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MINISTRY OF HEALTH
**NATIONAL INSTITUTE OF MALARIOLOGY-
PARASITOLOGY AND ENTOMOLOGY**

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**THE COMPOSITION, DISTRIBUTION, BEHAVIOUR,
MALARIA TRANSMISSION ROLE OF *Anopheles* AND
EFFECTIVENESS OF THE NIMPE MOSQUITO
REPELLENT CREAM, THE NIMPE MOSQUITO COIL
IN DONG XUAN DISTRICT, PHU YEN PROVINCE,
2017 - 2019**

Major: Entomology
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THESIS SUMMARY

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The thesis can be found at:

- The national library;
- The library of the National Institute of Malariology,
Parasitology, and Entomology.

INTRODUCTION

In spite of malaria cases reduced year by year in Viet Nam. But, malaria persisted in some areas of the Central - Hight Land and South East of Viet Nam. Couses, residents were sleeping in the forest and the farm.

It were hyperendemic malaria areas in Xuan Quang 1 and Phu Mo communes, Dong Xuan district, Phu yen province. Residents have the habit of sleeping in the forest and the farm. The density of *An. dirus* was usually hight in the forest and the farm. Besides, vector control measures were mainly indoor spray residue and impregnation bed net, additional measures such as repellent cream and coil to control malaria vector, that should be studied to contribute to promoting the elimination of malaria. For the reason, we conducted the study: “Research on composition, distribution, behavior, role of malaria transmission of Anopheles and effectiveness of NIMPE repellent cream, NIMPE mosquito coil in Dong Xuan district, Phu Yen province in 2017 - 2019” with the following objectives:

1. Determined composition, distribution, behavior and the role of malaria transmission of Anopheles in Xuan Quang 1 and Phu Mo communes, Dong Xuan district, Phu Yen province, 2017.
2. Evaluated the personal protection effectiveness and acceptance of community with NIMPE mosquito repellent cream at the study site, 2018.
3. Evaluated the personal protection effectiveness and community acceptance with NIMPE mosquito coil at the study site, 2019.

NOVELTY, SCIENTIFIC AND PRACTICAL SIGNIFICANCE OF THE THESIS

1. The study determined the distribution of Anopheles and the behavior of malaria vectors in 3 landscape as the forest, the farm, the village in malaria-endemic areas in Phu Yen province.
2. For the first time, assessing the effectiveness of personal protection and community acceptance with NIMPE mosquito repellent cream and NIMPE mosquito coil that were products of the National Institute of Malariology, Parasitology and

Entomology to control malaria vectors for the residents to the sleeping in forest and the farm.

THESIS STRUCTURE

The thesis consists of 127 pages divided into the following sections: Introduction (02 pages), literature review (33 pages), study subjects and methods (24 pages), study results (32 pages), discussions (33 pages), conclusions (02 pages), and recommendations (1 page). There are 31 tables, 23 figures, and 118 references.

CHAPTER 1.

LITERATURE REVIEW

1.1. Study on composition and distribution of malaria vectors

1.1.1. Study on composition and distribution of malaria vectors in the world

Sinka et al (2012) listed 465 species of Anopheles in the world, of which 41 species were the main malaria vectors. In the different regions had the other malaria vectors. Of the 41 main vectors, *An. dirus*, *An. minimus* and *An. maculatus* distributed in the forest and mountain areas in Phu Yen province, Vietnam.

An. dirus was a complex of 7 sibling species. It distributed in the Orient Countries. The density often were higher in the forests and the farm than the village. The density was high in the rainy season.

An. minimus was a complex of 3 sibling species. It distributed in the Orient Countries. The density often were higher in the farm than the village. The density was high in the dry season.

An. maculatus was a complex of at least 9 sibling species. It distributed in the Orient Countries. The density often were higher in the forests and the farm than village. The density was high in the rainy season.

1.1.2. Study on composition and distribution of malaria vectors in Vietnam

Vietnam (2011) was identified 63 Anopheles species. Of which, 3 main vectors were *An. dirus*, *An. minimus*, *An. epiroticus*. The secondary vectors were *An. aconitus*, *An. jeyporiensis*, *An.*

maculatus. The vectors distributed in the forest and the mountain areas in Phu Yen province, Vietnam.

An. dirus was a complex of 2 sibling species. It was only found in the mountainous areas from the 20 North latitude (South Thanh Hoa) to the Southern of Viet Nam. The density often was higher in the forests and the farm than the village. The density was high in the rainy season.

An. minimus was a complex of 2 sibling species. It distributed mainly in the mountainous, highland and midland. The density often was higher in the village than the farm. The density was high in the dry season.

An. maculatus was a complex of at least 7 sibling species. It distributed mainly in the mountainous, highland. The density often was higher in the forests and the fields than the village. The density was high in the rainy season.

1.2. Study on behaviors of *An. dirus*, *An. minimus*, *An. maculatus*

1.2.1. Behaviors of *An. dirus*

An. dirus preferred biting humans to animals

An. dirus biting density was higher outdoor than indoor. The biting activity resed from 18h, the peak at 20h - 22h.

An. dirus was a long life time relation to the forest, garden. Rest and blood degestion in outdoor.

An. dirus larvae were found in the puddles in the stream under the shape of the plant.

1.2.2. Behaviors of *An. minimus*

Previously, *An. minimus* preferred biting humans to animals. Now, depending on the locality, it bited different types of hosts.

Previously, *An. minimus* bited mainly indoor. After long periods of using DDT, mosquitoes tend to biting outdoor. The biting activity was over the night, the peak at 20h - 22h.

