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National Institue of Malariology Parasitology and Entomology

TRAN THI THUONG

STUDY ON THE ECOLOGY, BIOLOGICAL CHARACTERISTICS, DISTRIBUTION, INSECTICIDE SUSCEPTIBILITY OF *AEDES* MOSQUITOES, THE EFFECTIVENESS OF NIMPE MOSQUITO REPELLENT CANDLES IN HANOI AND THANH HOA

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Scientific advisors:

1. …….

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Reviewer 1:

Reviewer 2:

Reviewer 3:

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INTRODUCTION

1. Urgency of the study

Dengue hemorrhagic fever (DHF) is a severe infectious disease caused by the Dengue virus. If not diagnosed and treated promptly, DHF can result in high morbidity and mortality rates. It is a significant public health concern, with four strains of the Dengue virus transmitted through the Aedes mosquito vector. The global incidence of dengue fever has increased 30-fold in the past 50 years. According to the World Health Organization (WHO), dengue fever is most prevalent in Southeast Asia and the Pacific, hotspots for mosquito-borne diseases. These regions account for approximately 75% of global dengue morbidity and mortality, with Vietnam having one of the highest disease burdens.

Hanoi and Thanh Hoa cities have seen a rise in dengue cases in recent years, with more frequent outbreaks. Currently, there is no specific treatment for dengue, and vaccines are not widely available. Therefore, disease prevention mainly relies on controlling the mosquitoes that transmit the disease. It is crucial to have up-to-date information on the distribution, habit, biology, and ecology of mosquitoes to propose appropriate prevention measures for each region. This includes using insecticides such as repellent scents, creams, and candles to prevent the spread of dengue hemorrhagic fever effectively. Therefore, we conducted this study.

2. Objectives of the study

- *Determine the biological, ecological characteristics, and distribution of Aedes aegypti and Aedes albopictus mosquitoes in selected districts of Hanoi City and Thanh Hoa Province in 2019-2020.*

- *Assess the sensitivity of Aedes aegypti and Aedes albopictus mosquitoes to insecticides at the research sites.*

- *Evaluate the effectiveness of NIMPE mosquito repellent candles against Aedes aegypti and Aedes albopictus mosquitoes in laboratory and field settings in Thanh Hoa province in 2020.*

3. Novelty, scientific and practical significance of the thesis

The project applies scientific research design methods that are currently used in Vietnam and internationally. Data is processed using modern biomedical statistics software. The research techniques used follow the procedures outlined by the USCCD, WHO, and the National Institute of Malariology Parasitology and Entomology (with SOPs). By combining modern laboratory techniques with routine techniques in accordance with WHO guidelines, the research produces highly accurate and reliable results.

This study presents updated data on the distribution, biological characteristics, and ecological characteristics of *Aedes aegypti* and *Aedes albopictus* mosquitoes in specific districts of Hanoi City and Thanh Hoa Province from 2019 to 2020.

For the first time in Vietnam, USCDC sensitivity testing techniques were used to assess the sensitivity and resistance of Aedes mosquitoes to chemicals. Additionally, by monitoring the biting activity of *Aedes* mosquitoes in the laboratory for 24 hours, it has been established that this predominant mosquito species is active both during the day and at night. The project findings demonstrate that NIMPE candles effectively prevent Aedes mosquitoes in Vietnam.

CONTENT OF THE THESIS

The thesis consists of 135 pages divided into the following sections: Rationale (2 pages), literature review (37 pages), subjects and methods (27 pages), results (36 pages), discussions (30 pages), conclusions (2 pages), recommendations (1 page), and 1 page for novelty, scientificity, and practicality. There are 27 tables, 26 figures, 18 appendices included, and 165 references.

CHAPTER 1: LITERATURE REVIEW

1.1. Dengue hemorrhagic fever

DENV-1, DENV-2, DENV-3, and DENV-4 are the four types of dengue virus (also known as serotypes). This disease is prevalent in more than a hundred tropical and subtropical countries. The warm and humid climate in these regions provides ideal conditions for mosquitoes, which carry the virus, to breed. The virus responsible for causing dengue hemorrhagic fever (DHF) belongs to the Flavivirus genus, which is part of the Flaviviridae family. It is primarily spread through the blood of female *Aedes* mosquitoes, with *Aedes aegypti* playing a secondary role.

1.1.2. Dengue hemorrhagic fever situation

1.1.2.1. In the world: The number of dengue cases reported globally in 2019. In Asia, the highest numbers of cases were reported in Bangladesh (101,000), Malaysia (131,000), the Philippines (420,000), and Vietnam (320,000). Projections indicate that in 2022, there will be over 3 million cases of dengue fever worldwide, resulting in more than 3 thousand deaths. South America and Asia have the highest number of cases, with dengue disease present on all 5 continents. The highest number of dengue cases was recorded in 2023, with Thailand (150,000) and Vietnam (369,000) reporting a significant number of cases. In 2023, there was an unexpected surge in dengue cases, with over 6.5 million reported cases and more than 7,300 dengue-related deaths, reaching a historic high. The Southeast Asia and Western Pacific regions are the most severely affected, with Asia accounting for approximately 70% of the global disease burden.

1.1.2.2. In Vietnam: DFH is always prevalent in Vietnam but changes from year to year or region to region due to climate change, urbanization, etc. The disease is still distributed from North to South, in which the disease develops mainly in the summer and autumn months in the North, while the South is hot all year round, so the disease is scattered throughout the year but concentrated in the months of June - November. In the period from 1998 - 2020 in the North, an average of 8,683 cases were recorded each year, of which only 1 - 2 cases died. 2017 had the highest

number of cases in the history of the surveillance system with 55.531 cases and 7 deaths. In the Southern region from 2001 to 2020, the average annual number of dengue fever cases was 64.153 cases/year, compared to the period from 1986 to 1998, the average number of dengue fever cases increased by more than 25%. From 2014 to 2019, the number of cases continuously increased at a rapid rate, peaking in 2019 with 440 cases/100.000 people, 6.1 times higher than in 2014.

1.1.2.3. Dengue fever situation at the study site

In 2017, Hanoi experienced a significant rise in dengue fever cases, with a fourfold increase compared to the previous year. Hospitalizations also saw a notable 11.2% increase during this time. The outbreaks revealed the presence of both *Ae. aegypti* and *Ae. albopictus* mosquitoes. Additionally, Hanoi faced several complex outbreaks at the commune and ward level, leading to a high number of affected patients. In Thanh Hoa, a total of 3.374 dengue fever cases were reported in 2017. Of these cases, 349 were domestic (accounting for 10.34% of the total), and 3.025 were imported (accounting for 89.66% of the total). The domestic cases were concentrated in 10 dengue fever hotspots, while the remaining cases were spread across 115 communes in 21 districts, towns, and cities.

1.1.3. Classification of Aedes aegypti and Aedes albopictus mosquitoes:

There are approximately 3,500 mosquito species that can be found all over the world. These species are classified into 39 genera and 135 subgenera. Out of these, the *Aedes* genus, which belongs to the Diptera: Culicidae family, is made up of around 700 species. In Vietnam, researchers have identified 205 mosquito species, and out of these, 67 species are part of the *Aedes* genus. Two particularly noteworthy species within the *Aedes* genus are *Ae. aegypti* and *Ae. albopictus*.

1.2. Distribution of *Aedes aegypti* and *Aedes albopictus* mosquitoes

1.2.2. Distribution of Aedes aegypti and Aedes albopictus mosquitoes in the world

Aedes aegypti is currently present in 167 countries and territories, which makes it a major carrier of human pathogens due to its proximity to humans.

