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

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STUDY ON SPECIES COMPOSITION, DISTRIBUTION, ROLE IN MALARIA TRANSMISSION OF *ANOPHELES* AND THE ZeroFly® APPLICATION AGAINST VECTORS IN EA SO NATURE RESERVE, DAK LAK (2020-2023)

SUMMARY OF THESIS

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INTRODUCTION

Ea So Nature Reserve is a protected forest with good growth vegetation and water body divesity create favorable conditions for the growth and development of *Anopheles* mosquitoes.

Local people have been moved to live in the buffer zone, which create distinct habitats including the residential area, the forest edge and the deep forest. Therefore, the distribution of vectors and malaria transmission at these habitats are also different. Malaria patients are mainly the local people and forest rangers who enter the forest to labor and exploit the forest products [3], [4]. Malaria prevention for high-risk people related to Ea So Nature Reserve has different characteristics, so malaria in this area is still complicated. The effective vector control measures should be selected appropriately for each habitat's characteristics and the habits and practices of local people and forest rangers.

To maintain and reinforce the achieved results and to move towards malaria elimination, more and more complementary and alternative interventions are being used, including ZeroFly®, the plastic sheet treated with deltamethrin 4.5g/kg, the residual efficacy is from 2-3 years [5], [6].

There are some studies on malaria and vectors at Ea So Nature Reserve area. However, these studies are just carried out in the forest edge and the residential habitat [7].

Simultaneously, whether the composition, distribution and transmission role of malaria vectors are different among habitats and which appropriate and effective measures to control vectors in the current context, especially for high-risk groups such as forest goers, farm sleepers, forest rangers.

Therefore, I carry out the project: *"**Study on species composition, distribution, role in malaria transmission of anopheles and the Zerofly® application against vectors in Ea So Nature Reserve, Dak Lak (2020-2023)***". The aims of the study are:

1. Determination of species composition, distribution, some ecological characteristics and role in malaria transmission of Anopheles in Ea So Nature Reserve, Dak Lak 2020-2021.

2. Evaluation of application effectiveness of ZeroFly® against malaria vectors in the laboratory and field.

NOVELTY, SCIENTIFIC AND PRACTICAL SIGNIFICANCE OF THE THESIS

This is the first time the role in malaria transmission of *Anopheles* vectors in the deep forest habitat in Ea So Nature Reserve was determined and updating species composition and distribution characteristics of *Anopheles* mosquitoes in general and malaria vectors in particular by habitat and season is the basis for concentration of malaria vector control in the deep forest and forest edge habitats in the study area where is high malaria endemic area and the localities with similar conditions.

The data on the insecticide susceptibility of the main malaria vector *An. dirus* to actively contribute to the insecticide selection to control vectors in the study area.

This is the first time the deltamethrin treated sheeting, ZeroFly® has been successfully trialed in the field at ranger stations in Ea So Nature Reserve with positive results, opening up the potential to supplement and replace the traditional residual spraying method in areas where the walls and conditions are not suitable.

THESIS STRUCTURE

The thesis consists of 134 pages divided into the following parts: Introduction 2 pages; literature review 37 pages; Study methods: 27 pages; Study results 34 pages; Discussions: 31 pages; Conclusion 2 pages; Recommendation 1 page. There are 14 figures, 33 tables of data and 117 references.

Chapter 1: LITERATURE REVIEW

1.1. Malaria situation in the world, Vietnam and study area.

According to the World Health Organization Report (WHO, 2022), globally, there were an estimated 247 million malaria cases in 2021, an increase 2 million from 2020 and the number of malaria deaths were estimated 619,000 in 2021 [8]. In Vietnam, in 2022, malaria cases were found mainly in 3 areas: the Central Highlands (48.79%), the Northern Mountains (23.52%), and the Central Region (16.48%).

Dak Lak was one of the six focal provinces in the last five years and one of the seven provinces with the highest number of malaria cases all over the country in 2022 [9]. In which, Ea Kar district had reported a

large number of malaria cases, which was concentrated in 2 communes Ea Sar and Ea So. In 2014, Ea Sar and Ea So were located in Zone 4, a moderate malaria-endemic areas. However, the two communes changed to highly endemic malaria regions, Zone 5 in 2019 [10].

1.2. Species composition, distribution, role in malaria transmission of *Anopheles* mosquitoes

1.2.1. Species composition and distribution of Anopheles mosquitoes in the world

Based on the data on 465 *Anopheles* species and more than 50 unnamed species complexes in the world by Harbach (2011) [14] and the data on more than 70 *Anopheles* species have the capacity to transmit human malaria parasites by Service (2002) [15] , together with the data on more than 41 species are considered to be dominant vector species/species complexes by Hay SI (2010), Sinka et al. (2012) [16] had been synthesized and mapped the distribution of 41 species/species complexes dominant malaria vectors in the world, of which 9 species/vector species complexes in the Americas, 6 species/vector species complexes in Europe and the Middle East, 7 species/species complexes in Africa and 17 species/species complexes in Asia.

1.2.2. Species composition, distribution of Anopheles mosquitoes in Vietnam

By 2015, Vietnam had identified 64 species of *Anopheles* mosquitoes, 15 species were capable of transmitting malaria. In which, the three main vectors were *An. dirus, An. minimus* and *An. epiroticus*. The secondary vectors included *An. aconitus, An. jeyporiensis, An. maculatus, An. sinensis, An. subpictus, An. vagus*. In addition, some species were suspected malaria vectors such as *An. lesteri, An. nimpe, An. interruptus, An. culicifacies* [24], [25].

In Vietnam, *An. dirus* is distributed in mountainous areas from the south of Thanh Hoa to the South of Vietnam, including some islands such as Con Dao and Phu Quoc. In recent years, the natural forest has been narrowed due to deforestation for cultivation land, so the distribution area of this species has also been reduced. In some high malaria endemic areas such as Thanh Hoa, Nghe An, this species has a high density but until now they are almost not found [31]. *An. minimus* is distributed in mountainous areas across the country [31].