Previously, *An. minimus* was the rest and blood degestion in indoor. Now, depending on the locality, it was rest in outdoor or indoor.

An. minimus larvae were found in clear streams, ditches, slow-flowing fresh water with vegetation both side.

1.2.3. Behaviors of *An. maculatus*

An. maculatus preferred biting animals to humans.

An. maculatus biting density was higher outdoor than indoor. The biting activity resed 18h, the peak at 20h - 22h.

An. maculatus was the rest and blood degestion in outdoor.

An. maculatus larvae were found in the well, the puddles with the direct sunlight.

1.3. The role of malaria transmission of *An. dirus*, *An. minimus*, *An. maculatus*

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

An. dirus played an important role of malaria transmission in the forests and the farm.

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

An. minimus was the main vector for malaria transmission in all areas where it was present.

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

An. maculatus was the main vector in Malaysia, Thailand, Lao. But, it was the secondary vector in Vietnam.

1.4. Study on malaria vector control measures

1.4.2. Personal measures

- Repellent cream: DEET was used maily repellent.
- Mosquito coil: Pyrethroids was used repellent.

CHAPTER 2

SUBJECTS AND METHODS

2.1. The first objective: Determined the composition, distribution, behavior and the role of malaria transmission of Anopheles in Dong Xuan district, Phu Yen province, 2017

2.1.1. Subjects, sites, duration of study

2.1.1.1. Study subjects

- Anopheles mosquitoes and larvae at the study site.
- Host blood in midgut of the malaria vectors.
- Plasmodium in vectors.

2.1.1.2. Study sites

- Xuan Quang 1 and Phu Mo communes, Dong Xuan district, Phu Yen province.

- Departement of Entomology and Molecular Biology, Institute of Malariology, Parasitology and Entomology

2.1.1.3. Study duration

- From May to December 2017. Investigated once in June and once in September 2017.

2.1.2. Methods

2.1.2.1. Study design

- Cross-sectional surveys in the field.
- Experimental research at the laboratory.

2.1.2.2. Sample size

- Sample size to determine composition, density of Anopheles mosquitoes and larvae:

- + In the forest: Choose 3 sites for human biting collection.
- + In the farm: Choose 3 houses for human biting collection, 5 houses to set light traps in indoor.
- + In the village: Choose 3 houses for human biting collection, 5 houses to set the light traps in indoor, 30 houses for the resting indoor collection, 4 households had cattle sheds to Anopheles collection at night.

- Sample size to determine host blood: All blood - fed malaria vectors collected by the light traps and the resting indoor.

- Sample size to determine the disease transmission role of vectors of malaria: The vector of malaria collected by human baiting, the light traps and the resting indoor.

2.1.3. Study contents

- Determined composition, distribution and behaviour of Anopheles in the village, the forests and the farm.
- Determined host blood of malaria vectors.
- Determined the role of malaria transmission of vectors.

2.1.5. Research indices

- The density of Anopheles collected by the human baiting and cattle collection.
- Percentage of mosquitoes and larvae (%).
- Percentage of host blood (%).
- Plasmodium rate (%).
- Annual Entomological Inoculation Rate (AEIR).

2.2. The second objectives: Evaluated the effectiveness of personal protection and acceptance of community with NIMPE mosquito repellent cream at the study site, 2018

2.2.1. Subjects, sites, duration of study

2.2.1.1. Study subjects

- NIMPE mosquito repellent cream was manufactured by the National Institute of Malariology, Parasitology and Entomology.
- Residents sleeping in the forest and the farm in Xuan Quang 1 commune, Dong Xuan district, Phu Yen province.
- The malaria vectors in the farm of Phu Mo commune.

2.2.1.2. Study sites

- Xuan Quang 1 and Phu Mo communes, Dong Xuan district, Phu Yen province.

2.2.1.3. Study duration

- From September to December 2018. Investigated once in October and once in December 2018.

2.2.2. Methods

2.2.2.1. Study design

- Experimental research with control in the field.

2.2.2.2. Sample size

- Sample size to evaluate the effectiveness of NIMPE mosquito repellent cream: Choose 4 huts in the farm, 2 volunteers collected mosquitoes by human biting outdoor at each house.
- Sample size to evaluate the side effects and community acceptance: Choose 390 people sleeping in the forest or the farm in Xuan Quang 1 commune, they used NIMPE mosquito repellent cream.

2.2.3. Study contents

- Assessed the personal protective effectiveness of NIMPE mosquito repellent cream: According to WHO (2009).
- Assessed the side effects and acceptance of the community with NIMPE mosquito repellent cream: According to Circular N^o 22/2015/TT-BYT.

2.2.5. Research indices

- Density of Anopheles species.
- Percentage of the NIMPE mosquito repellent cream against malaria vectors by WHO (2009).

- Percentage of households and people used NIMPE mosquito repellent cream (%).
- Percentage of tubes of NIMPE mosquito repellent cream were used by residents (%).
- Percentage of side effects of NIMPE mosquito repellent cream (%).

2.3. The third objectives: Evaluated the effectiveness of personal protection and acceptance of community with NIMPE mosquito coil at the study site, 2019

2.2.1. Subjects, sites, duration of study

2.2.1.1. Study subjects

- NIMPE mosquito coil was manufactured by the National Institute of Malariology, Parasitology and Entomology.
- Residents sleeping in the forest and the farm in Xuan Quang 1 commune, Dong Xuan district, Phu Yen province.
- The malaria vectors in the farm of Phu Mo commune.

2.2.1.2. Study sites

- Xuan Quang 1 and Phu Mo communes, Dong Xuan district, Phu Yen province.