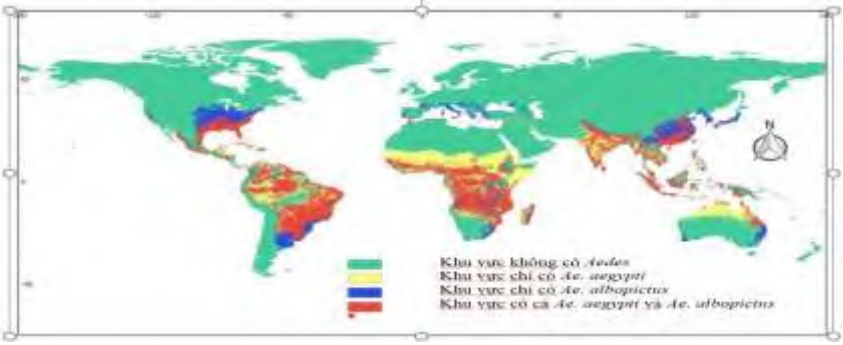

Fig. 1.1. Distribution map of *Aedes* mosquitoes in the world

1.2.3. Distribution of Aedes aegypti and Aedes albopictus mosquitoes in Vietnam

Ae. aegypti is primarily found in the South and Central regions, while *Ae.albopictus* is more prevalent in the Northern region. This distribution can be attributed to climatic factors such as temperature and rainfall. *Aedes* mosquitoes are also commonly found in densely populated areas with numerous water containers and regular vehicular traffic. Hanoi and Thanh Hoa are a few examples of these areas.

1.3. Biological and ecological characteristics of *Aedes* mosquitoes

1.3.1. Growing season and reproductive characteristics of Aedes mosquitoes

The *Aedes* mosquito, like other mosquito species, goes through four stages in its life cycle. Once hatched, female *Aedes* mosquitoes mate and take their first blood meal within approximately 48 hours. They continue to feed on blood throughout their reproductive cycles. After feeding, the mosquito looks for a suitable location to lay eggs. Female *Ae. aegypti* mosquitoes commonly choose clean water sources such as jars, tanks, flower vases, water pipes, flower pots, bottles, coconut shells, and water troughs. They also lay eggs in shaded areas in and around homes. Similarly, female *Ae. albopictus* mosquitoes lay their eggs in water containers, just like Ae. aegypti mosquitoes.

1.3.2. Host preference, resting and blood-feeding behaviors of Aedes Mosquitoes

Female *Ae. aegypti* mosquitoes primarily live near humans, often resting indoors and seeking human blood for nourishment. In contrast, female *Ae. albopictus* mosquitoes have a more semi-wild behavior. They feed on both human and animal blood and typically rest outdoors. After feeding, *Aedes* mosquitoes search for a suitable place to rest and digest. Although they may not have strong flying abilities and tend to fly slowly, they can cover distances of up to 100-400m from their larval habitat. The distance they can fly depends on factors such as wind, humidity, temperature, rainfall, terrain, and vegetation. These flights serve the purpose of finding mates, locating hosts for blood feeding, and identifying suitable sites for egg-laying.

1.3.3. Research situation on the biological and ecological characteristics of Aedes mosquitoes in Vietnam and the study area

Ae. aegypti and *Ae. albopictus* mosquitoes are active year-round in Vietnam, but they thrive especially during the rainy season. Mosquitoes in the North thrive from May to October, in the Central region from August to November, and in the South from May to August. The amount of rainfall directly affects mosquito development, but excessive rain can have a negative impact on their life and reproduction. Since the mosquito development season aligns with the spread of mosquito-borne diseases like dengue fever, it is crucial for the community to adopt habits that prevent the spread of these diseases, primarily by eradicating *Ae. aegypti* mosquitoes when the rainy season begins.

Several research studies have been conducted on the biology and ecology of *Ae. aegypti* and *Ae. albopictus* mosquitoes. For example, in 1987, Vu Duc Huong and Do Thi Hien investigated the biological characteristics of mosquitoes that

transmit dengue hemorrhagic fever. In 1995, Tran Vinh Hien, Tran Khanh Tien, Tran Huu Hoang, et al. explored the ecological characteristics of *Ae. aegypti* in the Mekong Delta. Vu Sinh Nam examined the biological and ecological characteristics of dengue fever vectors and proposed prevention and control measures in certain regions of Northern Vietnam in 1995. In 2008, Do Thi Phuong Bac examined the quantity and some biological characteristics of *Ae. aegypti* mosquitoes in the suburbs of Hanoi. From 2016 to 2017, Tran Cong Hien investigated the ecological characteristics of adult *Aedes* mosquitoes in various locations, including Hanoi, Hai Phong, Thanh Hoa, and Ha Tinh.

1.4. Insecticides and chemical resistance of *Aedes aegypti* and *Aedes albopictus* mosquitoes in the world and Vietnam

1.4.1 The Global Chemical Resistance Situation of Ae. aegypti and Ae. albopictus Mosquito Species

Organochlorine, organic phosphorus, carbamate, and pyrethroid are the main chemical groups used in insecticides. In addition to these, other chemical groups are used to enhance the effectiveness of insecticides, such as neonicotinoids (acetamiprid, clothianidin, imidacloprid, nitenpyram, nithiazine, thiacloprid, and thiamethoxam), growth inhibitors (diflubenzuron, pyriproxyfen), adjuvants, and resistance enzyme inhibitors (piperonyl butoxide). However, mosquito control faces significant challenges due to the increasing resistance of mosquitoes to insecticides. Specifically, the mosquito species *Ae. aegypti* is resistant to most of the chemical groups mentioned above, and it is widely distributed worldwide. This highlights the current status of resistance to the chemical groups commonly used in vector control programs.

1.4.2. Insecticide resistance of Ae. aegypti and Ae. albopictus mosquitoes in Vietnam

In Vietnam, the resistance of mosquitoes to pyrethroid chemicals varies depending on the location. Specifically, in the Central Highlands and the South, some mosquitoes have developed resistance to these chemicals. However, in the Central and Northern regions, *Ae. aegypti* mosquitoes are still susceptible to pyrethroids. In contrast, in the South and Central Highlands, these mosquitoes display higher resistance to insecticides. Additionally, in certain northern provinces, *Ae. aegypti* mosquitoes demonstrate resistance to 0.75% permethrin, increased tolerance to 0.05% deltamethrin, and are only sensitive to 5% malathion. As for *Ae. albopictus* mosquitoes, they are resistant to pyrethroid chemicals.

1.4.3. Status of chemical resistance in Aedes mosquitoes at the research site

A study conducted by Pham Thi Khoa (2015) in Hanoi found that the mosquito species *Ae. aegypti* was resistant to certain pyrethroid chemical groups but sensitive to the organic phosphorus group malathion 5%. On the other hand, *Ae. albopictus* showed resistance to pyrethroids. A more recent study in Hanoi by Tran Cong Hien (2019) revealed that *Ae. aegypti* mosquitoes were resistant to alphacypermethrin, deltamethrin, permethrin, and lambdacyhalothrin at all study sites. They also showed resistance to malathion at 4 out of 5 study sites.

In Thanh Hoa province, Tran Cong Hien's (2019) research showed that *Ae. aegypti* mosquitoes were sensitive to alphacypermethrin at 4 out of 6 research sites but resistant to this chemical in Hai Ha commune, Tinh Gia district. They were also found to be sensitive to alphacypermethrin at 1 out of 7 study sites.

1.5. Mechanisms of insecticide resistance in *Ae. aegypti* and *Ae. albopictus* mosquitoes

Insecticide resistance, as defined by the World Health Organization, refers to the ability of insects to survive exposure to insecticides that would have previously been lethal to them. This resistance can occur through natural selection and mutation. Several biological and ecological factors influence the development of resistance to insecticides, including gene flow between populations, the strength of chemicals used, and the duration and dosage of chemical exposure. The entrance of chemicals into mosquitoes can occur through various routes and can have different effects on their survival. There are primary types of resistance mechanisms determined by how chemicals interact with a mosquito's body and whether they can be broken down directly. These include changes in the target site, metabolic resistance, hypoosmotic resistance, and behavioral resistance.

1.5.1. Resistance caused by variations in target position

This resistance occurs when there is a change in the target site of action of the insecticide. Changes in enzymes and nerve receptors targeted by different insecticide classes have been observed. Three forms of insecticide resistance result from modifications in the sensitive target sites.

1.5.2. Metabolic resistance: This resistance mechanism is highly significant. It occurs when enzymes undergo structural changes, enhancing their capacity to detoxify or increasing the quantity of enzymes to eliminate insecticide toxins more effectively. Metabolic resistance involves a specific group or family of enzymes rather than being caused by a single enzyme alone. Once chemical molecules enter the mosquito's body, they undergo breakdown through different processes such as oxidation, hydrolysis, hydrogenation, dechlorination, and alkylation. Ultimately, this breakdown leads to the formation of non-toxic substances.