1.2.3. Species composition, distribution of adult mosquitoes and larvae of An. dirus, An. minimus in Ea So Nature Reserve

Nguyen Xuan Quang (2012) showed that there were 17 *Anopheles* species, including two main vectors *An. dirus, An. minimus* at Ea So Nature Reserve [7]. 16 species were collected in the residential area, including both main vectors *An. dirus, An. minimus* and two secondary vectors *An. aconitus, An. maculatus*. 17 species were found near the forest (the forest edge), including two main vectors *An. dirus, An. minimus* and two secondary vectors *An. aconitus, An. maculatus.*

Nguyen Xuan Quang (2012) also sampled 7 species of *Anopheles* larvae at Ea So Nature Reserve, larvae of *An. dirus* was collected in fields and puddles (including puddles near streams, fields, around houses, and in the forest). In small puddles, the number of larvae of *An. dirus* was caught in large numbers 122 larvae/total 126 larvae of *An. dirus* were found in water bodies near and far from the forest [7].

1.3. The role in malaria transmission of Anopheles

1.3.1. Infection rate of malaria parasite in Anopheles

An. dirus has a high parasitic infection rate, mainly *P. falciparum* and *P. vivax*. *An. dirus* play a major role in malaria transmission [29], [30].

An. minimus is the main malaria vector in all regions where they are present. Nguyen Xuan Quang (2012) studied malaria-infected mosquitoes collected in the forest edge habitat in 3 National parks/Nature reserves: In Chu Mon Ray, *An. minimus* was not infected with parasites; In Kon Ka Kinh malaria infected rate of *An. minimus* was 2.3% and in Ea So, malaria infected rate of *An. minimus* was 3.33% [7].

1.3.2. Some ecological characteristics related to malaria transmission role of Anopheles

1.3.2.1. Host preference behavior of An. dirus and An. minimus

An. dirus prefers to feed on human blood than cattle. In the forest, *An. dirus* mainly feeds on the blood of primate species. When humans appear, *An. dirus* switch to bite humans, and this species is considered to be extremely fond of biting people [24].

An. minimus prefers to feed on human blood than cattle blood, but the rate of human and animal bites varies in different regions. In Vietnam, *An. minimus* likes to bite humans. However, some studies have demonstrated that the density of *An. minimus* bite humans less than cattle [56].

1.3.2.2. Biting activity of An. dirus và An. minimus

In Vietnam, *An. dirus* bite people not only indoors but also outdoors. The rate of indoor and outdoor bites changes at different sites [24]. Biting activity of *An. dirus* occurs throughout the night, and the peak activity time varies by region and by season. The common activity peak is from 20h to 24h [29], [30].

Biting activity of *An. minimus* occurs throughout the night with the common activity peak is from 22h to 3h [24], [28].

1.3.3. The role of malaria transmission of some secondary vectors in Vietnam

In Vietnam, there are evidences have demonstrated that secondary vectors also contribute to malaria transmission because of their positive results of ELISA tests such as *An. aconitus* (0.46%), *An. jeyporiensis* (0.15%), *An. maculatus* (8.1%), *An. vagus* (1.09%) [24]. When environmental conditions change, these secondary vectors can have favorable conditions for growth and mosquitoes have more opportunities for malaria transmission.

1.4. Study on insecticide susceptibility of Anopheles

Studies in Vietnam show that *An. dirus* is susceptibe to most insecticides. However, *An. dirus* is possibly resistant to some insecticides in some places. In general, most of *An. minimus* is susceptibe to insecticides. However, *An. minimus* is resistant to insecticides at some sites and also possibly resistant in many places [33].

1.5. Main interventions for malaria vector control

Following by WHO guideline (2019) for malaria vector control, core interventions including residual spraying and insecticide-treated nets/ long-lasting insecticidal nets, supplementary interventions using biological and chemical larvicides, personal protection measures and other interventions and including the use of repellents, insecticide-treated clothing or space repellents should be used to control malaria vectors [74].

In Vietnam, millions of people living in malaria-endemic areas are protected by interventions such as residual spraying and insecticide-treated bed nets, especially providing long-lasting insecticidal nets [9].

1.6. Measure of using ZeroFly® for *Anopheles* control

In Vietnam, a deltamethrin-treated sheet (4.4 g/kg ± 15% active ingredient) was evaluated entomological efficacy and household acceptability by Messenger in Phuc Tien commune, Ky Son, Hoa Binh in

2012. The results showed that the average time to install the sheets was 81.7 minutes (30 standard deviation) in the living room and 77.5 minutes (40 standard deviation) in the bedroom. While indoor residual spraying application time was 60 minutes for approximately 60 minutes for an individual to spray one house. After 15 months, sheeting bioefficacy remained at 100%. For indoor residual spraying, average mosquito mortality fell to 60% after only one month and to 40% by three months post-intervention. Adverse reactions were reported from households (7/28) [102].

Studies on insecticide-treated sheeting for control malaria vectors are few and there are still no studies on the bioefficacy of sheets with a field population of *An. dirus* and human protection from mosquito bites when using sheets. Zerofly® is currently used in agriculture to control pets and protect products. There have also been some places applying ZeroFly® to control malaria, but currently, there is no specific study on ZeroFly®. Therefore, research on the ZeroFly® application against *Anopheles* in the laboratory and field to control malaria is very necessary in order to have an effective alternative approach for malaria vector control in areas where conventional vector control measures such as indoor residual spraying and insecticide-treated bed nets are difficult to apply.

Chapter 2: STUDY METHODS

2.1. Objective 1: *Determination of species composition, distribution, some ecological characteristics and role in malaria transmission of Anopheles in Ea So Nature Reserve, Dak Lak 2020-2021*

2.1.1. Subjects, location and time of the study

- Research subjects:

Adults and larvae of *Anopheles*; Host blood meals in malaria vectors; Species of malaria parasites in malaria vectors.

- Research location:

Ea So nature reserve area and laboratory of Entomology department of National Institute of Malaria, Parasitology and Entomology.

- Research time: From June 2020 to December 2021.

2.1.2. Methods

- Study design: Cross-sectional descriptive study and laboratory experimental research.

- Sample size: To identify species composition: All 2,958 adult mosquitoes and 152 larval samples of *Anopheles* collected in the study area; To observe the human biting activities: All 183 *An. dirus* samples collected by human-baited double net trap method in 2020 and 2021 by every hour; To determine the blood meals by host: All 15 *An. dirus* và 2 *An. maculatus* which had blood in the gut, collected by human-baited double net traps method and CDC light traps in residental area, the forest edge and the deep forest; To determine the malaria infection rate: All 395 *Anopheles* samples collected by human-baited double net trap method and CDC light traps in the forest edge and the deep forest.