2.2.1.3. Study duration

- From September to December 2019. Investigated once in October and once in December 2019.

2.2.2. Methods

2.2.2.1. Study design

- Experimental research with control in the field.

2.2.2.2. Sample size

- Sample size to evaluate the effectiveness of NIMPE mosquito coil: Choose 3 huts in the farm, 1 volunteer collected mosquitoes by human biting indoor at each house.

- Sample size to evaluate the side effects and community acceptance: Choose 80 households in Xuan Quang 1 commune, of which had residents sleeping in the forest or the farm used NIMPE mosquito coil.

2.2.3. Study contents

- Assessed the personal protective effectiveness of NIMPE mosquito coil: According to WHO (2009).

- Assessed the side effects and acceptance of the community with NIMPE mosquito coil: According to Circular N^o 22/2015/TT-BYT.

2.2.5. Research indices

- Density of Anopheles species.
- Percentage of NIMPE mosquito coil against malaria vectors by WHO (2009).
- Percentage of households and people used NIMPE mosquito coil (%).
- Percentage of packet of NIMPE mosquito coil were used by residents (%).
- Percentage of side effects of NIMPE mosquito coil (%).

2.5. Data input and analysis

2.5.1. Data input

Data input by Microsoft Excel and EPI DATA 3.1 and all data converted into SPSS 16.0.

Processing results using Microsoft Excel and SPSS 16.0. The average value was compared by χ^2 (chi-square test) to determine the

difference of the two rates.

2.5.2. Data analysis

Calculated frequency, percentage (%) of variables.

Calculated the density of Anopheles mosquitoes and larvae

Compared the density of malaria vectors biting indoor to outdoor.

Compared the density of malaria vectors for biting in control huts to test huts

2.6. Ethical clearance

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

CHAPTER 3 RESULTS

3.1. Composition, distribution, behavior, role of malaria transmission of Anopheles in Xuan Quang 1 and Phu Mo communes, Dong Xuan district, Phu Yen province, 2017

3.1.1. Composition, distribution of Anopheles

Table 3.1. Composition, distribution of Anopheles by landscapes in Xuan Quang 1 and Phu Mo communes in 2017

N ^o	Species	Landscapes					
		Village		Farm		Forest	
		M	L	M	L	M	L
Subgenus Anopheles Meigen, 1818							
1	<i>An. barbirostris</i> Van Der Wulp, 1884	-	-	+	-	-	-
2	<i>An. crawfordi</i> Reid, 1953	-	-	-	-	+	-
3	<i>An. peditaeniatus</i> (Leicester, 1908)	+	-	+	-	-	-
4	<i>An. sinensis</i> Wiedemann, 1828	+	+	-	-	-	-
Subgenus Cellia Theobald, 1902							
5	<i>An. aconitus</i> Doenitz, 1902**	-	-	+	-	-	-
6	<i>An. dirus</i> Peyton & Harrison, 1979*	-	-	+	+	+	+
7	<i>An. jeyporiensis</i> James, 1902**	+	+	+	+	-	-
8	<i>An. kawari</i> (James, 1903)	+	-	-	-	-	-
9	<i>An. maculatus</i> Theobald, 1901**	+	+	+	+	+	+
10	<i>An. minimus</i> Theobald, 1901*	+	+	+	+	-	-
11	<i>An. philippinensis</i> Ludelow, 1902	+	+	+	-	-	-
12	<i>An. splendidus</i> Koidzumi, 1920	+	-	+	-	-	-
13	<i>An. vagus</i> Doenitz, 1902	+	+	+	+	-	-
	Total of species	9	6	10	5	3	2

The results collected 13 species of Anopheles belonging to two subgenus that were Anopheles Meigen, 1818 and Cellaia Theobald, 1902. Anopheles was 4 species, Cellaia was 9 species. 10 Anopheles species, of which 5 species of larvae was collected in the farm, 9 Anopheles species, of which 6 species of larvae was collected in the village and 3 Anopheles species, of which 2 species of larvae was collected in forest.

3.1.2. Percentage of Anopheles by landscapes

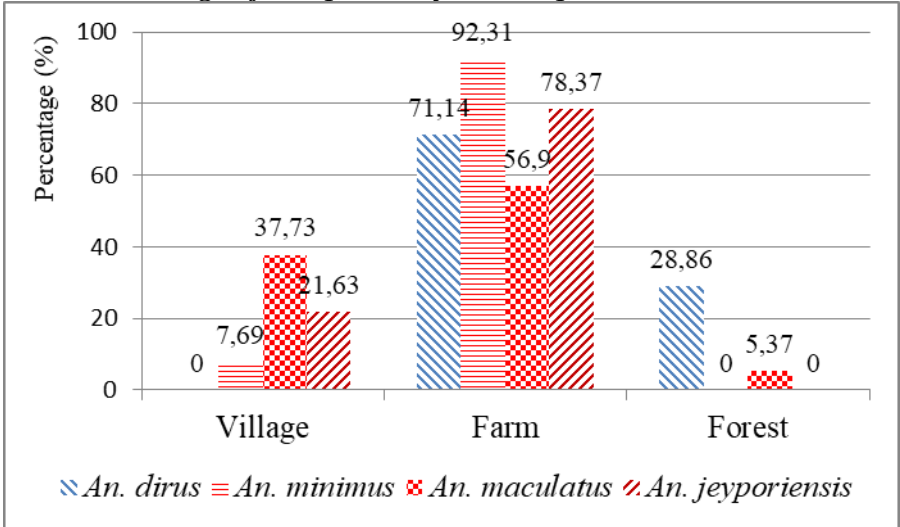


Fig 3.1. Percentage (%) of malaria vectors by landscapes in Xuan Quang 1 and Phu Mo communes in 2017

An. dirus collected from the farm and the forests, accounted for 71.14% and 28.86%, respectively. *An. minimus* collected in the village and the farm, accounted for 7.69% and 92.31%, respectively. *An. maculatus* collected from the village, the farm and the forests, accounted for 37.73%; 56.90% and 5.37%, respectively. *An. jeyporiensis* collected in the village and the farm, accounted for 21.63% and 78.37%, respectively.