1.5.3. Resistance to hypoosmosis: Hypoosmotic resistance in insecticides is a mechanism in which the chemical insecticide is not directly decomposed. Instead, resistance is developed due to reduced permeability.

1.5.4. Resistance due to changes in behavior: To minimize exposure to insecticides, mosquitoes change their feeding or resting behavior. This mechanism is typically divided into two categories: direct contact stimulation and non-contact spatial repellence.

1.5.5. Method for detecting insecticide resistance in mosquitoes: Current methods used to detect chemical resistance in mosquitoes include biological testing methods, biochemical methods, and molecular biological methods.

1.6. The use of mosquito repellent candles for insect prevention

Extensive research and testing have been conducted worldwide on mosquito-repellent candles. Currently, incense and candles are the most commonly used products to deter mosquitoes effectively.

In a 2016 study by Vu Duc Chinh, mosquito repellent candles containing 3% transfluthrin, produced in Malaysia, were highly effective. The study showed that these candles were 71.92% effective in preventing *An. epiroticus* mosquitoes and 76.36% effective in preventing *Cx. vishnui* mosquitoes. Notably, a high % of households, precisely 98%, use candles as a preventive measure [139].

Furthermore, in 2018, the Department of Entomology, National Institue of Malariology Parasitology and Entomology successfully researched and developed NIMPE Mosquito Repellent Candles. These candles have demonstrated remarkably high efficacy in repelling and killing mosquitoes in laboratory tests [140] (Figure 1.3).

1.7. General information about the research area

1.7.1. Hanoi city

Hanoi, the capital of the Socialist Republic of Vietnam, is the country's largest centrally-governed city and the second most populous locality since the merger of Ha Tay province. As of 2019, its population is over 8 million, making it a city with a notably high population density. Unfortunately, this high population density has negatively affected the city's overall epidemic, particularly concerning dengue fever. The influx of people migrating to Hanoi for employment has further exacerbated this issue.

1.7.2. Thanh Hoa province

Thanh Hoa, situated in the North Central region of Vietnam, acts as a transitional urban area connecting the economically booming North region to the North Central region. It holds significant importance as a hub for the entire country, playing a vital role in security and defense. Thanh Hoa is also witnessing rapid development in various sectors and services, emphasizing the growth of high-tech, clean industries. However, it has recently faced a challenging situation due to the dengue fever epidemic.

CHAPTER 2: RESEARCH METHODOLOGIES

2.1. Target 1: Determining the distribution and biological/ecological characteristics of *Aedes aegypti* and *Aedes albopictus* mosquitoes in selected districts of Hanoi City and Thanh Hoa Province in the years 2019-2020

2.1.1. Survey objects: Mosquitoes *Ae. aegypti* and *Ae. albopictus* collected at the study site

2.1.2. Survey time: from 01/2019 to 12/2020. A number of investigations times: 01 times per year, in October 2019 and July 2020.

2.1.3. Survey locations:

- In the field: The survey locations (where Dengue hemorrhagic fever often occurred) from 2016 to 2019.

Table 2.1. Survey locations

Province/City	District	Commune/ward	Notes
Hanoi	Hai Ba Trung	Bach Mai	Inner city
		Vinh Tuy	
	Ha Dong	Phu Luong	Bordering
		Kien Hung	
	Hoai Duc	Son Dong	Suburbs
		La Phu	
Thanh Hóa	Thanh Hoa city	Nam Ngan	Urban area
		Thieu Duong	
	Hoang Hoa	But Son town	Rural area
		Hoang Phu	
	Tinh Gia	Tinh Gia town	Coastal area
		Hai Thanh	

- At the Laboratory: Carried out at the laboratory of the National Institute of Malariology Parasitology and Entomology.

2.1.4. Research design: The research topic was developed through the use of cross-sectional descriptive methodologies and laboratory experiments.

2.1.5. Sample size of study

- This refers to all mosquitoes and larvae that are collected from households.
- In terms of households, the number of households to be investigated for mosquito collection in the study must comply with the regulations set by the Ministry of Health. These regulations are outlined in "Decision No. 3711/QD-BYT dated September 19, 2014, of the Ministry of Health, which provides guidelines for the surveillance, prevention, and control of Dengue hemorrhagic fever".

2.1.6. Research content

This study aims to investigate the presence and distribution of two mosquito species, *Ae. aegypti* and *Ae. albopictus*, in Hanoi and Thanh Hoa. Additionally, it seeks to examine the ecological traits of these mosquitoes in their natural habitats and assess their biological and environmental characteristics under laboratory conditions.

2.1.7. Variables used in the study

Density and distribution of two mosquito species, *Ae. aegypti* and *Ae. albopictus*; the water containers with larvae; the behaviors of these species and their development cycle in the laboratory.

Indicators for target 1 relating to these two mosquito species, *Ae. aegypti* and *Ae. albopictus*. These indicators include the mosquito density index (DI), the percentage of houses with mosquitoes, the percentage of houses with larvae (HI), the index of water containers with larvae (CI), and the Breteau Index (BI).

2.1.8. Techniques used in the study

- Techniques for collecting adult mosquitoes and larvae.
- Techniques for identifying mosquitoes and *Aedes* larvae: Based on the course on identifying mosquitoes and *Aedes* larvae in Vietnam.
- Techniques to determine specific biological characteristics of *Ae. aegypti* and *Ae. albopictus* mosquitoes in the laboratory: This includes determining their

life cycle, reproductive ability, and the timing of their feeding/sucking activity in the laboratory.

2.2. Target 2: ***Determining the sensitivity of Aedes aegypti and Aedes albopictus mosquitoes to insecticides at research sites***

2.2.1. Research object

Mosquitoes belonging to the F1 generation, specifically *Ae. aegypti* and *Ae. albopictus*, were raised from larvae collected from the field until they reached adulthood and could lay eggs. The chemicals used for testing were alphacypermethrin (10 µg/bottle), lambdacyhalothrin (10 µg/bottle), deltamethrin (10 µg/bottle), and permethrin (10 µg/bottle). The testing method followed the US-CDC method (2019).

2.2.2. Research location

At the Laboratory of the Department of Entomology, Department of Molecular Biology, National Institute of Malariology Parasitology and Entomology

2.2.4. Research time

From 10 / 2019 to 12 / 2020.

2.2.5. Research design

The study is focused on describing laboratory experiments.

2.2.6. Research sample size

Each chemical will be tested using a total of 125 female *Ae. aegypti* mosquitoes and 125 female *Ae. albopictus* mosquitoes, all aged between 2-5 days old. The mosquitoes used in this study were raised from larvae collected from the field, and they had not fed on blood prior to the survey. Before testing, the mosquitoes were given a 10% glucose solution for one day. Only healthy female mosquitoes with intact legs, wings, and a normal perching position will be selected for the actual testing. These selected mosquitoes will be allowed to rest and stabilize in the cage for 1 hour before the testing begins. The sample size for detecting resistance genes will include all female *Ae. aegypti* and *Ae. albopictus* mosquitoes that were used in the susceptibility testing, regardless of whether they were alive or dead.

2.2.7. Research content

Test mosquito sensitivity using the bottle test method recommended by the United States Centers for Disease Control (US-CDC) (2019).

Analyze Kdr resistance genotypes using the qPCR technique as described by Alden S. Estep et al. (2018).

2.2.8. Techniques used in research

- Sensitivity testing technique according to the USCDC bottle method, 2019
- Technique for extracting total DNA from adult mosquitoes using the alcohol precipitation method.
- qPCR technique to determine Kdr resistance genotype according to Alden S.Estep (2018).

2.2.9. Evaluation indicators

The sensitivity levels to four tested chemicals of *Ae. aegypti* and *Ae. albopictus* were determined using the US-CDC method. This method is based on the mosquito mortality rate and the frequency of occurrence of Kdr gene mutations.

2.3. Target 3: ***Evaluating the effectiveness of NIMPE mosquito repellent candles against Aedes aegypti and Aedes albopictus in the laboratory and the field in Thanh Hoa province, 2020***

2.3.1. Research object

- NIMPE mosquito repellent candle with active ingredient transfluthrin 2%, soft palm wax form, dosage 70g/cup of candle (Figure 1.3)
- *Aedes* mosquitoes at the laboratory.
- *Aedes* mosquitoes at the field sites.
- Officials and households participating in the test.

