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CHARACTERISTICS OF DENGUE FEVER, *AEDES* MOSQUITOES IN SOME DISTRICTS OF HANOI, AND THE REPELLENT EFFICACY OF *LEMONGRASS* AND *MELALEUCA* ESSENTIAL OILS

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SUMMARY OF DOCTORAL THESIS

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LIST OF PUBLISHED ARTICLES

No	Article name	Year of publication	Author
1	Nguyen Thi Van, Le Tran Anh, Nguyen Khac Luc (2023). The epidemiological characteristics of the Dengue virus serotype causing Dengue hemorrhagic fever in some districts of Hanoi (2017-2019). *Journal of 108- Clinical Medicine and Pharmacy*. 3(18), 56-60.	2023	First author
2	Nguyen Thi Van, Le Quoc Tuan, Nguyen Khac Luc (2023). Study on *Melaleuca cajuputi* essential oil in protecting from *Aedes aegypti* mosquitoes. *Vietnam Journal of Community Medicine*. 64(topic), 42-49.	2023	First author
3	Nguyen Thi Van, Le Quoc Tuan, Nguyen Khac Luc (2021). Study on composition and habits of *Aedes* mosquito in some districts in Hanoi. *Ho Chi Minh City Journal of Medicine.* 25 (2), 104 – 111.	2021	First author

INTRODUCTION

Dengue hemorrhagic fever (DHF) is an acute infectious disease caused by the Dengue virus (DENV), transmitted by *Aedes* mosquitoes. According to the World Health Organization (WHO), Dengue fever has evolved into a global health issue, with over 100 countries and half of the world's population at risk of contracting the disease. In Vietnam, DHF is prevalent throughout the country, including Ho Chi Minh City, with Hanoi being one of the two cities experiencing the highest number of cases.

In Vietnam, the primary vector for Dengue fever is *Ae. aegypti*. Since 2008, there has been a rapid increase in area, population, and urbanization rate in Hanoi, significantly influencing the distribution and composition of *Aedes* mosquito species. Meanwhile, the WHO recommends vector control as the most effective way to prevent Dengue fever. Researchers worldwide search for natural essential oils with insecticidal and repellent properties to prevent mosquito bites and insecticide resistance to replace chemicals that affect health and habitat.

Building upon these considerations, we initiate this project with the following objectives:

1. Describing some clinical and paraclinical characteristics and serotypes of the Dengue virus in patients with Dengue fever in some districts of Hanoi (2017 – 2019).
2. Identifying the species composition and distribution of *Aedes aegypti* and *Aedes albopictus* mosquitoes in some districts of Hanoi.
3. Evaluating the repellent efficacy against adult *Aedes aegypti* and *Aedes albopictus* mosquitoes of laboratory and field strains using *Lemongrass* and *Melaleuca* essential oils.

NEW CONTRIBUTIONS OF THE THESIS TOPIC

This thesis constitutes a comprehensive research endeavor focusing on DHF, its vectors, and the efficacy of vector control measures in mitigating the prevalence of this significant infectious disease in recent years in Vietnam. The project's research results have new contributions to science, academia, and life: Clinical and paraclinical characteristics of Dengue fever and serotypes causing the disease have been described and updated the picture of *Aedes* mosquito distribution in some districts of Hanoi in 2017 - 2019. The thesis also evaluated

L*emongrass* essential oil and *Melaleuca* essential oil mixed in *Coconut* oil solvent with a concentration of 2. 5%, effectively repelling mosquitoes that transmit Dengue. The time to repel *Aedes aegpti* mosquitoes from laboratory and field strains is 213 minutes. *Aedes albopictus* laboratory strain is 183 minutes; field strain is 153 minutes.

Structure of the thesis

The thesis has 128 pages, including the following sections: Introduction - two pages; Literature Review - 33 pages; study subjects and Methods - 24 pages; Result - 38 pages; Discussion-27 pages; Conclusion: 2 pages; one page of suggestion; list of related research - 1 page; a total of 164 reference documents, including 76 documents updated in 5 years (2019 - 2023), 62 Vietnamese documents, 102 foreign language documents; 50 tables; 18 figures; 7 appendices.