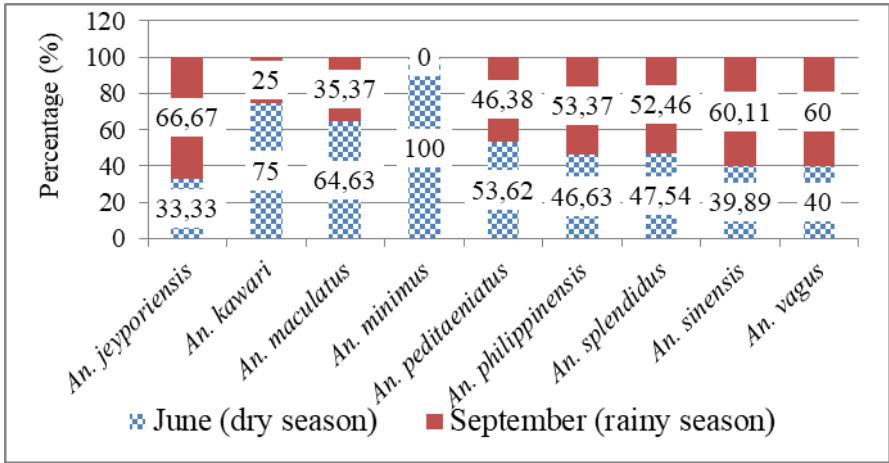


Fig 3.2. Percentage (%) of Anopheles in the village of Xuan Quang 1 and Phu Mo by season in 2017

The results showed that 9 Anopheles species were collected in the June (dry season), 8 Anopheles species were collected in September (rainy season) in village. *An. minimus* was only collected in June. Percentage of *An. maculatus* was 64.63% in June higher than 35.37% in September. Percentage of *An. jeyporiensis* was 66.67% in June higher than 33.33% in September.

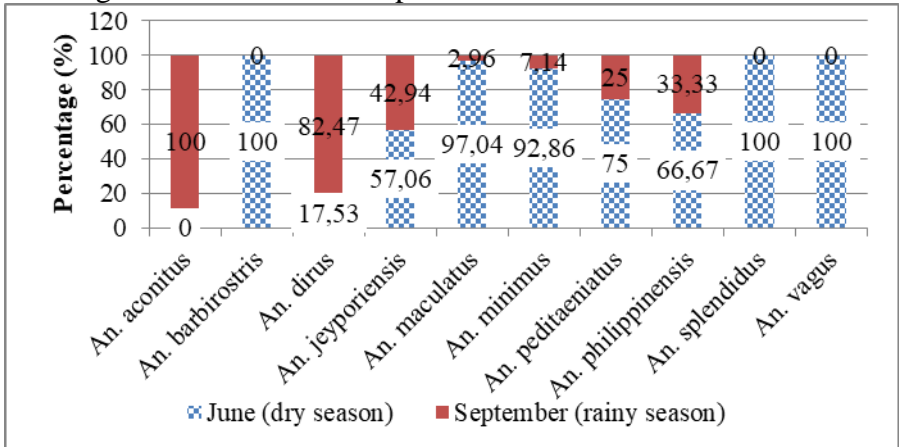


Fig 3.3. Percentage (%) of Anopheles in the farm of Xuan Quang 1 and Phu Mo by season in 2017

The results showed that 9 Anopheles species were collected in the June (dry season), 7 species Anopheles were collected in September (rainy season) in the farm. *An. aconitus* was only collected in September. Percentage of *An. dirus* was 82.47% in September higher than 17.53% in June. Percentage of *An. minimus* was 92.86% in June higher than 7.14% in September. Percentage of *An. maculatus* was 97.04% in June higher than 2.96% in September. Percentage of *An. jeyporiensis* was 57.06% in June higher than 42.94% in September.

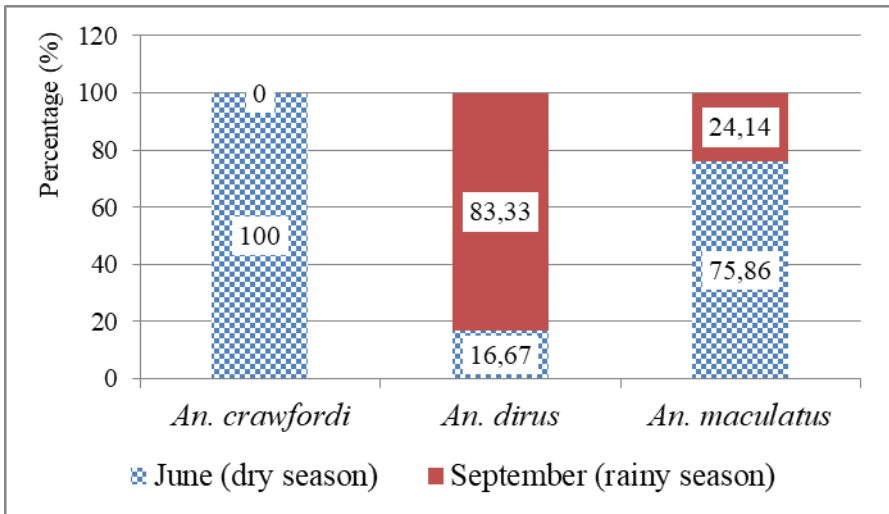


Fig 3.4. Percentage (%) of Anopheles in the forest of Xuan Quang 1 and Phu Mo by season in 2017

The results showed that 3 Anopheles species were collected in the June (dry season), 2 species Anopheles were collected in September (rainy season) in the forest. Percentage of *An. dirus* was 83.33% in September higher than 16.67% in June. Percentage of *An. maculatus* was 75.86% in June higher than 24.14% in September.