2.3.2. Research time and location

- Research time: 4 - 12/2020.
- Research location: At the Laboratory of the Department of Entomology, National Institute of Malariology Parasitology and Entomology; At the field site: Hai Thanh commune, Tinh Gia district, Thanh Hoa province.

2.3.3 Research design

The study is focused on describing laboratory experiments.

2.3.4. Research sample size

- At the laboratory: use 100 individuals/1 experiment for a test chamber sized 70cm x 70 cm x 70cm and 300 individuals/1 experiment for a test chamber sized 180cm x 180 cm x 180 cm.
- In the field, 200 households agreed to participate in the study, and 200 household heads were interviewed (2 cups of NIMPE mosquito repellent candles were provided to each household).
- All mosquitoes *Ae. aegypti* and *Ae. albopictus* obtained at the test and control sites were counted and compared.

2.3.5. Research content

- Density of *Aedes* mosquitoes at control and test sites before, during, and after the test; Effectiveness of candles to repel mosquitoes.
- Evaluate the unwanted effects of NIMPE mosquito repellent candles on test participants.

2.3.6. Variables and measurement of variables

- Percentage of live and dead *Aedes* mosquitoes after 24 hours (criteria for assessing life and death according to US-CDC, 2019); Questionnaire and research data collection form: Appendix 1, 2, 3; Symptoms of side effects experienced by participants; KT50, KT95; Determining the effectiveness of NIMPE(P) mosquito repellent candles.
- Symptoms experienced by participants in the repel candle test.

2.3.7. Evaluation indicators

Effectiveness of repellent candles against *Aedes* mosquitoes (P %)

$$P\ (\%) = \frac{C - T}{C} \times 100$$

Where T is the number of female *Aedes* mosquitoes caught at the test site; C is the number of female *Aedes* mosquitoes caught at the control site; P is the percentage of unwanted effects occurring in people participating in the test.

2.3.8. Techniques used in the research

- Aedes mosquito investigation techniques: According to the Ministry of Health's procedures [145].

- Aedes mosquito identification technique: According to the SOP of the National Institue of Malariology Parasitology and Entomology

- Technique for evaluating the killing effectiveness of NIMPE candles in the laboratory: Use a test chamber measuring 70 cm x 70 cm x 70 cm alongside a Peet Grady chamber to conduct tests involving *Ae. aegypti* and *Ae. albopictus*. In the Peet Grady test chamber, monitor the intervals of candle burning time at 1h, 2h, 4h, 6h, 8h, 12h, and 24h. Proceed with the testing in five steps.

- Techniques to evaluate the effectiveness of repelling candles in the field

2.4. Data analysis and processing methods

- Collected data is cleaned and then imported using Excel software.
- Process data using SPSS 26.0 software.

2.5. Methods to control numerical noise and errors in research

- Comply with the steps according to the research procedures and regulations.
- Train the research team to conduct investigations and interviews.

2.6. Ethics in research

The research content has been approved by the Ethics Committee in Biomedical Research of the National Institute of Malariology Parasitology and Entomology, according to Decision No. 225/QD-VSR dated March 11, 2019.

CHAPTER 3: RESEARCH RESULTS

3.1. Distribution and the biological and ecological characteristics of *Aedes aegypti* and *Aedes albopictus* mosquitoes in selected districts of Hanoi and Thanh Hoa province from 2019 to 2020

3.1.1. Distribution of Aedes aegypti and Aedes albopictus mosquitoes in selected districts of Hanoi, 2019 – 2020

Table 3.1. Indices of Ae. aegypti and Ae. albopictus mosquitoes at inspected sites in Hanoi, 2019 – 2020

District	Commune/ Ward	Mosquito density (individuals/house)		Positive houses (%)	
Phase1 (10/2019)					
		Ae.aegypti	*Ae. albopictus*	*Ae.aegypti*	*Ae.albopictus*

Hoai Duc	Son Dong	0.18	0.66	8.0	42.0
	La Phu	0.22	0.60	14.0	44.0
Hai Ba Trung	Bach Mai	0.56	0.24	22.0	14.0
	Vinh Tuy	0.48	0.30	18.0	20.0
Ha Dong	Kien Hung	0.24	0.36	14.0	26.0
	Phu Luong	0.32	0.48	16.0	38.0
Phase 2 (7/2020)					
Hoai Duc	Son Dong	0.14	0.50	6.0	34.0
	La Phu	0.16	0.56	8.0	44.0
Hai Ba Trung	Bach Mai	0.42	0.22	12.0	10.0
	Vinh Tuy	0.28	0.28	18.0	18.0
Ha Dong	Kien Hung	0.22	0.40	14.0	32.0
	Phu Luong	0.20	0.36	10.0	26.0
Average ± SD		0.29±0.13	0.41±0.15	13.3± 4.77	29±11.83

The mean density index of *Ae. aegypti* mosquitoes in Hanoi is 0.29 ± 0.13 individuals per house, with a mean of cheerful houses of 13.3 ± 4.77%. On the other hand, the mean density index of *Ae. albopictus* mosquitoes in Hanoi are 0.41 ± 0.15 individuals per house, with a mean of cheerful houses of 29 ± 11.83%

Table 3.2. Indices of Ae. aegypti and Ae. albopictus larvae at inspected sites in Hanoi, 2019 – 2020

District	Commune/Ward	BI (Breteau Index)		HI (House Index) (%)		CI (% Container Index)	
		Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae albopictus*
Phase 1 (10/2019)							
Hoai Duc	SonDong	38	48	18.0	36.0	10.7	13.5
	La Phu	28	68	14.0	50.0	7.0	16.9
Hai Ba Trung	BachMai	32	16	16.0	10.0	17.4	8.7
	Vinh Tuy	46	24	24.0	12.0	20.9	10.9
Ha Dong	KienHung	42	40	30.0	28.0	17.2	16.4
	PhuLuong	50	50	28.0	38.0	18.8	18.8
Phase 2 (7/2020)							
Hoai Duc	SonDong	30	56	18.0	46.0	12.1	22.6
	La Phu	40	52	28.0	24.0	14.5	18.8
Hai Ba Trung	BachMai	44	18	32.0	10.0	16.4	6.70
	Vinh Tuy	52	26	38.0	14.0	15.8	7.90
Ha Dong	KienHung	34	36	24.0	24.0	17.5	18.6
	PhuLuong	40	46	32.0	34.0	13.9	16.0
Average ±SD		39.7±7.67	40.0±16.2	25.27±7.46	27.2±13.9	15.2±3.83	14.6±5.0

In Hanoi, the Breteau Index (BI) of *Ae. aegypti* larvae had a mean value of 39.7±7.67. The mean percentage of houses positive for larvae (HI) was 25.27±7.46, and the mean percentage of containers positive for larvae (CI) was

15.2±3.83. In contrast, the mean BI of *Ae. albopictus* larvae in Hanoi was 40.0±16.2. The mean HI was 27.2, and the mean CI was 14.6±5.0.

3.1.2. Distribution of Aedes aegypti and Aedes albopictus mosquitoes in selected districts of Thanh Hoa, 2019 - 2020

Table 3.3. Indices of Ae. aegypti and Ae. albopictus mosquitoes at inspected sites in Thanh Hoa, 2019 – 2020

District	Commune/Ward	Mosquito density (individuals/house)		Positive houses (%)	
Phase 1 (10/2019)					
		Ae. aegypti	*Ae. albopictus*	*Ae.aegypti*	*Ae.albopictus*
Thanh Hoa city	Nam Ngan	0	0.28	0	18.0
	Thieu Duong	0.20	0.32	14.0	24.0
Tinh Gia	Tinh Gia town	0.52	0.30	44.0	20.0
	Hai Thanh	0.70	0.34	50.0	16.0
Hoang Hoa	But Son town	0	0.46	0	30.0
	Hoang Phu	0	0.38	0	28.0
Phase 2 (7/2020)					
Thanh Hoa city	Nam Ngan	0	0.40	0	32.0
	Thieu Duong	0.16	0.48	12.0	36.0
Tinh Gia	Tinh Gia town	0.40	0,26	22.0	12.0
	Hai Thanh	0.64	0.42	48.0	22.0
Hoang Hoa	But Son town	0	0.34	0	26.0
	Hoang Phu	0	0.36	0	240
Average ± SD		0.29±0.28	0.36±0.07	21.1±21.07	24.0±6.93

The mean density index of *Ae. aegypti* mosquitoes in Thanh Hoa province is 0.29 ± 0.28 individuals per house. The mean index of houses favorable for mosquitoes is 21.1 ± 0.28. The mean density index of *Ae. albopictus* mosquitoes in Thanh Hoa is 0.36 ± 0.07 individuals per house. The mean index of houses favorable for mosquitoes is 24.0 ± 6.93%.