CHAPTER 1: LITERATURE REVIEW

Dengue, according to the Ministry of Health's 2019 guidelines, exhibits diverse clinical manifestations progressing from mild to severe. Categorized into DHF, DHF with warning signs, and severe DHF, the disease's dynamic nature requires careful clinical classification for prognosis and treatment planning.

Diagnosing Dengue virus infection involves simultaneous NS1, IgM, and IgG tests, crucial for distinguishing primary and secondary infections. A positive result for NS1 or IgM and negative IgG indicates a primary infection, while positivity for all three suggests a secondary infection. A virus typing test to identify the specific serotype causing the infection is recommended for enhanced disease prognosis. Additionally, complementary tests, including hematology, biochemical assessments, ultrasound, and X-rays, play a crucial role in disease monitoring and treatment.

The *Aedes aegypti* mosquito is the primary carrier of the Dengue virus. This disease can occur throughout the year, but cases tend to increase during the rainy season when the mosquito population grows. Both children and adults can be affected by Dengue. Symptoms include a persistent high fever, bleeding, and fluid leakage from blood vessels, which can lead to shock and organ failure. Without prompt treatment, Dengue can be fatal.

Dengue fever is a disease that is transmitted all year round in Vietnam, but it tends to develop more strongly in the summer and autumn months in the North and during the rainy season in the South. The disease is more prevalent in urban areas, as compared to rural areas. Hanoi has seen a rapid rate of urbanization that has not been matched by the development of infrastructure, leading to a change in the behavior of *Aedes* mosquitoes. This has resulted in a significant number of cases of Dengue fever that negatively impact public health.

The WHO emphasizes vector control to prevent Dengue, highlighting concerns about chemical impacts on health and the environment. Globally, there's a shift toward exploring natural alternatives. Vietnam's climate supports diverse vegetation, including *Lemongrass* and *Melaleuca* trees, praised for repellent properties. However, optimal concentrations of essential oils for mosquito repellency lack sufficient study in Vietnam.

CHAPTER 2: STUDY SUBJECTS AND METHODS

2.1. Study subjects: The study involves 3,084 Dengue patients, *Aedes* mosquitoes, *Lemongras*s, and *Melaleuca* essential oils, and four volunteers.

Selection Criteria:

- Dengue Patients: Participants diagnosed with Dengue according to the guidelines outlined in Ministry of Health Decision No. 3705. Eligibility requires a positive result in at least one of the following tests: NS1, IgM, or determination of the pathogenic serotype of Dengue virus.

- Mosquitoes and *Aedes* Larvae: Selection follows the classification table of mosquitoes devised by Vu Duc Huong and Leopoldo M. Rueda.

- Essential Oils: *Lemongrass* and *Melaleuca* essential oils are sourced from the state-level project with the code KC10.20/16-20.

- Volunteers: The trial involves four volunteers, two men and two women.

Exclusion Criteria:

- Patients who have dengue hemorrhagic fever and also suffer from other acute diseases or advanced blood diseases.

- Mosquitoes do not have enough legs and wings, are not old enough, and mosquitoes sucked blood

2.2. Time of research

- Recruit Dengue patients from January 1, 2017, to December 31, 2019.
- Collect mosquitoes and larvae once in August 2017.

+ Research on mosquito identification in the laboratory from August to December 2017.

+ Testing the mosquito repellent effectiveness of essential oils from October 2017 to December 2021.

2.3. Location of research

- Four districts in Hanoi: Dong Da District (Lang Thuong Ward, Lang Ha Ward), Hoang Mai District (Dinh Cong Ward, Dai Kim Ward), Thuong Tin District (Tan Minh Ward, Tien Phong Ward), Hoai Duc District (La Phu Ward, Van Canh Ward).
- CDC Hanoi, National Hospital for Tropical Diseases, Insect Lab – Vietnam Military Medical University.

2.4. Research Methodology

2.4.1. Research Method for Objective 1

2.4.1.1. Research Design

A descriptive cross-sectional and retrospective study

2.4.1.2. Study Sample Size

- 3084 Dengue patients were included for the analysis of disease characteristics.
- 180 Dengue patients treated at the National Hospital for Tropical Diseases in 2019 were analyzed for clinical and paraclinical characteristics.
- 270 Dengue patients with positive typing tests were included to analyze the characteristics of the serotype causing the disease.