3.1.3. Behaviors of Anopheles

3.1.3.1. Preferring to host feeding of Anopheles

Table 3.8. Result of determining host blood of malaria vectors in Xuan Quang 1 and Phu Mo communes in 2017 (n = 90)

Species	Z ₀	Number and percentage (%) of host blood									
		Human		Animal		Poultry		Dog		Other	
		N ^o	Per (%)	N ^o	Per (%)	N ^o	Per (%)	N ^o	Per (%)	N ^o	Per (%)
<i>An. dirus</i>	5	5	100	0	0	0	0	0	0	0	0
<i>An. jeyporiensis</i>	16	4	25.00	10	62.50	0	0	0	0	2	12.50
<i>An. maculatus</i>	44	10	22.73	31	70.45	0	0	0	0	3	6.82
<i>An. minimus</i>	25	1	4.00	24	96.00	0	0	0	0	0	0

The percentage of human blood of *An. dirus* accounted for 100%. *An. minimus* accounted for 4%. *An. jeyporiensis* accounted for 25.00%. *An. maculatus* accounted for 22.73%.

3.1.3.2. Biting activity of Anopheles

Table 3.9. The density of malaria vectors at the indoor and outdoor in the farm in Xuan Quang 1 commune and Phu Mo commune in 2017

Site	Number and density of malaria vectors							
	<i>An. dirus</i>		<i>An. jeyporiensis</i>		<i>An. maculatus</i>		<i>An. minimus</i>	
	N ^o	Density	N ^o	Density	N ^o	Density	N ^o	Density
Indoor	25	0.17	38	0.26	33	0.23	3	0.02
Outdoor	50	0.34	61	0.42	43	0.30	3	0.02
χ^2	8.33		5.34		1.32		0	
p	< 0.01		< 0.05		> 0.05		> 0.05	

Density of *An. dirus* and *An. jeyporiensis* collection by human biting was higher at the outdoor than at the indoor, 0.34 and 0.17 sample/hour/person; 0.42 and 0.26 sample/hour/person respectively, the difference was significant (p < 0.05).

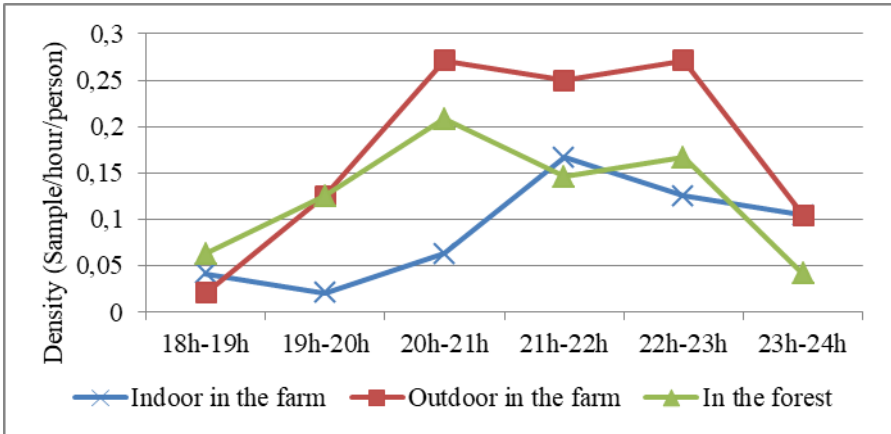


Fig 3.9. Density of *An. dirus* by human biting by the hour in the farm and the forest of Xuan Quang 1 and Phu Mo communes in 2017

The density of *An. dirus* was the highest at the outdoor in the farm, followed by the forests and the indoor in the farm. Peak of biting activity of the first half of the night of *An. dirus* was 21h - 22h at the indoor, and 20h - 22h at the outdoor and the forest.

3.1.3.3. Breeding habitats of Anopheles

Table 3.13. The percentage (%) of Anopheles larvae collected at Xuan Quang 1 and Phu Mo communes in 2017

Larvae	Habitats									
	Rive		Stream		Puddle in stream		Puddle next road		Drain	
	N ⁰	Per (%)	N ⁰	Per (%)	N ⁰	Per (%)	N ⁰	Per (%)	N ⁰	Per (%)
<i>An. dirus</i>	0	0	0	0	16	11.94	0	0	0	0
<i>An. jeyporiensis</i>	0	0	13	15.48	47	35.08	0	0	0	0
<i>An. maculatus</i>	0	0	56	66.66	71	52.98	0	0	2	8.33
<i>An. minimus</i>	0	0	15	17.86	0	0	0	0	0	0
<i>An. philippinensis</i>	0	0	0	0	0	0	0	0	10	41.67
<i>An. sinensis</i>	0	0	0	0	0	0	0	0	6	25.00
<i>An. vagus</i>	0	0	0	0	0	0	17	100	6	25.00
Total	0	0	84	100	134	100	17	100	24	100

The result collected 7 species of Anopheles larvae. In the stream collection 3 species of larvae such as *An. minimus*, *An. jeyporiensis* and *An. maculatus* accounts for 17.86%; 15.48% and

66.66%, respectively. By the puddle in stream collection 3 species of larvae such as *An. dirus*, *An. jeyporiensis* and *An. maculatus* accounts for 11.94%; 35.08% and 52.98%, respectively.