Table 3.4. Indices of Ae. aegypti and Ae. albopictus larvae at inspected sites in Thanh Hoa, 2019 – 2020

District	Commune /Ward	BI (Breteau Index)		HI (House Index)		CI (Container Index)	
		Ae.aegypti	*Ae.albopictus*	*Ae.aegypti*	*Ae.albopictus*	*Ae.aegypti*	*Ae.albopictus*
Phase 1 (10/2019)							
Thanh Hoa city	NamNgan	0	46	0	28.0	0	22.3
	ThieuDuong	24	52	18.0	36.0	10.8	23.4
TinhGia	Tinh Gia	52	24	34.0	18.0	13.1	6.1
	HaiThanh	64	32	48.0	20.0	13.7	6.8
HoangHoa	But Son	0	28	0	16.0	0	11.3
	HoangPhu	0	20	0	10.0	0	6.1
Phase 2 (7/2020)							

Thanh Hoa city	Nam Ngan	0	36	0	26.0	0	18.4
	ThieuDuong	16	42	12.0	32.0	6,10	16.0
Tinh Gia	Tinh Gia	38	30	24.0	22.0	15.4	12.2
	Hai Thanh	50	38	32.0	30.0	12.4	9.4
HoangHoa	But Son	0	34	0	20.0	0	15.2
	Hoang Phu	0	28	0	24.0	0	11.5
Trung bình ± SD		27.1±24.9	34.2±6.24	18.7±17.3	23.5±7.3	7.9±6.5	13.2±5.96

In Thanh Hoa, the mean Breteau Index (BI) of *Ae. aegypti* larvae was 27.1±24.9, with the mean house larval index (HI) at 18.7±17.3 and the mean container larval index (CI) at 7.9. On the other hand, the mean BI of *Ae. albopictus* larvae was 34.2±6.24, with the mean house larval index (HI) recorded at 23.5±7.3 and the mean container larval index (CI) at 13.2±5.96.

3.1.3. Source larvae nests of Aedes mosquitoes in Hanoi and Thanh Hoa in 2019 and 2020

In 10 water containers with *Aedes* larvae concentration rates ranging from 15% to 50%, we identified 26 source larvae nests. Source larvae nests in Hanoi and Thanh Hoa areas all contained flower pots and discarded tools.

3.1.4. Biological and ecological characteristics of Aedes aegypti and Aedes albopictus mosquitoes in Hanoi and Thanh Hoa

Table 3.7. Number and landing rate of Ae. aegypti and Ae. albopictus mosquitoes indoors and outdoors were measured at the inspected sites.

Species	Location	Total mosquito count	Indoors		Outdoors	
			Amount	Rate (%)	Amount	Rate (%)
Ae. aegypti	Hanoi	171	164	95.9	7	4.1
	ThanhHoa	131	121	92.4	10	7.6
Total/Overall ratio (%)		302	285	94.4	17	5.6
Ae.albopictus	Hanoi	248	8	3.2	240	96.8
	ThanhHoa	217	11	5.1	206	94.9
Total		465	19	4,15	446	95.9

Ae. aegypti mosquitoes are primarily active and rest indoors, accounting for 94.4% of the population. On the other hand, *Ae. albopictus* mosquitoes are predominantly active and rest outdoors, accounting for 95.9%.

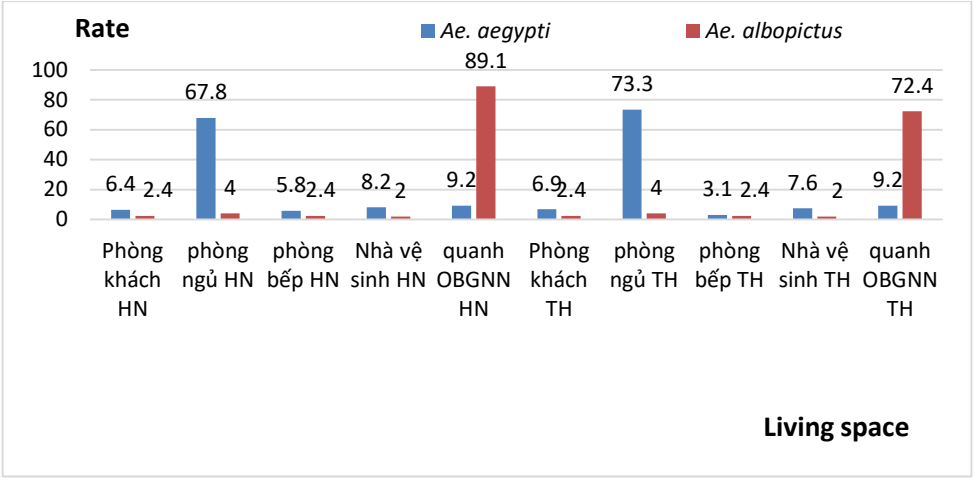

Figure 3.13. Landing rates of Aedes aegypti and Aedes albopictus mosquitoes

In Hanoi, *Ae. aegypti* mosquitoes accounted for 67.8% of those found resting in bedrooms, while in Thanh Hoa, this number was 73.3%. As for *Ae. albopictus* mosquitoes, they were found resting around larval nests in Hanoi at a rate of 89.1%, and in Thanh Hoa at a rate of 72.4%.

- The resting rate of Ae. aegypti and Ae. albopictus mosquitoes on substrates in Hanoi and Thanh Hoa in 2019 - 2020

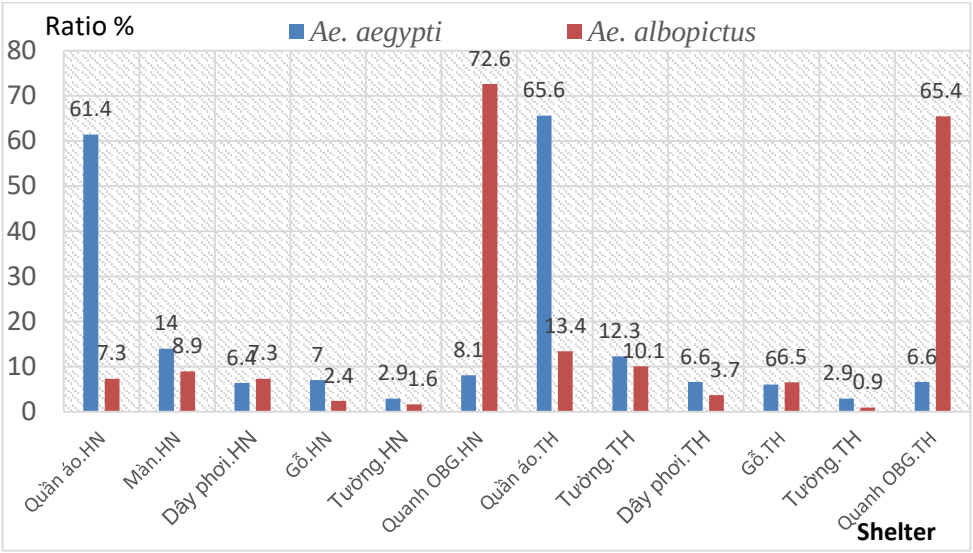

Figure 3.14. The resting rate of Ae. aegypti and Ae. albopictus mosquitoes on substrates in Hanoi and Thanh Hoa in 2019 - 2020.

The rate of *Ae. aegypti* mosquito landing on clothes was found to be up to 61.4% in Hanoi and 65.64% in Thanh Hoa.