- Sampling methods:

+ Purposive sampling the study sites for mosquito surveys to determine species composition, density of adult and larvae of *Anopheles*.

+ Purposive sampling adult and larvae of *Anopheles* to carry out laboratory experimental researchs.

- Study content:

Investigation of species composition and density of *Anopheles* mosquitoes by habitat and by season; Identification of parasite species in *Anopheles* by realtime PCR method; Determination of the blood meals by host in *Anopheles* by agar diffusion assay method; Biting activity: observation the number, density of *Anopheles* by human-baited double net trap method by every hour; Determination of the entomological inoculation rate of malaria vectors follow by WHO (2013) [106].

- Research variables:

Including the number of each *Anopheles* species; the number of *Anopheles* species; Density of *Anopheles* mosquito species; Percentage (%) of *Anopheles* mosquito species; The number of larvae of *Anopheles*; Percentage (%) of larvae of *Anopheles*; The number of blood meals by host; Percentage of blood meals by host (%); The number of infected mosquitoes; Percentage of infected mosquitoes (%); The entomological inoculation rate.

- The techniques used in study:

The techniques used in study include: Human-baited double net trap; Mosquito collections around cattle sheds; CDC light trap; Collection indoor daytime; Investigate the larvae; Mosquito morphological identification technique; larval morphological identification technique; Agar diffusion assay by host; Mapping the presence of malaria vectors

technique; Real time PCR for detection of malaria parasites.

- Indicators:

Density of *Anopheles* mosquito species; Percentage (%) of *Anopheles* mosquito species; Percentage (%) of larvae of *Anopheles*; Percentage of blood meals by host (%); Percentage (%) of infected mosquitoes, The entomological inoculation rate [106].

2.2. Objective 2: *Evaluation of application effectiveness of ZeroFly® against malaria vectors in the laboratory and field*

2.2.1. Subjects, location and time of the study

- Research subjects:

An. dirus laboratory strain and field population; Insecticide: alphacypermethrin, deltamethrin; ZeroFly®; forest rangers.

- Research location:

Ea So Nature Reserve and laboratory of Entomology Department of National Institute of Malaria, Parasitology and Entomology.

- Research time: From June 2020 to November 2022.

2.2.2. Methods

- Study design: Laboratory experimental research; Field trial with control; Cross-sectional descriptive study.

- Sample size:

- To evaluate the susceptibility of *Anopheles* mosquitoes to insecticide: 150 female *An. dirus* aged 2-5 days old reared in laboratory or field population of *An. dirus*.

- To evaluate the application effectiveness of ZeroFly® against malaria vectors in the laboratory and field:

+ 200 susceptible, 2-5 days old female *An. dirus* reared in laboratory (100 for control and 100 for testing; 240 female *An. dirus* susceptible laboratory strain or field population.

+ 3 ranger stations, each has at least 3 rooms.

+ All of 6 participants tested ZeroFly® in laboratory and 30 participants lived in treated ranger stations.

***- Study content*:**

+ Evaluation of the susceptibility of *An. dirus* (laboratory strain and field population) to deltamethrin, alphacypermethrin follow by WHO (2018) [59].

+ Determination of residual efficacy of ZeroFly® on *An. dirus* in the laboratory and field.

+ Evaluation of human protection efficacy from mosquito biting of ZeroFly® after 6 months of use in November 2021.

+ Evaluation of side effects and the user acceptance of ZeroFly®.

- Research variables:

Number of mosquitoes knock down; Percentage (%) of mosquitoes knock down; Number of dead mosquitoes; Motality rate (%) after 24 hours test; Number of human side effects; Rate of human side effects; the number of *An. dirus*; Density of *An. dirus*; Acceptance rate of users (%); Human protection efficacy from mosquito biting (%).

- The techniques used in study:

WHO susceptibility bioassay*;* WHO cone bioassay; Evaluation of the human protection efficacy from mosquito biting of ZeroFly® by human-baited double net trap. Interviewing techniques to evaluate side effects and the user acceptance of ZeroFly®.

- Các chỉ số đánh giá:

Rate of mosquitoes knock down (%); Motality rate (%) of mosquitoes after testing 24h; Density of *An. dirus*; Rate of human side effects (%); Acceptance rate of users (%); Human protection efficacy from mosquito biting (%).

2.3. Data processing and analysis methods

- Data was entered, calculated and analyzed on Microsoft Excel software.

- Data was analyzed by using Independent t-test on SPSS software to compare average of mosquito densities between 2 seasons and between treated rangers and control.

2.4. Error and reducing error

There may be two types of error in research: systematic error because of study design; random error due to the conduct of research.

Reducing error: Strictly follow technical procedures, study design, clean data before analysis.

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. Species composition, distribution, some ecological characteristics and role in malaria transmission of *Anopheles* in Ea So Nature Reserve

3.1.1. Species composition, distribution, some ecological characteristics of Anopheles in Ea So Nature Reserve

3.1.1.1. Species composition, distribution of Anopheles by habitat

Bảng 3.1. Species composition, distribution of *Anopheles* by habitat in Ea So Nature Reserve area in 2020-2021 (n=2,985)

No.	Species of *Anopheles*	Residental area		In Forest edge		In deep forest	
		N	P (%)	N	P (%)	N	P (%)
I	**Subgenus *Anopheles* Meigen,1818**						
1	*An. barbirostris* Van derWulp, 1884	0	0	3	11.11	1	0.26
2	*An. peditaeniatus* (Leicester, 1908)	10	0.39	0	0	0	0.00
3	*An. separatus* Leicester, 1908	0	0	0	0	2	0.52
4	*An. sinensis* Wiedemann, 1828	451	17.54	0	0	0	0
II	**Subgenus *Cellia* Theobald,1902**						
5	*An. aconitus* Doenitz, 1902**	39	1.52	7	25.93	3	0.77
6	*An. dirus* Peyton & Harrison, 1979*	0	0	9	33.33	363	93.80
7	*An. jamesii Theobald, 1901*	25	0.97	0	0	0	0
8	*An. jeyporiensis* James,1902**	0	0	2	7.41	5	1.29
9	*An. maculatus* Theobald, 1901**	36	1.40	4	14.81	8	2.07
10	*An. minimus* Theobald, 1901*	0	0	0	0	1	0.26
11	*An. philippinensis* Ludelow,1902	1,716	66.74	0	0	1	0.26
12	*An. tessellatus* Theobald, 1901	167	6.50	2	7.41	0	0
13	*An. vagus* Donitz, 1902	127	4.94	0	0	3	0.77
Total individuals		**2,571**	**100**	**27**	**100**	**387**	**100**
Total species		**8**		**6**		**9**	