3.1.4. The role of malaria transmission of vectors

An. dirus infected with 0.52% *P. falciparum*. Other mosquitoes did not detect Plasmodium.

Density of *An. dirus* biting human in the forest, the farm was 1.38 sample/human/night. Annual Entomology Incubate Rate (AEIR) of *An. dirus* was 2.62.

3.2. Personal protection effectiveness and community acceptance with NIMPE mosquito repellent in 2018

3.2.1. Personal protection effectiveness of NIMPE mosquito repellent cream

Table 3.19. Density of *An. dirus*, *An. jeyporiensis*, *An. maculatus* biting human at control and test house

Species	Control		Test		p
	N ^o (sample)	Density (s/h/p)	N ^o (sample)	Density (s/h/h)	
<i>An. dirus</i>	56	0.58	7	0.07	< 0.01
<i>An. jeyporiensis</i>	1	0.01	0	0	-
<i>An. maculatus</i>	7	0.07	0	0	< 0.05
Total	64		7		

The biting density of *An. dirus* was 0.58 sample/hour/person at the control sites higher than was 0.07 sample/hour/person at the test sites, the difference was significant ($p < 0.01$).

Table 3.20. The percentage (%) of effectiveness of NIMPE mosquito repellent cream against *An. dirus*, *An. jeyporiensis* and *An. maculatus*

Time (hour)	Control		Test		Effect (%)
	N ^o (sample)	Density (s/h/p)	N ^o (sample)	Density (s/h/p)	
18 - 19	9	0.56	1	0.06	88.89
19 - 20	14	0.88	1	0.06	92.86
20 - 21	21	1.31	2	0.13	90.48
21 - 22	12	0.75	2	0.13	83.33
22 - 23	5	0.31	1	0.06	80.00
23 - 24	3	0.19	0	0	100
Total	64	0.66	7	0.09	89.06

The personal protective effectiveness of NIMPE mosquito repellent creams by reducing the density of *An. dirus*, *An. jeyporiensis*, *An. maculatus* for 6 hours was 89.06%, mosquito repellent cream was good to control malaria vectors.

3.2.2. Community acceptance with NIMPE mosquito repellent cream

Table 3.21. Number, percentage (%) of households and NIMPE mosquito repellent creams using in Xuan Quang 1 in 2018

Village	Number of household distributed NIMPE MRC	Number of household using NIMPE MRC	Number of person using NIMPE MRC	Number of NIMPE MRC distributed	Number of NIMPE MRC using	Percentage (%) of NIMPE MRC used/distributed
Ky Lo	100	100	192	300	179	59.67
Suoi Coi 1	50	50	101	150	85	56.67
Suoi Coi 2	50	50	97	150	83	55.33
Total	200	200	390	600	347	57.83

100% of households with 390 people used NIMPE mosquito repellent cream. The number of NIMPE mosquito repellent creams used 347 tubes, accounting for 57.83%.

A total of 390 people and 8 volunteers used NIMPE mosquito repellent cream. NIMPE mosquito repellent creams did not occur the side effects, but 25 (6,28%) participants felt bad smell to the mosquito repellent cream.

3.3. Personal protection effectiveness and community acceptance with NIMPE mosquito coil in 2018

3.2.1. Personal protection effectiveness of NIMPE mosquito coil

Table 3.24. Density of *An. dirus*, *An. jeyporiensis*, *An. maculatus* biting human at control and test house

Species	Control (1)		Positive control (2)		Test (3)		p		
	N ^o (s)	Density (s/h/p)	N ^o (s)	Density (s/h/p)	N ^o (s)	Density (s/h/p)	(1)	(1)	(2)
							to (2)	to (3)	to (3)
<i>An. dirus</i>	30	0.83	3	0.08	3	0.08	< 0.01	< 0.01	> 0.05
<i>An. jeyporiensis</i>	11	0.31	0	0.00	1	0.03	< 0.01	< 0.01	> 0.05
<i>An. maculatus</i>	4	0.11	0	0	0	0	-	-	-
Total	45		3		4				

The density of *An. dirus* and *An. jeyporiensis* in the control house was 0.83 and 0.31 sample/hour/person higher than the positive control house was 0,08 and 0 sample/hour/person and the testing house were 0.08 and 0.03 sample/hour/person, respectively, the difference was significant ($p < 0.01$).

Table 3.25. The percentage (%) of effectiveness of NIMPE mosquito coil against *An. dirus*, *An. jeyporiensis* and *An. maculatus*

Time (hour)	Control		Test		Effect (%)
	N ^o (sample)	Density (s/h/p)	N ^o (sample)	Density (s/h/p)	
20 - 21	13	1.44	1	0.01	92.31
21 - 22	16	1.78	1	0.01	93.75
22 - 23	12	1.33	1	0.01	91.67
23 - 24	4	0.4	1	0.01	75.00
Total	45	1.25	4	0.01	91.11

The personal protective effectiveness of NIMPE mosquito coil by reducing the density of *An. dirus*, *An. jeyporiensis*, *An. maculatus* for 4 hours was 91.11%; mosquito coil was good to control malaria vectors.