3.1.5.4. Feeding activity of Ae. aegypti and Ae. albopictus mosquitoes in the laboratory

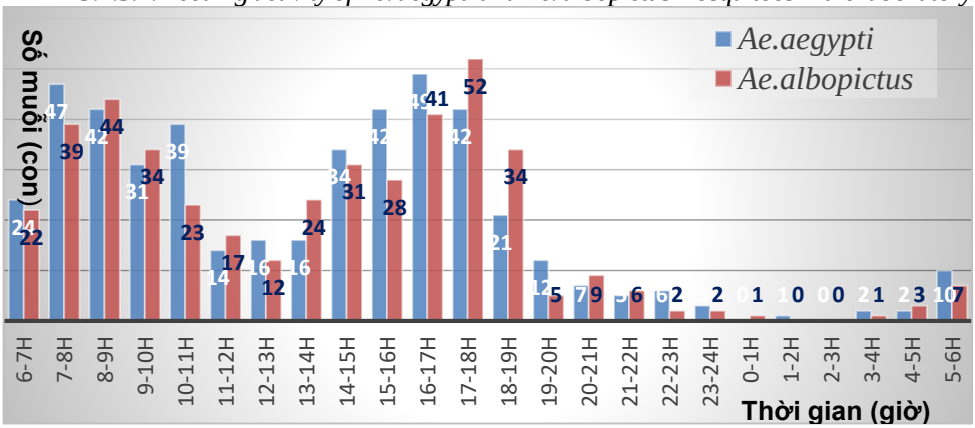

Figure 3.18. Feeding activity of Ae. aegypti and Ae. albopictus mosquitoes in the laboratory for 24h.

Aedes aegypti mosquitoes are active both day and night, from 6 a.m. to 6 a.m. the following day. The most significant number of these mosquitoes is captured in the morning (7-8 a.m.) and afternoon (4-5 p.m.).

3.2. Results of determining the sensitivity of *Aedes aegypti* and *Aedes albopictus* mosquitoes to insecticides at the inspected sites

3.2.1. Results of sensitivity testing conducted on field-collected Aedes aegypti and Aedes albopictus mosquitoes towards various insecticides.

- For laboratory strains

Table 3.10. Test results for the sensitivity assessment of laboratory strains of Ae. aegypti and Ae. albopictus mosquitoes.

Type of chemical	Mosquito species	Lot	Number of tested mosquitoes	The number of mosquitoes that die after 24 hours	Death rate after 24 hours (%)
Alphacypermethrin	*Ae. aegypti*	Experimental	100	99	99
		Control	50	0	0
	Ae.albopictus	Experimental	100	100	100
		Control	50	0	0
Lambdachalothrin	*Ae. aegypti*	Experimental	100	100	100
		Control	50	0	0
	Ae.albopictus	Experimental	100	98	98
		Control	50	0	0
Deltamethrin	*Ae. aegypti*	Experimental	100	100	100
		Control	50	0	0
	Ae.albopictus	Experimental	100	100	100
		Control	50	0	0

Type of chemical	Mosquito species	Lot	Number of tested mosquitoes	The number of mosquitoes that die after 24 hours	Death rate after 24 hours (%)
Permethrin	*Ae. aegypti*	Experimental	100	100	99
		Control	50	0	0
	Ae.albopictus	Experimental	100	9	99
		Control	50	0	0

Both species of *Aedes* mosquitoes tested showed sensitivity to four pyrethroid chemicals, resulting in mortality rates ranging from 98% to 100%.

- *For field-collected strains*

Table 3.11. Percentage of mosquito mortality following sensitivity testing of Ae.aegypti mosquitoes with various insecticides in Hanoi and Thanh Hoa.

Commune/Ward	District	Percentage of dead mosquitoes after sensitivity test			
		Del	Per	Alph	Lamd
Hanoi					
Hoai Duc	Son Dong	83	91	94	92
	La Phu	92	85	91	87
Hai BaTrung	Bach Mai	24	10	93	69
	Vinh Tuy	24	35	76	76
Ha Đong	Kien Hung	69	47	92	49
	Phu Luong	71	82	78	65
Thanh Hoa					
Thanh Hoa	ThieuDương	84	77	99	93
Tinh Gia	Tinh Gia	78	80	85	94
	Hai Thanh	58	12	16	50

Aedes aegypti mosquitoes in Hanoi were found to be resistant to all tested chemicals. Similarly, in Thanh Hoa, these mosquitoes were also resistant to the tested chemicals, with the exception of alphacypermethrin in Thieu Duong, where they showed sensitivity.

3.2.2. Results of determining Kdr resistance genotypes of Aedes mosquitoes collected at research sites treated with insecticides.

The Kdr resistance genotype of *Aedes* mosquitoes was determined on the Vgsc gene at two point mutation positions: 1016 and 1534. At position 1016, the homozygous susceptible genotype (valine) has a melting peak at 86.47±0.3^{0}C. The mutant genotype, which replaces valine with isoleucine, has a melting peak at 77.0±0.3^{0}C. The heterozygous genotype exhibits both of the melting peaks mentioned above.

At position 1534, the homozygous genotype for phenylamine exhibits a melting peak at 79.8±0.3^{0}C, while the mutant genotype for cysteine produces a

melting peak at 84.65±0.2^{0}C. The heterozygous genotype displays both of these melting peaks. After conducting tests, it was observed that all 21 mosquito populations of *Ae. aegypti* and *Ae. albopictus* in Hanoi and Thanh Hoa possess the homozygous genotype 1016VV/1534CC.

3.3. Results of testing the effectiveness of NIMPE mosquito repellent candles against *Aedes* mosquitoes

Table 3. 15. Test results were conducted to evaluate the effectiveness of NIMPE repellent candles against Aedes mosquitoes in a test chamber measuring 70cm x 70cm x 70cm

Number order	Duration	Number of mosquitoes falling over time/ 60 tested mosquitoes		
		Ae. aegypti	*Ae.albopictus*	Reference
		Test	Test	
1	30'	1	0	0
2	1'	3	4	0
3	1'30"	3	4	0
4	2'	4	12	0
5	2'30"	4	15	0
6	3'	4	22	0
7	3'30"	7	33	0
8	4'	7	41	0
9	4'30"	12	54	0
10	5'	17	57	0
11	5'30"	32	60	0
12	6'	44	60	0
13	6'30"	53	60	0
14	7'	58	60	0
15	7'30"	60	60	0
16	8'	60	60	0
17	9'	60	60	0
18	10'	60	60	0
19	15'	60	60	0
20	20'	60	60	0
Number of dead mosquitoes after 24 hours		60	60	0
Percentage (%) of mosquitoes dead after 24 hours		100	100	0
KT_{50}(minute) (95%CI)		5.17 (4.98-5.34)	3.18 (2.9-3.36)	0
KT_{95}(minute) (95%CI)		7.47 (7.13-7.93)	5.28 (4.9-6.7)	0

The mortality rate of *Ae. aegypti* and *Ae. albopictus* after 24 hours was 100%. The KT_{50} and KT_{95} in *Ae. aegypti* were 5.17 minutes and 7.47 minutes, respectively.

Table 3.2. Results from testing the effectiveness of NIMPE mosquito repellent candles against Aedes mosquitoes in the Peet Grady test chamber

Number order	Duration	Number of mosquitoes falling over time/ 60 tested mosquitoes		
		Ae. aegypti	*Ae. albopictus*	Control
		Test	Test	
1	1	3	15	0
2	2	15	37	0
3	3	39	113	0
4	4	67	169	0
5	5	81	185	0
6	6	168	254	0
7	7	251	287	0
8	8	269	289	0
9	9	283	292	0
10	10	289	297	0
11	11	291	300	0
12	12	298	300	0
13	13	300	300	0
14	14	300	300	0
15	15	300	300	0
16	20	300	300	0
17	30	300	300	0
18	40	300	300	0
19	50	300	300	0
20	60	300	300	0
Number of dead mosquitoes after 24 hours		300	300	0
Percentage (%) of mosquitoes dead after 24 hours		100	100	0
KT50(minute) (95%CI)		5.13 (4.96-5.29)	3.43 (3.26-3.6)	0
KT95(minute) (95%CI)		9.97 (9.15-9.81)	8.24 (7.78-8.78)	0

The mortality rate of *Ae. aegypti* and *Ae. albopictus* mosquitoes after 24 hours was 100%. The KT_{50} and KT_{95} times for *Ae. aegypti* were 5.13 minutes and 9.97 minutes, respectively, while for *Ae. albopictus*, they were 3.43 minutes and 8.24 minutes, respectively.