Sampling Method:

From the 16,977 reported cases of Dengue hemorrhagic fever patients by Hanoi CDC between 2017 and 2019 in four districts (see 2.3), a subset of 3,084 patients meeting the selection criteria (see 2.1) were selected for specific analysis of Dengue patient characteristics.

All 3,084 patients reported by Hanoi CDC, treated at the National Hospital for Tropical Diseases in 2019, were selected for in-depth analysis of clinical and paraclinical Dengue characteristics, with a subset of 180 patients for detailed examination. Similarly, those with pathogenic serotype testing were chosen to analyze the serotype characteristics. Our study selected 270 patients.

The method of data collection:

Retrospective statistical data reported at CDC Hanoi.

Retrospective review of medical records of Dengue patients at the National Hospital for Tropical Diseases.

2.4.1.3. Research content

Research on characteristics of Dengue patients; Clinical and paraclinical characteristics of Dengue disease; circulation of pathogenic serotypes of Dengue virus; distribution of DENV types according to research subjects, over time, and according to ecological regions.

2.4.2. Research Methods for Goal 2

2.4.2.1. Research Design: A descriptive cross-sectional

2.4.2.2. Research Sample Size: In adherence to the Ministry of Health's instructions, a minimum of 30 houses were surveyed at each research site.

Research in the Laboratory: All 646 mosquitoes captured at the research site underwent identification based on the *Aedes* mosquito species identification table of Vu Duc Huong and Leopoldo M. Rueda.

Sampling Method:

The sampling method involved purposefully selecting four districts, with each district intentionally choosing two communes/wards for an in-depth investigation into the Dengue disease vector. Within each selected commune/ward, a minimum of 30 households were randomly chosen to survey mosquitoes and larvae. This approach facilitated the analysis of the characteristics of the Dengue disease vector.

2.4.2.3. Research content

Study the vector characteristics of Dengue, focusing on *Ae. aegypti* and *Ae. albopictus* species composition, distribution, and behavior indoors and outdoors. Examine perching substrate height, color preferences, and reproductive behavior based on water container types. Explore larvae nest characteristics, including flower vases, yard ornamental pots, tires, waste, and other sources identified during the investigation.

2.4.3. Research methods for goal 3

2.4.3.1. Research design: experimental method (in the laboratory).

2.4.3.2. Study sample size

Determine the research sample size according to the guidance of the Ministry of Health in Circular 22 issued on July 27, 2015, and according to author Klun JA, author Phasomkusolsil S.

+ Effective dose test: 05 female *Aedes mosquitoes* / 01 concentration/ 01 experiment. Each experiment was repeated three times. Thus, each test dose was repeated three times.

+ Repellent time test: 250 female *Aedes* mosquitoes / 01 experiment. Each experiment was repeated three times.

Criteria for selecting mosquitoes and essential oils:

Lemongrass and *Melaleuca* essential oils, compliant with expiration dates and storage conditions, underwent quality testing. Test mosquitoes were healthy female *Aedes aegypti* and *Aedes albopictus*, 5-7 days old, unfed for 12 hours, belonging to the F1 generation field strain. Laboratory strains were provided by the Department of Entomology, National Institute of Malariology Parasitology and Entomology

Volunteers, instructed on the experimental procedure, were healthy and had no history of mosquito bite allergies or essential oil sensitivity. They refrained from using perfume, mosquito repellent, smoke, or oils on their skin 12 hours before the experiment.

2.4.3.3. Research content

- Testing the effectiveness of citronella and *Melaleuca* essential oils in repelling adult mosquitoes from laboratory and field strains.

+ Test the repellent effect of essential oils on mosquitoes.

+ Test the mosquito repellent time of essential oils diluted in ethanol and *Coconut* oil.

- Evaluate unwanted effects of *Lemongrass* essential oil and *Melaleuca* essential oil in volunteers participating in the test.

2.4.3.5. Techniques used in research

- Technique for testing the mosquito-repellent effect of essential oils

+ Preparing mosquitoes: 05 female mosquitoes, 5-7 days old, healthy, with legs and wings, can fly, do not feed 12 hours before the test. Mosquitoes were transferred to test boxes and left in a quiet place for 30 minutes.

+ Preparing tools: dose test box, waterproof cotton, adhesive tape, notebook, pen, thermometer, hygrometer, and stopwatch.

+ Test solution: Absolute ethanol (for control and dilution), *Lemongrass* essential oil, and pure *Melaleuca* essential oil dilution at different concentrations.