3.2.2. Community acceptance with NIMPE mosquito coil

Table 3.26. Number, percentage (%) of households and NIMPE mosquito coils using in Xuan Quang 1 in 2019

Number of household distributed NIMPE mosquito coil	Number of household using NIMPE mosquito coil in the farm		Number of NIMPE mosquito coil distributed	Number of NIMPE mosquito coil used	Percentage (%) of NIMPE mosquito coil used/ distributed
	N ^o	Percentage %			
80	80	100	800	249	31.11

The percentage of households using NIMPE mosquito coil in the farm was 100%. The number of NIMPE mosquito coil used 249 packets, accounting for 31.11%.

In 80 households with 392 people and 3 volunteers used NIMPE mosquito coils. NIMPE mosquito coils did not occur the side effects.

CHAPTER 4 DISCUSSIONS

4.1. Composition, distribution, behavior, role of malaria transmission of *Anopheles* in Xuan Quang 1 and Phu Mo communes, Dong Xuan district, Phu Yen province in 2017

4.1.1. Composition, distribution of *Anopheles*

Results collected 13 *Anopheles* mosquitoes, of which 7 species of larvae in the village, the farm and the forests. Some *Anopheles* mosquitoes collected to low density, *Anopheles* larvae were not collected sample, because we could not found all breeding habitats of *Anopheles* by the short time.

4.1.2. Percentage of *Anopheles* by landscape

An. maculatus, *An. philippinensis*, *An. sinensis*, *An. vagus* were the dominant species in the village. Among these species, *An. maculatus* was the secondary vector, therefore National Control and Elimination Strategy concerned in this species. Because of recent cases of malaria transmission in village, it did not collect the main vector, only collected *An. maculatus*. This suggests that the high density of secondary vectors contributed to the transmission of malaria.

Most of the main vectors *An. dirus*, *An. minimus* and the secondary vectors *An. jeyporiensis*, *An. maculatus* collected in the forest and the farm. The high density of two main vectors and two secondary vectors shows that the high risk of malaria transmission by sleeping people in the forest or the farm. Therefore, it was necessary to study control measures to sleeping people in the forest or in the farm.

Our study, the season of *An. dirus* and *An. minimus* was unchanged compared to other studies in Vietnam and the world. *An. dirus* developed in the rainy season, decreased at the end of the dry season. *An. minimus* developed at the end of the dry season, decreased in the rainy season. The season of *An. maculatus* was different from previous studies. *An. maculatus* developed at the end of the dry season, decreased in the rainy season.

4.1.3. Behaviors of *Anopheles*

4.1.3.1. Preferring of host feeding of *Anopheles*

Our study, *An. dirus* biting people accounted for 100%. *An. dirus* tested only 5 samples, less than the minimum sample size of 30 samples. However, the collected density of *An. dirus* by human baiting was higher than other methods. This result showed that *An. dirus* preferred to biting human than cattle. So, we should study control measures to *An. dirus*.

Our research, *An. minimus* biting animal accounted for 96%. It may be *An. harrisoni*, the sibling species of *An. minimus*, this mosquito preferred biting animal to human.

4.1.3.2. *Biting activity of Anopheles*

Density of *An. dirus* biting outdoor was high, so vector control measures such as spraying residues indoors, impregnating bed nets were ineffective. Especially, when people were still working in the forests and the farm. Therefore, it was necessary to study some personal measures such as mosquito repellent cream, mosquito coil against malaria vectors in forests and farm to supplement the measures by the National Control and Elimination Strategy in Viet Nam.

Biting activity of *An. dirus* in our study has not changed compared to previous studies in Vietnam and in the world, *An. dirus* biting was from 18h - 19h, the peak of the first half of the night was from 20h - 22h.

4.1.3.3. *Breeding habitats of Anopheles*

Our study collected *An. dirus* in puddles in the stream in the farm and the forest. Breeding habitats of *An. dirus* has not changed to the previous study.

An. minimus collected in clear water springs, water flows slowly with grass on the both side and the sunlight shines on. Breeding habitats of *An. minimus* did not change previous studies.

An. maculatus collected mainly in streams and puddles in streams. Breeding habitats of *An. maculatus* did not change to previous studies.

4.1.4. *The role of malaria transmission by Anopheles*

In our study, only *An. dirus* infected with *P. falciparum* at the rate of 0.52%, other *Anopheles* did not detect Plasmodium. The results also confirmed the role of malaria transmission of *An. dirus*.

Annual Entomological Inoculation Rate (AEIR) of *An. dirus* was 2.62. It means that a person could be infected 2.62 times with malaria, if they sleep continuously in the forest or the farm for a year. Therefore, people sleeping in the forest or the farm in Xuan Quang 1 and Phu Mo communes were at high risk of biting malaria vector and malaria infection.

4.2. Personal protection effectiveness and community acceptance with NIMPE mosquito repellent cream in 2018

4.2.1. Personal protection effectiveness of NIMPE mosquito repellent cream

The results on the personal protection effectiveness of NIMPE mosquito repellent cream after 6 hours was 89.06%, NIMPE mosquito repellent cream was good to control malaria vector effectiveness.

NIMPE mosquito repellent cream was good mosquito prevention effect, reducing the density of mosquito bites in the experimental house compared to the control house. NIMPE mosquito repellent can be an additional measure to help people prevent mosquitoes while sleeping in the forest or on the farm.

4.2.2. Community acceptance with NIMPE mosquito repellent cream

There were 390 people using NIMPE mosquito repellent cream, the number of NIMPE mosquito repellent tubes used 57.83%. The mosquito repellent cream was suitable for people in the study area, so all households had users and acceptance of this mosquito repellent cream.

390 users and 8 volunteers did not occur the side effects. This results shows that NIMPE mosquito repellent cream was safe for users.