Table 3.3. The efficacy of NIMPE Mosquito Repellent Candle in repelling Aedes mosquitoes over time was tested in the Peet Grady test chamber

Candle burning time (hour)	Number of tested mosquitoes	KT50 (minute) (95% CI)	KT95 (minute) (95% CI)	Number of dead mosquitoes after 24 hours	Percentage of mosquitoes dead after 24 hours (%)
***Ae. aegypti* pecies**					
1	300	5.13 (4.96-5.29)	9.97 (9.15-9.81)	300	100
2	300	5.20 (4.43-5.92)	10.26 (8.76-13.02)	300	100
4	300	5.31 (4.58-6.0)	10.06 (8.64-12.68)	300	100
6	300	4.93 (4.3-5.53)	10.03 (8.70-12,24)	300	100
8	300	5.08 (4.57-5.57)	10.60 (9.45-12.28)	300	100
10	300	4.87 (4.29-5.42)	9.79 (8.56–1.78)	299	99.67
12	300	5.42 (4.81-5.99)	10.49 (9.24–2.1)	296	98.67
24	300	5.13 (4.43-5.79)	10.51(9.06-12.99)	300	100
Ae. albopictus pecies					
1	300	3.43 (3.26-3.61)	8.24 (7.78- 8.78)	300	100
2	300	3.36 (3.19-3.53)	8.19 (7.73- 8.74)	300	100
4	300	3.61 (3.45-3.77)	8.73 (8.29- 9.23)	300	100
6	300	3.54 (3.39-3.69)	8.68 (8.28-9.15)	299	99.67
8	300	3.38 (3.24-3.52)	8.12 (7.73-8.56)	300	100
10	300	3.35 (3.19-3.51)	8.39 (7.96-8.89)	298	99.33
12	300	3.30 (3.18-3.42)	8.13 (7.80-8.50)	296	98.67
24	300	3.25 (3.13-3,36)	7.74 (7.43-8.09)	300	100

For *Ae. aegypti* and *Ae. albopictus*, candles with 2% transfluthrin were highly effective in killing mosquitoes at all 8 test times. After 24 hours, the mosquito mortality rate ranged from 98% to 100%.

3.3.4. Efficacy of NIMPE Mosquito Repellent Candle against Aedes mosquitoes in the fields

Table 3.4. Efficacy of NIMPE Mosquito Repellent Candle

Lot	Time 1		Time 2		Average±SD	Efficiency (%)
	Ae.aegypti	*Ae.albopictus*	*Ae.aegypti*	*Ae.albopictus*		
Control	166	69	172	78	121.25±55.31	89.48
Test	16	10	14	11	12.75 ± 2.75	
($p < 0.05$)						

The NIMPE mosquito repellent candle has been proven to be 89.48% effective in repelling *Aedes* mosquitoes and reducing mosquito bites. There was a statistically significant difference ($p < 0.05$) in the number of mosquitoes collected from the comparison (control) and experimental groups in both categories. Specifically, the experimental group demonstrated an impressive effectiveness of 89.48% in preventing *Aedes* mosquitoes from biting humans.

3.3.5. Safety and community acceptance of mosquito repellent candles

Table 3.20. Undesirable effects of test participants

Interview content	Interview number	The answer		Percentage who answered yes (%)
		Yes	Not	
Does the test sample have any odor? Are you feeling uncomfortable?	6	0		0
Do you experience the following symptoms when undergoing testing? Specifically:				
Sneeze	6	0	6	0
Itchy	6	0	6	0
Cough	6	0	6	0
Runny nose	6	0	6	0
Dizzy	6	1	5	17
Nausea	6	0	6	0
Eye itching	6	0	6	0
Stuffy nose	6	0	6	0
Headache	6	0		0

Unwanted symptoms were reported, including cough, runny nose, nausea, itchy eyes/nose, stuffy nose, headache, and one case of dizziness (1/6, accounting for 17%).

Table 3.22. Undesirable effects of field-tested candles

Interview content	Interview number	The answer		Percentage who answered yes (%)
		Yes	Not	
Does the test sample have any odor	200	0	200	0
Do you experience the following symptoms when undergoing testing? Specifically:				
Sneeze	200	2	198	1.0
Itchy	200	0	200	0
Cough	200	0	200	0
Runny nose	200	0	200	0
Dizzy	200	0	200	0
Nausea	200	0	200	0
Eye itching	200	0	200	0
Stuffy nose	200	0	200	0
Headache	200	0	200	0
Other side effects	200	0	200	0
Does the act of lighting candles have an impact on your everyday activities?	200	0	200	0
Do you want to use the candles if they are available?	200	200	0	200

The test subjects did not experience any adverse effects from the results.

CHAPTER 4: DISCUSSION

4.1.1. The distribution of Aedes aegypti and Aedes albopictus mosquitoes

The results of this study, conducted in October 2019 and July 2020, revealed

the presence of both *Ae. aegypti* and *Ae. albopictus* mosquitoes in Hanoi and Thanh Hoa. However, their distribution varied across the research sites. *Ae. aegypti* was found at 9 out of 12 research sites, whereas *Ae. albopictus* was collected at all 12 sites. In our study on distribution in Thanh Hoa, we found *Ae. aegypti* was present at all research sites except for the Hoang Hoa district. This finding contradicts the findings of Tran Cong Hien (2018), who reported no collection of *Ae. aegypti* in Thanh Hoa province.

4.1.2. The biological and ecological characteristics of Aedes aegypti and Aedes albopictus mosquitoes

Landing behavior

The indoor resting rate of *Ae. aegypti* mosquitoes in Hanoi is higher than in Thanh Hoa (95.9% compared to 92.4%). The outdoor resting rate of *Ae. albopictus* mosquitoes in both Hanoi and Thanh Hoa is the same (Table 3.9). Our results consistently show that *Ae. aegypti* mosquitoes primarily rest indoors, which is in line with domestic and foreign studies conducted by Pham Van Minh (2012) and Tran Thanh Duong (2019). These studies also support the view that *Ae. aegypti* mosquitoes prefer to rest indoors and breed in man-made water containers near human dwellings, such as flower vases, water tanks, jars, and flower pots. As a result, they are commonly found in urban and inner-city areas. On the other hand, *Ae. albopictus* mosquitoes rest outdoors and breed in man-made water containers such as tree holes, waste, and stagnant leaf crevices. Therefore, they are more prevalent in gardens in rural areas and among ornamental plants in cities. The resting habits of both *Aedes* mosquito species align entirely with the studies conducted by Tran Thanh Duong and Tran Cong Hien.

Biting and sucking blood behavior

Our laboratory study on the biting and blood-sucking activity of *Aedes* mosquitoes revealed that *Ae. aegypti* mosquitoes were active throughout the day, from 0:00 to 24:00. However, higher numbers of *Ae. aegypti* were caught in the morning (7:00 to 11:00) and afternoon (15:00 to 19:00) compared to noon and night. The peak activity occurred from 7:00 to 8:00 in the morning and 4:00 to 5:00 p.m., while the lowest numbers were observed from 21:00 to 5:00. Therefore, it is evident that *Ae. aegypti* mosquitoes display two distinct periods of intense blood-sucking activity: early morning and late afternoon (Figure 3.19).

4.2.1. Sensitivity to insecticides of *Aedes aegypti* and *Aedes albopictus* mosquitoes at study sites

At the time of the study, it was found that *Ae. aegypti* mosquitoes in the Hai Ba Trung district, including Bach Mai ward and Vinh Tuy ward, had developed resistance to pyrethroid chemicals such as deltamethrin, permethrin, alphacypermethrin, and lambdacyhalothrin. Specifically, *Ae. aegypti* mosquitoes in this area were entirely resistant for deltamethrin. In contrast, *Ae. albopictus* mosquitoes were mostly susceptible to pyrethroid chemicals. Over the past three decades, synthetic pyrethroid chemicals like alphacypermethrin, deltamethrin,

lambdacyhalothin, and permethrin have been widely used in Vietnam for mosquito control and prevention of diseases such as malaria and dengue fever. These pyrethroids have been proven effective in controlling mosquito populations.