*Ghi chú: *: Main vectors, **: Secondary vectors; N: Number of mosquitoes; P: Percentage*

A total of 13 species of *Anopheles* mosquitoes were collected in three habitats, including the residential area, forest edge, and deep forest at Ea So Nature Reserve, Ea So commune. *An. dirus* was collected in the forest edge and the deep forest accounting for 2.42% and 97.58%, respectively, and *An. dirus* was not captured in the residential area.

3.1.1.2. Species composition, distribution of Anopheles by season

In the residential area, 6 species of *Anopheles* mosquitoes were

collected at the beginning of the rainy season in June 2020 and May 2021 and 7 species of *Anopheles* mosquitoes were captured in November 2020 (the end of the rainy season) and December 2021 (the beginning of the dry season). The number and density of *Anopheles* mosquitoes at the beginning of the rainy season are lower than at the end of the rainy season and the beginning of the dry season.

In the forest edge, in two surveys, at the beginning of the rainy season, 3 species *Anopheles* were collected. However, all of these species were secondary vectors. The main vector was not captured. At the end of the rainy season and the beginning of the dry season not only 3 secondary vector species but also the main vector species *An. dirus*.

In forest habitats, the number of vector species at the beginning of the rainy season is lower than at the end of the rainy season and the beginning of the dry season. There were two species of vectors captured at the beginning of the rainy season while five vector species were caught at the end of the rainy season - the beginning of the dry season. The dominant malaria vector *An. dirus* was found at the beginning and end of the rainy season, *An. minimus* was also collected at the beginning of the dry season with low density (0.0017 individuals/hour/person).

3.1.1.3. Species composition, distribution of larvae of Anopheles

Bảng 3.9. The number and percentage of larvae of *Anopheles* in the water bodies at Ea So Nature Reserve area in 2020-2021

No.	Larvae of *Anopheles* species	Types of water bodies									
		River		Stream		PS		PR		Ditch	
		N	P %	N	P %	N	P %	N	P %	N	P %
1	*An. aconitus*	0	0	0	0	3	7.14	0	0	0	0
2	*An. dirus*	0	0	0	0	26	61.91	0	0	0	0
3	*An. jeyporiensis*	0	0	0	0	3	7.14	0	0	0	0
4	*An. maculatus*	0	0	1	100	10	23.81	0	0	0	0
5	*An. philippinensis*	0	0	0	0	0	0	0	0	42	56.76
6	*An. sinensis*	0	0	0	0	0	0	0	0	15	20.27
7	*An. vagus*	0	0	0	0	0	0	35	100	17	22.97
Total individuals		0	0	1	100	42	100	35	100	74	100
Total species		0		1		5		1		3	

Ghi chú: N: Number of mosquitoes; P: Percentage PS: puddles at the forest stream edge, PR: puddles at roadside.

The results of Table 3.9 showed that the larvae of *An. dirus*, *An.*

aconitus and *An. jeyporiensis* were collected only in puddles at the forest stream edge. The larvae of *An. dirus* accounted for 61.91% of this habitat.

3.1.2. Role in malaria transmission of vectors at Ea So Nature Reserve

3.1.2.1. Results of determination of malaria parasites in Anopheles vectors

Table 3.12. The rate of malaria parasite infection of vectors by habitat

Time	*Anopheles* vector species	The rate of infected vectors					
		The forest edge			The deep forest		
		Number of samples analyzed	Number of infected samples	P (%)	Number of samples analyzed	Number of infected samples	P (%)
In 2020	*An. dirus*	4	0	0	189	3	1.58
	An. jeyporiensis	2	0	0	5	0	0
	An. maculatus	2	0	0	6	0	0
In 2021	*An. aconitus*	7	0	0	2	0	0
	An. dirus	5	0	0	168	1	0.59
	An. maculatus	2	0	0	2	0	0
	An. minimus	0	0	0	1	0	0
Total		22	0	0	373	4	1.07

Note: P: Percentage

In 2020 and 2021, malaria parasites were detected in *An. dirus* was caught in the forest with infection rates of 1.587% and 0.595%, respectively (Table 3.12).

Table 3.13. The entomological inoculation rate (EIR) index of *An. dirus* at Ea So Nature Reserve in 2020 and 2021

Habitats	Time	Density of *An. dirus* collected by HDN (m/p/n)	No. of infected mosquitoes	EIR
The forest edge	June 2020	0 (0/6/4)	-	-
	November 2020	0,125 (3/6/4)	0/3	0
	May 2021	0 (0/6/4)	-	-
	December 2021	0,208 (5/6/4)	0/5	0
The deep forest	June 2020	0,50 (12/6/4)	0/12	0
	November 2020	7,125 (171/6/4)	3/171	0.125
	May 2021	0,083 (3/6/4)	0/2	0
	December 2021	6,708 (161/6/4)	1/161	0.042

Note: HDN: human-baited double net trap m/p/n: Mosquitoes/person/night

The entomological inoculation rate index at the end of the rainy season in November 2020 was 0.125 and the index at the beginning of the dry season in December 2021 was 0.042.

3.1.2.2. Some ecological characteristics related to the role in malaria transmission of malaria vectors

- Host preference of *Anopheles* vectors:

Table 3.14. Results of determination of host blood meals in vectors at Ea So in 2021

Vector species	Total	Number and percentage of host blood meals									
		Human		Cattle		Poultry		Dog		Others	
		N	P (%)	N	P (%)	N	P (%)	N	P (%)	N	P (%)
An. dirus	15	15	100	0	0	0	0	0	0	0	0
An. maculatus	2	0	0	2	100	0	0	0	0	0	0

Ghi chú: N: Number, P: Percentage

The results showed that *An. dirus* fed on humans accounted for 100% and *An. maculatus* fed on cattle accounted for 100%. However, because of a small sample size with 15 *An. dirus* and 2 *An. maculatus*, these results only partially described the host preference of malaria vectors.