4.3. Personal protection effectiveness and community acceptance with NIMPE mosquito coil in 2018

4.3.1. Personal protection effectiveness of NIMPE mosquito coil

The results on the personal protection effectiveness of NIMPE mosquito coil after 6 hours was 91.11%, NIMPE mosquito coil was good to control malaria vector effectiveness.

4.3.2. Community acceptance with NIMPE mosquito coil

100% household with 392 people used mosquito coil, the number of used mosquito coil accounted for 31.11%. The rate of using households of NIMPE mosquito coil was high in the study area because the mosquito coil was easily to use, because when burning a stick of mosquito coil can control mosquitoes for all people in the family.

392 people and 3 volunteers used mosquito coil, all of them did not occur the side effects. This result shows that NIMPE mosquito coil was safe for users.

CONCLUSIONS

1. Composition, distribution, behavior and the role of malaria transmission of *Anopheles* in Xuan Quang 1 and Phu Mo communes

- Collected 13 species of *Anopheles*, including 2 main vector *An. dirus*, *An. minimus*, 3 secondary vector *An. aconitus*, *An. jeyporiensis*, *An. maculatus*.

- *An. dirus*, *An. maculatus* were collected in the forest. *An. dirus*, *An. minimus*, *An. maculatus* were collected in the farm. *An. minimus*, *An. maculatus* were collected in the village.

- Percentage of *An. dirus* was higher in the rainy season than in the end of the dry seasons, accounted for 83.33% in the forest and 82.47% in the farm of the total of *An. dirus* collection at these areas.

- Percentage of *An. minimus* was higher the end of the dry seasons than in the rainy season, accounted for 92.86% in the farm, 100% in village of the total *An. minimus* collection at these areas.

- Percentage of *An. maculatus* was higher the end of the dry seasons than in the rainy season, accounted for 75.86% in the forest, 97.04% in the farm, 64.63% in the village of the total *An. maculatus* collection at these areas.

- Season of *An. dirus* and *An. minimus* unchanged to previous research. Season of *An. maculatus* changed to previous research. The density of *An. maculatus* was higher in the end of dry season than in the rainy season.

- Percentage of human blood in midgut of *An. dirus* was 100%, *An. minimus* was 4%, it was probably *An. harrisoni* preferring biting animal to humans; *An. maculatus* was 22.73%.

- The density of *An. dirus* was 0.34 sample/hour/person at the outdoor higher than 0.17 sample/hour/person at the indoor in the farm.

- The peak of biting activity of the first haft of the night to *An. dirus* was 21h - 22h at the indoor, and from 20h - 23h at the outdoor and the forest. *An. maculatus* was 21h - 22h at the indoor, and from 20h - 21h at the outdoor and the forest.

- *An. dirus* larvae were found in the puddles in the stream under the shape of the plant. *An. minimus* larvae were found in clear streams, ditches, slow-flowing fresh water with vegetation both side. *An. maculatus* larvae were found in the well, the puddles with the direct sunlight.

- The behavior of *An. dirus*, *An. minimus*, *An. maculatus* have not changed compared to previous studies.

- *An. dirus* infected with *P. falciparum* at the rate of 0.52%. Annual Entomological Inoculation Rate (AEIR) of *An. dirus* was 2.62. The role of *An. dirus* malaria transmission was unchanged to previous studies.

2. Personal protection effectiveness and community acceptance with NIMPE mosquito repellent cream

- Using NIMPE mosquito repellent cream to reduce the density of *An. dirus* from 0.58 sample/hour/person in the control house to 0.07 sample/hour/person at test house, the difference was significant ($p < 0.01$). The personal protection effectiveness of NIMPE mosquito repellent cream for 6 hours was 89.06%, the mosquito repellent cream against mosquitoes very well.

- 100% of people used NIMPE mosquito repellent cream.

- No side effects of NIMPE mosquito repellent cream, but 6.28% of people felt bad smell to the mosquito repellent cream.

3. Personal protection effectiveness and community acceptance with NIMPE mosquito coil

- Using NIMPE mosquito coil to reduce the density of *An. dirus* from 0.83 sample/hour/person in the control house to 0.08

sample/hour/person in the test house, the difference was significant ($p < 0.01$). Personal protection effectiveness of NIMPE mosquito coil in 4 hours was 90.11%, the mosquito coil against mosquitoes very well.

- 100% of households used NIMPE mosquito coil.
- No side effects of NIMPE mosquito coil.

RECOMMENDATIONS

1. Density of *An. dirus* were high in forests and outdoor of the hut, so it was necessary to strengthen the control of outdoor malaria vectors.
2. Malaria control and elimination program should distribute NIMPE mosquito repellent cream and NIMPE mosquito coil for personal protection against malaria vectors for residents who sleeping in the forest and the farm .

LIST OF PUBLICATIONS

1. Vu Viet Hung, Vu Duc Chinh et al (2018), “The composition and distribution of Anopheles species, the biting activity and host blood of malaria vectors in Dong Xuan district, Phu Yen province in 2017”, *Journal of Malaria and Parasite Diseases Control*, 5(107), pp. 57 - 64.
2. Vu Viet Hung, Nguyen Thi Anh, Hoang Thi Anh Tuyen, Thai Khac Nam, Nguyen Thi Huong Binh and Vu Duc Chinh (2019), “Evaluation of efficacy of the nimpe mosquito repellent cream (10% deet and cymbobogon citratus essential oil) against malaria vectors in Dong Xuan district, Phu Yen province in 2018” *Vietnam Journal of Preventive Medicine*, 29(4), pp. 74 - 78.