+ Procedure: Volunteer applies 70º alcohol to thigh area, draws rectangular boxes with a pen, and drops 40µl of control and test solutions. Let dry for 5 minutes, place mosquito boxes on the treated area, and expose to mosquitoes for 3 minutes. Record land/bite observations, repeat the experiment three times per dose, and calculate repellent effectiveness.

- *Technique to test the mosquito repellent effect time (time to protect the user from mosquito bites):*

+ Mosquitoes in the test: Select 250 healthy female *Aedes* mosquitoes aged 5-7 days with full legs and wings, exhibiting normal flight behavior. The mosquitoes have not been fed for 12 hours before the test. Transfer the mosquitoes to a test cage and allow them to rest in a quiet place for 30 minutes.

+ Test solution:

Control: absolute ethanol and *Coconut* oil

The solution used in the experiment was *Lemongrass* essential oil and *Melaleuca* essential oil diluted in ethanol and *Coconut* oil.

+ Essential oils are tested on mosquitoes using test cages, and the efficacy is determined by observing the number of times mosquitoes typically land or bite. The testing involves *Aedes* mosquitoes and is conducted from 8:00 a.m. to 4:00 p.m. when mosquitoes typically seek blood meals.

+ Volunteer Preparation: The volunteer designates the left arm for testing and the right arm for control. Both arms are covered with rubber gloves, leaving a 3x10 cm rectangle on the forearm exposed. Using a pipette, 60 µl of the control solution or test solution is applied to the designated test skin area, ensuring even distribution, and allowed to dry naturally for 5 minutes (refer to Figure 2.3).

Test on the control arm first. Put one arm with the control solution in the test cage (a cage measuring 30x30x30 cm containing 250 hungry female mosquitoes, 5-7 days old) for 3 minutes (observe the number of times the mosquitoes land/bite), then remove the arm. If within 3 minutes, 2 or more mosquitoes are landing or biting, stop the test. If in

3 minutes less than 2 mosquitoes land/bite, wait 30 minutes and repeat the experiment.

After stopping the test on the control arm, conduct the test with the arm smeared with essential oil. The procedure is like that of the control arm. After waiting 30 minutes each time, change the mosquito cage to another one when repeating the experiment. Stop testing when two or more mosquitoes land/bite within 3 minutes.

The time of the repellent effect is calculated from the time the essential oil is applied until the test is stopped. Each experiment was repeated three times.

2.4.3.6. Evaluation indicators

- Mosquito repellent effectiveness is evaluated by the rate of mosquito bite reduction according to the formula

Percentage (%) of mosquito bite reduction = $\frac{K - \lambda}{K}$x 100

Where: - K is the number of mosquitoes biting in the control plot.

- λ is the number of mosquitoes biting in the test plot

According to the guidance of the Ministry of Health in Circular No. 22/2015/TT-BYT issued on July 27, 2015, it is possible to determine the effective dose to repel mosquitoes as the dose that reduces the percentage of mosquito bites to 90 - 100%.

- Mosquito repellent time (time to protect the tester from mosquito bites) is the total time from the start of the test until the test stops.

2.5. Data collection

See appendices 1,2,3,4,5,6,7.

Mosquito collection: Mospack handheld mosquito vacuum cleaner; mosquito-catching type; collect larvae: larvae straw, larvae bottle, net rack, enamel tray; tools for making mosquito specimens; Test box tool determines mosquito repellent dose, test cage determines mosquito repellent time.

2.6. Data Analysis and Processing Method: Biomedical statistics will be conducted using SPSS 22 software and Excel 2016.

2.7. Methods to Control Noise and Errors in Research: During data analysis, it is imperative to decontaminate in SPSS before proceeding with the analysis.

2.8. Research Ethics: The study has obtained approval from the ethics council of the National Institute of Malariology Parasitology and

Entomology, as well as the medical ethics council of the Vietnam Military Medical University.

CHAPTER 3: STUDY RESULTS

3.1. Characteristics of Dengue hemorrhagic fever in some districts of Hanoi City (2017 - 2019)

3.1.1. Characteristics of Dengue hemorrhagic fever patients

The age range for the disease is 1 to 91 years, with an average age of 32.3 ± 16.9 years. The highest proportion of cases (33.9%) is in the 18 to 29-year-old age group. Dengue rates are balanced between women (50.2%) and men (49.8%).