- Biting activity of *Anopheles* vectors:

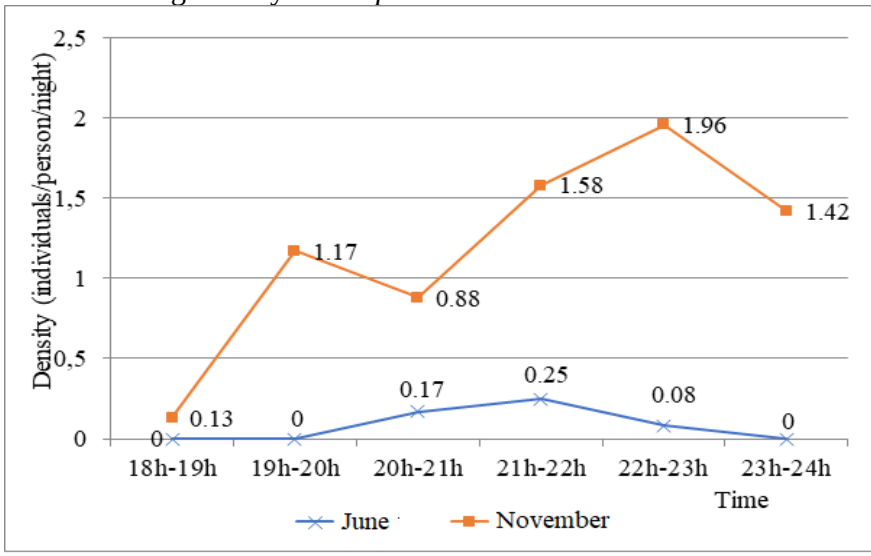

Figure 3.7. Biting activity of *An. dirus* in the deep forest at Ea So in 2020

The surveys in 2020 showed that the biting activity of *An. dirus* began from 17h-18h. The bitting time of *An. dirus* started at 18h and finished at 24h during the investigation periods, peaking between 22h - 23h with a density of 1.96 individuals/hour/person (Figure 3.7).

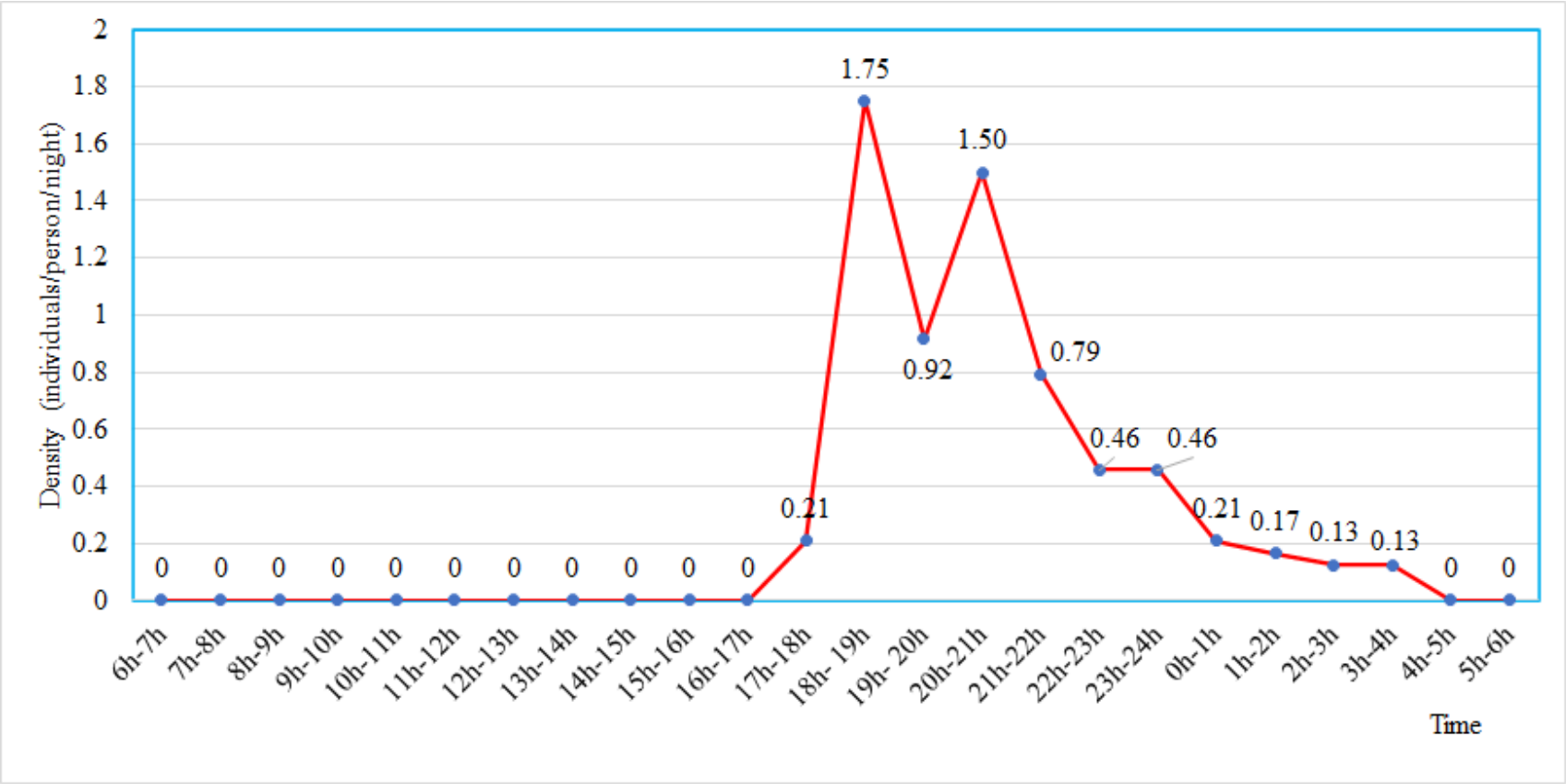

Figure 3.8. Biting activity of *An. dirus* in the deep forest at Ea So in December 2021

The surveys in 2021 showed that the biting activity of *An. dirus* started early from 17h-18h to 3h-4h am, peaking at 18h-19h. After 24 hours, the density of biting mosquitoes was low. The rate of mosquito bites from 17h to 24h accounted for 90.68% and from 0h to 6h only accounted for 9.32% (Figure 3.8).

