Hoa province

4.1.1. Malaria epidemiological situation in Son Thai commune

With data collected on malaria cases from Son Thai commune health station, Khanh Vinh district health center and longitudinal follow-up investigation by the research team, it shows that the malaria situation of Son Thai commune is maintained all year round. The composition of malaria parasite species is mainly *P. falciparum*, *P. vivax* accounts for a low proportion. The proportion of *P. falciparum* accounting for a high proportion in the malaria parasite structure is consistent with the 2014 WHO report on the composition of malaria parasite species in 9 countries in Southeast Asia [131]. In July, malaria parasites increased up to 31 parasites, much higher than in other months, but this includes 12 parasites that were actively detected by cross-sectional survey. This shows that in addition to the cases of passive detection (often with fever symptoms) visiting health facilities, there are still cases of parasites that exist in the community.

It is worth noting that the proportion of patients infected with parasites without symptoms is high, accounting for 58.33% (table 3.4.). Asymptomatic

parasite carriers, if not detected and treated promptly, will be an important source of pathogens and also one of the reasons why malaria persists.

4.1.2. Residual malaria transmission factors associated with malaria-transmitting mosquitoes in Son Thai commune, Khanh Vinh district, Khanh Hoa province

4.1.2.1. Anopheles species composition in 3 landscapes in Son Thai commune

In 3 landscapes of Son Thai commune, Khanh Vinh district, Khanh Hoa province, 13 species of mosquitoes were collected in village. The number of species collected is relatively abundant. Species *An. dirus* was only collected in the farms and in the forest, but not in village. This shows that the annual malaria control intervention in residential areas according to the provisions of the national program is carried out regularly and continuously for many years, including measures of indoor insecticide residual spraying, impregnating mosquito nets and long-lasting insecticide nets with high coverage with close supervision from central to district levels have achieved good results.

4.1.2.2. Density of Anopheles in 3 landscapes in Son Thai commune

The main malaria vector *An. dirus* collected in 3 surveys with both indoor and outdoor biting with the highest density in July, October and December, respectively 8.8 (individual/person/night); 3.0 (head/person/night) and 10.00 (head/person/night). This result has confirmed many previous research results that the population of *An. dirus* grows very strongly in the habitats in the forest and is very fond of human blood. The high-density of the main vector *An. dirus* and the secondary vector *An. maculatus* in the forests and farms shows that there is a very high risk of farm goers and forest goers being bitten by mosquitoes and infected with malaria parasites.

4.1.2.3. The biting activity of An. dirus

Biting activities of *An. dirus* in the farm showed that there was a change in the rate of mosquito bites inside and outside farm house of *An. dirus*, at the beginning of the rainy season, the rate of biting people inside and outside the house was similar (50.5% and 49.5%), but in the middle of the rainy season, the tendency to burn people inside the house was higher (88.2% and 11.8%), at the end of the rainy season, the rate of human burning reversed (30% and 70%).

Biting activities of *An. dirus* throughout the night from 18-6 hours, in which the strongest burning time both indoors and outdoors is from 20-23 hours. During

the December survey in winter, the cold nights and earlier evenings were extended to an earlier survey starting at 16 pm. that found that the biting activity of *An. dirus* in the farms and forest earlier lasted from 17 pm to 5 am. This is a new discovery about the biting activity behavior of *An. dirus* because previous studies in Vietnam have never published *An. dirus* works before 18 pm..

4.1.2.5. Role in malaria transmission of *Anopheles*

All 3 samples positive for malaria parasites identified as *An.dirus* collected during the survey in July, of which 1 sample positive for *P. falciparum* (+) was collected outside the farm house during the period from 3 -4 am., 1 sample positive for *P.vivax* (+) was collected outside the farm house between 23 -24 pm. and 1 sample *P.falciparum* (+) was collected in the forest during the period 19-20 pm..

The percentage of mosquitoes *An. dirus* infection with parasites is 1.11%.

AEIR index for *P. faciparum* and *P. vivax* of *An. dirus* outside the farm house is 21.47; AEIR index for *P. faciparum* of *An. dirus* in the forest is 26.07.

4.1.2.6. The factors related to ability exposure between people to mosquitoes in Son Thai commune

All of the farm houses observed in the farm in Son Thai commune are made of wood/bamboo, the house has a simple structure assembled from bamboo, the walls have gaps or lack walls (the house has 3 walls).

With such a farmhouse structure, the protective effect against mosquitoes flying into the house is very little. The open structure allows mosquitoes to enter freely from outside and inside. The density of mosquitoes collected inside and outside the swidden house through the surveys in this study was almost equal.

The time of using nets by people in the farm started at 19:00 with the rate of 55%, and only reached 64% when the observation time ended at midnight (Figure 3.8). The above results can be commented that the use of mosquito nets in the farms is not high.

4.1.2.7. The sleeping status of the participants in the farm and forest was monitored by GPS locator

The results through GPS tracking data show that the rate of nights sleeping in the farm is 21.4%, the rate of nights sleeping in the forest is 7% (table 3.15), the total rate of nights sleeping in the forest or sleeping in the

fields is 28.3%, accounting for 28.3%. nearly one-third of the total night sleep of GPS study participants.

The status of people going to the forest, sleeping in the farms with complicated travel routes through the locations where malaria is present is a challenge for malaria control for this population when they have not been adequately protected by vector control measures.