Residence analysis shows a significant difference between urban (82.7%) and rural (17.3%) areas ($p < 0.01$). Among occupational groups, students (25.2%) and freelancers (24.9%) have the highest Dengue cases.

Data trends reveal a peak in June 2017 with over 200 cases, surpassing peaks in 2018 and 2019. Dengue cases were consistently low and similar from December to April in all three years. Thus, from June to October 2017, there was an epidemic. The remaining months of 2017, as well as 2018 and 2019, were non-epidemic.

3.1.2. Clinical Characteristics of Dengue Hemorrhagic Fever Patients

DHF is the highest at 54% Dengue, followed by DHF with warning signs at 40%, and the lowest is severe DHF at 6%. During the dangerous phase, 90.6% were hospitalized, while the fever phase accounted for 9.4%. No hospitalizations occurred in the recovery phase. Most patients (65.0%) had a hospital stay of 4-6 days, consistent across severity groups 60.2%, 72.2%, and 60.0% for DHF, DHF with warning signs, and severe DHF. The average hospital stay was 4.84 ± 0.112 days, ranging from 1 to 9 days.

All Dengue patients experienced fever, lasting from 1 to more than 7 days, with an average duration of 5.14 ± 0.105 days. The group with fever lasting 4 to 7 days had the highest rates at all three severity levels (80.6%, 94.4%, and 60% for Dengue, Dengue with warning signs, and severe Dengue, respectively).

Table 3. 1. Symptoms of poisoning according to disease severity (n=180)

Symptom	DHF (98)		DHF with Warning sign (72)		Severe Dengue (10)		Total	
	No	Total (%)	No	Total (%)	No	Total (%)	No	Total (%)
Headache	67	68.4	48	66.7	9	90.0	124	68.9
Ocular pain	23	23.5	15	20.8	3	30.0	41	22.8
Myalgia/bone pain	98	100.0	72	100.0	10	100	180	100.0
Lethargy/restlessness	0	0	5	6.9	3	30.0	8	4.4

All Dengue patients in the study reported myalgia/bone pain. Headache symptoms were prevalent across all severity levels, with rates of 68.4%, 66.7%, and 90.0%. Ocular pain had a lower incidence, remaining below 25% at all disease levels. Fatigue and drowsiness symptoms were observed in 30% of patients in the severe Dengue group, affecting 3 out of 10 patients.

Table 3. 2. Hemorrhage manifestations according to disease severity (n=180)

Symptom	DHF (98)		DHF with Warning sign (72)		Severe Dengue (10)		Total	
	No	Total (%)	No	Total (%)	No	Total (%)	No	Total (%)
Bleeding under the skin	52	53.1	52	72.2	0	0.0	104	57.8
Mucosal bleeding	0	0	57	79.2	9	**90.0**	66	36.7
- Bleeding tooth	0	0	31	43.1	5	50.0	36	20.0
- Epistaxis	0	0	13	18.1	1	10.0	14	7.8
- Vomiting blood	0	0	6	8.3	0	0	14	7.8
-Black/bloody stools	0	0	4	5,6	0	0	6	3.3
- Hypermenorrhea	0	0	9	12.5	4	40.0	4	2,2
- Blood in urine	0	0	0	0	1	10.0	13	7.2

Manifestations of subcutaneous bleeding were prevalent, accounting for 57.8%, while mucosal bleeding occurred in 36.7% of cases. Among the warning signs, bleeding gums had the highest incidence at 43.1%, followed by epistaxis and hypermenorrhea at rates of 18.1% and 12.5%, respectively. Signs of vomiting blood and black/bloody stools were less common, each with a rate of less than

10%. In the severe Dengue group, as many as 90% of patients exhibited mucosal bleeding, characterized by various indicators.