3.2. Application effectiveness of ZeroFly® against malaria vectors in the laboratory and field

3.2.1. The insecticide susceptibility of Anopheles mosquitoes

Table 3.20. The results of the susceptibility of *An. dirus* collected in the forest at Ea So to insecticide in 2020-2021

Time	Insecticide	Batchs	Number	Rate of knockdown after 60 minutes (%)	Mortality rate after 24h (%)
November 2020	Alphacypermethrin 0,05%	Treatment	100	100	100
		Control	50	0	0
December 2021	Deltamethrin 0,05%	Treatment	100	100	100
		Control	50	0	0

The wild population of *An. dirus* was susceptible to alphacypermethrin and deltamethrin, showing 100% mortality after testing 24 hours.

3.2.2. The residual efficacy of ZeroFly® on Anopheles mosquitoes in the laboratory and field

Table 3.21. The residual efficacy of ZeroFly® on *An. dirus* (laboratory strain and field population) in laboratory and field

Mosquito strains	Time (After time of use)	Batch	Number	Knockdown (%)		Mortality rate after 24h (%)
				After 3 minutes	After 60 minutes	
An. dirus (laboratory train)	May 2021 (Start using)	Treatment	100	19	89	100
		Control	100	0	0	0
	November 2021 (After 6 months)	Treatment	120	11.67	87.5	100
		Control	120	0	0	0
	May 2022 (After 12 months)	Treatment	120	10.83	85	100
		Control	120	0	0	0
	Tháng 11/2022 (After 18 months)	Treatment	120	7.50	82.50	100
		Control	120	0	0	0
An. dirus (field population)	May 2021 (Start using))	Treatment	-	-	-	-
		Control	-	-	-	-
	November 2021 (After 6 months)	Treatment	120	10.83	84.17	100
		Control	120	0	0	0

Ghi chú: -: No test was conducted

The results revealed that ZeroFly® had a good effect on *An. dirus* (laboratory strain) after 18 months of use with a mortality rate was 100%. ZeroFly® had excellent residual efficacy after 6 months of use in the field with a mortality rate was also 100%.

2.2.3. Human protection efficacy from mosquito biting of ZeroFly®

Table 3.23. Human protection efficacy of ZeroFly®) on *An. dirus* in the field

Days of test	Number of mosquitoes collected		Human protection efficacy (%)
	Control (use ZeroFly®)	Treatment (not use ZeroFly®)	
1	7	3	57.14
2	4	1	75.00
3	3	1	66.67
4	7	3	57.14
5	3	1	66.67
6	10	4	60.00
Mean ± 1.96SD			**63.77 ± 13.72**

Table 3.23 showed that after 6 months of use, ZeroFly® could protect people from mosquito biting when they were in intervention rooms. The protection efficacy was 63.77 ± 13.72% (Mean ± 1.96SD).

3.2.4. Side effects and the user acceptance of ZeroFly®

Table 3.25. Summary of interview results about side effects of ZeroFly® with participants at intervention ranger stations

No.	Interview content	Number of people interviewed	Number of participants got side effects (Percentage %)		
			1 day	1 week	6 months
1	itchy	30	0(0)	7(23.33)	0(0)
2	Others side effects	30	0(0)	0(0)	0(0)

The results of interviewing 30 participants living at the intervention ranger stations showed that there were only 7 people

(23.33%) got itchy skin after direct exposure to ZeroFly®. However, these symptoms disappeared naturally as soon as 1 - 2 days after exposure. Others didn't have any side effects in testing time.

Table 3.26. The user acceptance of ZeroFly® in the field

Information	Total of interviewees	Number of answers were "yes"	Percentage (%)
Know the purpose of using Zerofly® to control mosquitoes	30	30	100
Zerofly® has a good effect on mosquitoes	30	30	100
Look forward to continuing using Zerofly®	30	30	100

The results of interviewing 30 forest rangers revealed that all interviewees (100%) believed that ZeroFly® had a good effect on mosquitoes and liked using the sheeting.

Chapter 4: DISCUSSIONS

4.1. Species composition, distribution, some ecological characteristics and role in malaria transmission of *Anopheles* at Ea So Nature Reserve, Dak Lak, 2020-2021

4.1.1. Species composition, distribution of Anopheles

4.1.1.1. Species composition, distribution of Anopheles by habitat

Our study collected 13 species out of 42 *Anopheles* species that had been recorded in the Central Highlands. In which, the species of *An. separatus, An. jeyporiensis* was first recorded in the forest habitat of Ea So commune with low density. The two main vector species of *An. dirus and An. minimus* and three secondary vector species of *An. aconitus, An. jeyporiensis* and *An. maculatus* were recorded in this study area.

The distribution of *Anopheles* species by habitat is markedly different. In our study, the number of *Anopheles* mosquitoes collected gradually decreased from the residential area,

the deep forest, and the forest edges. Thus, the results of our study and recent studies showed that the two main vectors *An. dirus* and *An. minimus* were not recorded in residential areas, they were often found in the forest edge with their density was lower than in the deep forest. In the forest edge and the deep forest, a large number of trees with canopy and streams are suitable for the growth and development of *An. dirus*. The distribution of *Anopheles* has changed between the results of this thesis as well as some recent studies and the previous studies. Orientation in vector control should focus on the deep forest and forest edges habitats to protect local people, forest rangers who work and travel in this area.

4.1.1.2. Species composition, distribution of Anopheles by season

The results of our study showed that the species composition, number and density of *Anopheles* mosquitoes at the end of the rainy season and the beginning of the dry season were higher than at the beginning of the rainy seasons. These results were similar to the study results of Nguyen Xuan Quang (2012) [7], showing that the density of vectors in farm areas in Ea So (both treatment and control sites) tended to increase at the end of the rainy season (October). Nguyen Van Tuan (2015) showed that at the beginning of the dry season in late December and early January, the density of *An. dirus* and *An. minimus* increased higher than at the end of the rainy season in October [27]. The high increasing density of *Anopheles* vectors at the end of the rainy season and the beginning of the dry season may be because of the seasonal transition time between the rainy season and to dry season. At this time, it is drizzling as well as the decreasing rainfall causing rivers and streams to dry up together with streamflows decline forming a large number of puddles on both sides of the water bodies which are suitable for *Anopheles* mosquitoes to lay eggs, grow and develop.