4.2. Efficacy of mosquito repellent candles in the laboratory and efficacy of mosquito repellent candles in malaria vector control for farm goers and forest goers

4.2.1. Efficacy of mosquito repellent candles in the laboratory

The obtained results showed that the mosquito mortality rate after 24 hours after testing with 0.04% transfluthrin candles with 2 mosquito species was 99-100%. The candle had a good mosquito killing effect on both tested mosquito species. Candles are further tested in the field.

4.2.2. Efficacy of mosquito repellent candles in malaria vector control for farm goers and forest goers in Son Thai commune, Khanh Vinh district, Khanh Hoa province

The efficacy of personal protection against mosquito bites inside and outside the house throughout the night ranged from 71.43% to 100%. The efficacy protection of candles tested indoor for an average of 12 h with *An. dirus* was 88.59% and the efficacy protection against outdoor mosquito bites was 85.15%. The results of our study show that mosquito repellent candles contain transfluthrin have a good protective efficacy against mosquitoes, which can be an additional tool to help people prevent mosquito bites in farm houses, especially during periods of time people are still active and have not yet gone to sleep and have not been protected by other vector control measures such as sleeping under nets.

4.2.3. Side effects and community acceptance of mosquito repellent candles

During the trial, test participant were interviewed for some side effects. The results showed that most of test participant and users did not unwanted symptoms. Thus, mosquito repellent candles are safe

Experimental candles were distributed to households living in stilt houses in the study area. Family members and test participants were interviewed about side effects during the use and testing of candles containing transfluthrin. The results show that the tested candles have almost no unwanted effects. There are only 01/30 cases of feeling dizzy (3.3%). Using candles does

not affect other activities of people in the swidden house. Mosquito repellent candles are safe for people in the study area.

CONCLUSIONS

1. Residual malaria transmission factors associated with malaria-transmitting mosquitoes in Son Thai commune, Khanh Vinh district, Khanh Hoa province

- Son Thai commune is a residual malaria endemic commune, monthly cases of malaria parasites are recorded. The rate of malaria parasite infection in 2016 was 1.71%, mainly *P. falciparum* accounted for 91.67% and *P. vivax* accounted for 8.33%, the proportion of patients infected with parasites without symptoms was 58.33% through cross-sectional study.

- In 3 habitats of Son Thai commune, Khanh Vinh district, Khanh Hoa province, 13 species of Anopheles mosquitoes were collected. Among them, the malaria vector *An. dirus* only collects in the fields and forests, not in villages ; Did not detect the presence of *An. minimus* at the study site.

- Mosquito *An. dirus* is active both indoors and outdoors and has seasonal changes: the rate of blood biting indoors/outside the house is 49.5/50.5 (early rainy season); 11.8/88.2 (mid-rainy season) and 30/70 (end-rainy season).

- The operating time of *An. dirus* throughout the night that begins at dusk from 5 p.m., peaks between 8 p.m. and 23 p.m., and declines toward the second half of the night. The biting rate of *An. dirus* was 48%, and *An. maculatus* was 100% before 21 p.m when most of forest and farm goers are still active

- Detect mosquito *An. dirus* infected with *P. falciparum* and *P. vivax* parasites, the infection rate of *P. falciparum* was 0.74%; *P. vivax* infection rate is 0.37%; Malaria transmission index of *An. dirus* outside the farm house is 21.47, in the forest is 26.07.

- Night activities of people in the farms are mainly from 18:00 to 20:00 when not protected by vector control measures. The time to use the nets of people in the fields started at 19:00 with the rate of 55%, and only reached 64% when the observation time ended at midnight.

- The activities of groups of people who go to the forest or the farms have complicated routes to many points in the forest and in the farms. The rate of night sleep in the farms of this group is 21.4% and in the forest is 7.0%.

- The vector control measures applied in villages is guaranteed but not in the farm and forest area:

- The average net coverage rate in villages is 1.72 people /net, the net sleeping rate of villagers is 95.6%. The structure of the house is mainly made of closed-wall bricks, suitable for insecticide residual spraying.

- The net coverage rate in the farms is low: 4.71 people/net on average. The farm houses in Son Thai commune are made of wood and bamboo, with a simple structure, the walls have gaps or lack walls that are easy for *Anopheles* mosquitoes to enter.

2. The efficacy and side effects of mosquito repellent candles contain transfluthrin in the laboratory and in the field

- Mosquito repellent candles containing 0.04% transfluthrin had good mosquito knockdown effect in the laboratory with both tested mosquito species *An. dirus*, *An. minimus*, with knockdown rate of 100%; The KT50 is 4.62; 4.52 (minutes), KT95 is 8.75; 9.35; (minutes). The mosquito mortality rate after 24 hours is 99-100%.

- Mosquito repellent candles reduce the number of *An. dirus* burned people of candles inside and outside the farmhouse with *An. dirus* with the effectiveness against 88.59% and 85.15%, respectively.

- Mosquito repellent candles containing 0.04% transfluthrin did not cause any adverse effects and was accepted by 100% of the interviewees.

RECOMMENDATIONS

1. Ensure malaria vector control measures for farm goers and forest goers by providing enough nets for them. Besides, additional personal protection for farm goers and forest goers to prevent mosquito bites. In which mosquito repellent candles are a highly effective measure of protection.

2. Annually, there should be a cross-sectional survey in areas with residual malaria transmission to actively detect asymptomatic malaria-infected people to reduce pathogens, especially farm goers and forest goers.