4.2.2. Genotypes at positions 1016 and 1534 on the Vgsc gene were analyzed in Aedes mosquito populations from Hanoi and Thanh Hoa in 2019-2020

In our study, we examined 21 mosquito populations, which included 12 populations of *Ae. albopictus* and 9 populations of *Ae. aegypti*. After conducting tests to determine insecticide sensitivity, we discovered that all 21 populations, whether susceptible or resistant to insecticides, possessed the homozygous valine-sensitive genotype, etc… and the mutant genotype (CC) with a substitution of phenylalanine to cysteine. Our findings differ from those of Kawada H (2009), who investigated point mutations in the voltage-gated sodium channel in pyrethroid-resistant *Ae. aegypti* populations in Vietnam. In Kawada H's study, the F1534C mutation was identified as the primary mutation responsible for high resistance in samples collected from the South. In contrast, the V1016G mutation, which occurred in a heterozygous state, had a shallow frequency. Although we detected low frequencies of the F1534C mutation in the North and a low homozygous rate (7.4%), the unrestricted use of persistent pyrethroids in the environment may have exerted selective pressure on this mutation, leading to a higher occurrence in the offspring [109]. In contrast, our study observed a 100% homozygous rate for the 1534C point mutation.

4.3. Effectiveness of repelling *Aedes* mosquitoes of NIMPE Mosquito Repellent Candle

4.3.1. In the laboratory, testing candles containing 2% transfluthrin resulted in a 100% mortality rate for *Ae. aegypti* and *Ae. albopictus* mosquitoes after 24 hours. Specifically, in a 70cm x 70cm x 70cm test chamber, the KT50 and KT95 times for *Ae. aegypti* were 5.17 minutes and 7.47 minutes, respectively. For *Ae. albopictus*, the times were 3.18 minutes and 5.28 minutes. In a Peet Grady test chamber, the KT50 and KT95 times for *Ae. aegypti* were 5.13 minutes and 9.97 minutes, respectively, while for *Ae. albopictus* they were 3.43 minutes and 8.24 minutes.

Our laboratory study on the effectiveness of NIMPE repellent candles against mosquitoes yielded results similar to those of the survey conducted by Dao Minh Trang and Associates (2018), both demonstrating a maximum efficacy of 100%. However, our KT50 and KT95 times were shorter. This difference may be attributed to the use of different mosquito species in our study compared to the survey conducted by Dao Minh Trang, who tested three types of *Anopheles* mosquito.

4.3.2. In Thanh Hoa, our 2020 study examined the effectiveness of NIMPE mosquito repellent candles against *Aedes* mosquitoes. The results showed that the candles had a success rate of 89.48%. The difference in the number of mosquitoes collected from the control and experimental groups was statistically significant ($p<0.05$) in both lots. Our findings exceeded those of Dao Minh Trang's study on mosquito repellent effectiveness, which reported a success rate of 85.15%. This

difference could be attributed to variations in the subjects and research locations: our study focused on *Aedes* mosquitoes, whereas Dao Minh Trang's study targeted *Anopheles* mosquitoes.

4.3.3. Undesirable effects and community acceptance of NIMPE mosquito repellent candles

During the field study, we interviewed 200 individuals from participating households. The results showed that most respondents did not experience any negative effects. However, two cases reported sneezing, which was only 2% of the total. It is essential to mention that all interviewees expressed a desire to use candles if they were provided, as they found that the candles did not disrupt their daily activities. The NIMPE mosquito repellent candles are user-friendly, as a single candle can effectively keep mosquitoes away for an entire family.

The acceptance of NIMPE mosquito-repellent candles in the community varies from studies conducted in other parts of the world. For example, Laksham et al. (2016) conducted a study in South India, while Hogarh et al. (2016) conducted a study in Ghana. These studies found that 52.6% of households using mosquito-repellent candles reported someone with a cough, compared to 46.1% of households not using these candles. However, in our study of 200 households where NIMPE mosquito repellent candles were distributed and used, only 1% of individuals reported sneezing as an unwanted effect of the candles. These findings indicate that NIMPE mosquito-repellent candles are safe for users.

CONCLUSION

1. In 2019-2020, we investigated the distribution, biological, and ecological characteristics of *Aedes aegypti* and *Aedes albopictus* mosquitoes in different districts of Hanoi city and Thanh Hoa province

Ae. aegypti mosquitoes were found in most research sites in Hanoi and Thanh Hoa, with the exception of the research site in Hoang Hoa District, Thanh Hoa Province, where this species was not detected. *Ae. albopictus* mosquitoes were present in all research sites in Hanoi and Thanh Hoa. *Ae. aegypti* mosquitoes were predominantly found indoors (94.4%), on clothes (65.6%), and in bedrooms (70.2%). On the other hand, *Ae. albopictus* mosquitoes preferred resting outdoors (95.9%), around larval nests (81.3%), and at a height of 1 - 2 meters (54.2%).

In the morning, from 7:00 to 8:00, and in the afternoon, from 17:00 to 18:00, *Ae. aegypti* is the most active. Similarly, *Ae. albopictus* is most active in the morning, from 8:00 to 9:00, and in the afternoon, from 18:00 to 19:00. On average, it takes 9.58 ± 0.15 days for an *Ae. aegypti* cycle to complete. *Ae. aegypti* lays an average of 3.9± 0.14 eggs, with each individual laying an average of 139.22± 4.62 eggs. The time it takes for an adult *Ae. albopictus* cycle to complete is 15.23±0.15 days. *Ae. albopictus* lays an average of 4.34±0.3 eggs, with each individual laying an average of 154.18±20.78 eggs. The average lifespan of adult female *Ae. albopictus* mosquitoes is 31.87 days.

2. The Sensitivity of *Aedes aegypti* and *Aedes albopictus* mosquitoes to insecticides at the study sites

In most locations in Hanoi and Thanh Hoa, *Ae. aegypti* mosquitoes were found to be resistant to various pyrethroid insecticides, including deltamethrin, permethrin, alphacypermethrin, and lambdacyhalothrin. However, *Ae. albopictus* mosquitoes in these exact research locations remained sensitive to pyrethroid chemicals. Both *Ae. aegypti* and *Ae. albopictus* mosquito populations had a homozygous genotype of 1016VV/1534CC. However, no association was discovered between this genotype and insecticide resistance in either mosquito population in the research locations.

3. Efficacy of NIMPE mosquito repellent candles against *Aedes aegypti* and *Aedes albopictus* in laboratory and field settings in Thanh Hoa province in 2020

The NIMPE candles, which contain 2% transfluthrin, exhibited a 100.0% efficacy in repelling laboratory strains of *Ae. aegypti* and *Ae. albopictus* within 24 hours. In real-world conditions, the NIMPE candles demonstrated an efficacy of 89.48% against *Aedes* mosquitoes. These candles were proven safe for both laboratory test subjects and community users. Additionally, all households within the community displayed a 100% acceptance rate of using NIMPE candles.

LIST OF THE SCIENTIFIC PUBLICATIONS OF THE AUTHOR THAT ARE RELATED TO THE THESIS

1. Tran Thi Thuong, Ho Dinh Trung, Nguyen Van Tuan, Dao Minh Trang, Nguyen Van Dung (2023), Some biological characteristics of *the Aedes aegypti* mosquito at the laboratory. *Journal of Community Medicine*, Volume 64, Special Issue, pp.109-114.

2. Tran Thi Thuong, Ho Dinh Trung, Nguyen Quang Thieu, Nguyen Thi Hong Ngoc, Nguyen Van Dung (2024), Evaluation of the effectiveness of *Aedes aegypti* and *Aedes albopictus* mosquito repellent candles in a narrow field in Thanh Hoa, 2020. *Hue Journal of Medicine and Pharmacy* - University of Medicine and Pharmacy, Hue University - Special Issue, No.25, pp.168 -173.

3. Tran Thi Thuong, Ho Dinh Trung, Nguyen Quang Thieu, Nguyen Thi Hong Ngoc, Nguyen Van Dung (2024). Evaluation of the effectiveness of *Aedes aegypti* and *Aedes albopictus* mosquito repellent candles in a narrow field in Thanh Hoa, 2020. *Hue Journal of Medicine and Pharmacy* - University of Medicine and Pharmacy, Hue University - Special Issue, April 2024, No.26, pp.174-179.