4.1.1.3. Species composition, distribution of larvae of Anopheles

The results of larval surveys in our study were consistent with the density of *Anopheles* mosquitoes in the different habitats. Larvae of *An. dirus* was collected in the deep forest with high density.

Larvae of *An. dirus* was not captured in the forest edge and residential area because of the absence or low density of *An. dirus.*

The results of our study showed that the water bodies with the main malaria vector *An. dirus* preferred to lay eggs were puddles at the stream edge in the deep forest. The puddles at the stream edge were also the water bodies where the secondary vectors such as *An. aconitus, An. jeyporiensis* and *An. maculatus* preferred to lay eggs at the forest edge and the deep forest habitats. These results provided the basis for control *An. dirus* and other malaria vectors through larval detection and investigation.

4.1.2. Role in malaria transmission of *Anopheles* vectors at Ea So Nature Reserve

4.1.2.1. The rate of malaria parasite infection of An. dirus và An. minimus

In our study, *P. vivax* was detected in *An. dirus* caught in the deep forest at a rate of 1.59% in 2020 and 0.595% in 2021. In comparison with the previous study at Ea So commune of Nguyen Xuan Quang (2012) [7], the distribution of infected mosquitoes by habitat had changed. Nguyen Xuan Quang (2012) improved that *An. dirus* was not infected with malaria parasites in the residential habitat while in our study, *An. dirus* was not sampled in residential habitat. In the study of Nguyen Xuan Quang, *An. dirus* was infected with malaria parasites with 4.96% in the forest edge habitat while in our study, malaria parasite was not detected in *An. dirus* collected at this habitat. The results showed that *An. dirus* was capable of transmitting malaria and it had demonstrated its ability to malaria transmition at the forest habitat. In which entomological inoculation rate was 0.125 in November 2020 and 0.042 in December 2021.

In conclusion, *An. dirus* plays a major role in malaria transmission in the forest edge and forest areas, especially the forest habitat.

4.1.2.2. The role of malaria transmission of secondary vectors

In our study, all 3 malaria secondary vectors, *An. aconitus, An. jeyporiensis* and *An. maculatus* were collected but malaria parasites were not detected in secondary vectors. Therefore, it is also necessary to pay attention to these mosquito species because

many studies have found these species infected with malaria parasites and they bite humans at habitats where present *An. dirus*.

4.1.2.3. Some ecological characteristics related to the role in malaria transmission of malaria vectors

- Host preference of *Anopheles* vectors:

Our research results were consistent with previous research results. The main vector *An. dirus* is a species that prefers to feed on humans than cattle. The rate of human per animal bites varies from place to place. When the human host does not appear, *An. dirus* mainly feed on livestock and primates, they switch to bite humans in the presence of humans. At Dong Xoai district, Binh Phuoc province, *An. dirus* preferred blood meals (83.33%) to human blood meals (16.67%) [56]. In Dong Xuan district, Phu Yen province, *An. dirus* human biting rate was 100% [30].

In our study, only 1 sample of *An. minimus* was collected by human-baited double net trap method, The host preference of this mosquito species was undetermined in our study, but previous studies in the world and Vietnam showed that *An. minimus* prefers to bite humans than cattle and this ratio varies in different regions.

- Biting activity of *An. dirus:*

The peak biting activity of *An. dirus* in 2021 (between 18h - 19h) was earlier than in 2020 (between 21h-22h) in our study and the previous study of Nguyen Xuan Quang (2012) with the peak biting activity of *An. dirus* in the forest edge and residential area of Ea So Nature Reserve was between 20h-21h, Chu Mom Ray National Park was between 20h-22h and Kon Ka Kinh National Park was between 21-22h [7]. Bui Le Duy et al. (2019) showed that the biting activity of *An. dirus* was all hours of investigation, peaking between 21h - 22h [40]. This difference may be due to the biting activity of mosquitoes is changed when the study time is different

4.2. Discussion on application effectiveness of ZeroFly® against malaria vectors in the laboratory and field

4.2.1. The insecticide susceptibility of Anopheles at Ea So Nature Reserve

In our study, the field population of *An. dirus* was susceptible to alphacypermethrin with a mortality rate of 100% while Nguyen Xuan Quang (2012) in Ea So showed that *An. dirus* was resistant to alphacypermethrin 30mg/m^2 (Mortality rate after 24 hours was 96%) and lambda-cyhalothrin 0.05% (Mortality rate after 24 hours was 97%). This difference may be due to *An. dirus* in the study of Nguyen Xuan Quang (2012) [7] were collected in a residential area (far from the forest) and in the forest edge (near the forest) where people lived and cultivated, they were exposed to pyrethroid insecticide used in medicine to control malaria and pyrethroid insecticide used in agriculture for a long time lead to insecticide resistance of mosquitoes. In contrast, *An. The dirus* population in our study was captured mainly in the deep forest, therefore they were less exposed to insecticides than mosquitoes in residential areas.

4.2.2. The residual efficacy of ZeroFly® on Anopheles mosquitoes in the laboratory and field

In our study, ZeroFly® showed a good effect on *An. dirus* (laboratory strain) after 18 months of use with a mortality rate was 100%. These results are consistent with the study results of Messenger (2012) in Equatorial Guinea, Ghana, Mali, South Africa and Vietnam. The residual efficacy of the deltamethrin treated sheet (4.4 g/kg ± 15% active ingredient) against *Anopheles* mosquitoes was up to 12-15 months. By contrast, indoor residual spraying displayed a significant decrease in bioactivity by 6 months and full loss after 12 months. In Vietnam, after 15 months, sheeting bioefficacy remained at 100%. For indoor residual spraying, average mosquito mortality fell to 60% after only one month and to 40% by three months post-intervention [102].

4.2.3. Human protection efficacy from mosquito biting of ZeroFly®

The results of our study indicated that after 6 months of use, human protection efficacy from mosquito biting of ZeroFly® was 63.77 ± 13.72% with a 95% confidence interval when in intervention rooms. The results showed that the average mosquito density in the control rooms was 0.31 individuals/hour/person,

which was higher than in the experiment rooms with 0.12 individuals/hour/person. There was a significant difference between the two groups ($p = 0.028 < 0.05$). The results of our study were similar to previous studies. Sharma (2008) indicated that, in the ZeroFly plastic sheeting treated area, there was a significant reduction of 84.7% in the entry rate of total mosquitoes in comparison to the pre-intervention phase. The overall feeding success rate of mosquitoes in the trial village was only 12.5% in comparison to 49.7% and 51.1% in villages with untreated sheets and no sheets respectively [95].

4.2.4. Side effects and the user acceptance of ZeroFly®

The results of interviewing the user participants in our study showed that only 7 people reported itchy skin when directly exposured to the Zerofly® excluding the hands, accounting for 23.33%. This rate is lower than the proportion of people with symptoms of skin irritation and itching (43%) and eye irritation (23%) in the study of Mittal (2011) [96]. Meanwhile, the deltamethrin content of Zerofly® in our study was 4.5g/Kg higher than 2g/Kg in the study of Mittal (2011) [96]. Therefore, the difference in side effects of the 2 studies may be the result of our recommendation that the users should not be directly exposure to the Zerofly® and our subject interviewees are forest rangers, children and women were not included. All interviewees (100%) believed that ZeroFly® had a good effect on mosquitoes and liked using ZeroFly® in comparison with 82% of interviewees answerd ZeroFly® was effective against mosquitoes and 73% wished to be used sheeting in a survey in Indonesia [5].

In summary, ZeroFly® is proven the high efficacy against mosquitoes and is safe for humans. There were some complaints of irritation and itching skin but these were temporary, and no health side effects were reported by users. ZeroFly is highly accepted by forest rangers at Ea So Nature Reserve.

CONCLUSIONS

1. Species composition, distribution, some ecological characteristics and role in malaria transmission of *Anopheles* in Ea So Nature Reserve, Dak Lak, 2020-2021

13 species of *Anopheles* mosquitoes were collected at Ea So Nature Reserve area. In which, the two main vector species, *An. dirus and An. minimus* and three secondary vector species *An. aconitus, An. jeyporiensis* and *An. maculatus* were recorded in the deep forest. *An. dirus* and three secondary vector species *An. aconitus, An. jeyporiensis, An. maculatus* were collected in the forest edge. Only two secondary vector species *An. aconitus, An. maculatus* was captured in the residential area.

An. dirus was collected at the end of the rainy season and the beginning of the dry season is higher in forest habitats and forest edges. Only one *An. minimus* was collected in the deep forest at the end of the rainy season.

An. dirus larvae was discovered mostly in puddles at the forest stream edge.

P. vivax was detected in *An. dirus* captured in the deep forest at a rate of 1.59% in 2020 and 0.595% in 2021. *An. dirus* could prove their ability to transmit malaria at the deep forest habitat. In which entomological inoculation rate in November 2020 was 0.125 and in December 2021 was 0.042.

An. dirus prefered to feed on humans, accounting for 100% (15/15). Biting activity of *An. dirus* began very early from 17h-18h, peaking early between 18h-19h or 22h-23h.

2. The application effectiveness of ZeroFly® against malaria vectors in the laboratory and field

Laboratory strain as well as the wild population collected in Ea So Nature Reserve of *An. dirus* were susceptible to alphacypermethrin and deltamethrin, showing 100% mortality after testing 24 hours.

ZeroFly® showed a good effect against *An. dirus* after 18 months of use for laboratory strain and after 6 months of use for *An. dirus* collected in the field with 100% of mortality.

After 6 months of use, human protection efficacy from mosquito biting of ZeroFly® was 63.77 ± 13.72% (Mean ± 1,96SD).

There were some complaints of skin itchy (23.33%) but these were temporary, no adverse health effects were recorded by users. ZeroFly® was highly accepted by forest rangers in Ea So Nature Reserve with a rate of 100%.

RECOMMENDATIONS

1. Because of the high density and early biting activity of the main vector *An. dirus*, so it is recommended that people should use personal protection measures to prevent malaria vectors outside their homes before the biting time mosquitoes such as repellent cream, mosquito coil, and wearing long sleeved clothing... at the same time, it is necessary to maintain measures to control mosquitoes such as residual spraying and insecticide treated bed nets.
2. Malaria prevention and eradication programs can provide ZeroFly® to supplement or replace indoor residual spraying in areas where the indoor residual spraying method is difficult to apply or is ineffective such as farmhouse areas with many temporary houses, ranger stations... to improve the effectiveness of malaria vector control.
3. It is necessary to continue to study the application of ZeroFly® and other community protection measures in areas with similar conditions to Ea So Nature Reserve to control malaria vectors more effectively and comprehensively.

LIST OF AUTHOR'S PUBLICATIONS RELATED TO THE THESIS

No.	Name of publications
1	Pham Van Quang, Tran Thanh Duong, Vu Viet Hung, Nguyen Van Dung (2022), "Field evaluation of Zerofly®, an insecticide incorporated plastic sheeting against *Anopheles dirus* (the major malaria vector) for forest rangers in Ea So Nature Reserve, Dak Lak province, 2021", *Vietnam Journal of Preventive Medicine*, 32(6), pp. 29-36. DOI: https://doi.org/10.51403/0868-2836/2022/794.
2	Pham Van Quang, Hoang Dinh Canh, Tran Thanh Duong, Nguyen Van Dung (2023), "Species composition, distribution of *Anopheles* mosquito and *Anopheles dirus* biting activity at Ea So Nature Reserve, Ea So commune, Ea Kar district, Dak Lak province, 2020-2021", *Vietnam Journal of Community Medicine*, 63(4), pp. 227-233.DOI: https://doi.org/10.52163/yhc.v64i4.707
3	Pham Van Quang, Hoang Đinh Canh, Tran Thanh Duong, Nguyen Van Dung (2023), "The role in malaria transmission and host preference of vectors in Ea So Nature Reserve, Ea So commune, Ea Kar district, Dak Lak province, 2020-202", *Tạp chí y học cộng đồng*, *Vietnam Journal of Community Medicine*, 64(4), pp. 1-8. DOI: https://doi.org/10.52163/yhc.v64i4.723.