Table 3. 3. Digestive symptoms according to disease severity (n=180)

Symptom	DHF (98)		DHF with Warning sign (72)		Severe Dengue (10)		Total	
	No	Total (%)	No	Total (%)	No	Total (%)	No	Total (%)
Stomachache	7	7.1	14	19.4	4	40.0	25	13.9
Nausea, vomiting	11	11.2	24	33.3	4	40.0	39	21.7
Vomit is a warning sign	*0*	*0.0*	*3*	*4.2*	*0*	*0.0*	*3*	*1.7*
Diarrhea	12	12.2	12	16.7	4	40.0	28	15.6
Enlarged liver below the costal margin	2	2.0	2	2.8	0	0	4	2,2
< 2cm	*2*	*2.0*	*1*	*1.4*	*0*	*0*	*3*	*1.7*
≥ 2cm	*0*	*0*	*1*	*1.4*	*0*	*0*	*1*	*0.5*

The prevalent digestive symptoms among Dengue patients include nausea and vomiting, constituting 21.7% of cases. Additionally, abdominal pain, diarrhea, and hepatomegaly were observed at rates of 13.9%, 15.6%, and 2.2%, respectively.

Table 3. 4. Warning signs in study patients

Signal	Quantity	Ratio %
Struggling, lethargic, lethargic	3	4.2
Vomiting a lot	3	4.2
Mucosal bleeding	57	79.2
Liver enlarged ≥ 2cm below the costal margin	1	1.4
Pleural and peritoneal effusion	21	29.2
AST/ALT ≥ 400U/L	5	6.9

The predominant warning signs in the study patients were mucosal bleeding, observed in 79.2% of cases, followed by signs of pleural and

peritoneal effusion, which occurred at a rate of 29.2%. Other signs exhibited lower rates, all below 10%.

3.1.3. Paraclinical characteristics of Dengue hemorrhagic fever

Table 3. 5. Platelet characteristics according to disease severity (n=180)

Degree of thrombocytopenia (G/L)	DHF		DHF with Warning sign		Severe Dengue		Total	
	No	Total (%)	No	Total (%)	No	Total (%)	No	Total (%)
Mild: 101-149	9	9.2	6	8.3	0	0.0	15	8.3
Average: 51-100	36	36.7	13	18.1	4	40.0	53	29.4
Danger: ≤ 50	53	54.1	53	73.6	6	60.0	112	62.2
Total	98	100.0	72	100.0	10	100.0	180	100.0

Thrombocytopenia reached critical levels across the three disease stages, with rates of 54.1%, 73.6%, and 60.0%, respectively. Interestingly, none of the patients exhibited mild thrombocytopenia in the severe Dengue group.

Table 3. 6. Hematocrit characteristics according to disease severity (n=180)

Hematocrit value (L/L)	DHF		DHF with Warning sign		Severe Dengue		Total	
	No	Total (%)	No	Total (%)	No	Total (%)	No	Total (%)
Increase < 20%	41	41.8	26	36.1	2	20.0	69	38.3
Increase ≥ 20%	0	0.0	0	0.0	0	0.0	0	0.0
No increase	57	58.2	46	63.9	8	80.0	111	61.7
Total	98	100.0	72	100.0	ten	100.0	180	100.0

Hematocrit levels in Dengue patients modestly increased to 38.3% compared to normal values. Notably, the Dengue group had a higher incidence (41.8%) than the severe Dengue group (36.1%) and the severe Dengue group (20.0%). Across all Dengue disease levels, no cases showed a Hematocrit increase exceeding 20% relative to age and gender.

Most Dengue patients experienced a white blood cell count reduction (76.7%), with a low rate of increase (5%). Severe Dengue showed no instances of leukocytosis.

Approximately 53.3% of Dengue patients displayed no change in red blood cell values, while 31.7% exhibited an increased count, and 15% had a decreased red blood cell index.

Table 3. 7. AST and ALT enzyme activities according to disease severity (n=180)

Index (U/L)		DHF (98)		DHF with Warning sign (72)		Severe Dengue (10)		Total	
		No	Total (%)	No	Total (%)	No	Total (%)	No	Total (%)
AST	< 40	31	31.6	10	13.9	5	50.0	46	25.6
	40 - 399	67	68.4	57	79.2	2	20.0	126	70.0
	400 - 999	0	0.0	5	6.9	0	0.0	5	2.7
	≥ 1000	0	0.0	0	0.0	3	30.0	3	1.7
ALT	< 40	51	52.0	24	33.3	5	50.0	80	44.5
	40 - 399	47	48.0	46	63.9	3	30.0	96	53.3
	400 - 999	0	0.0	2	2.8	2	20.0	4	2,2
	≥ 1000	0	0.0	0	0.0	0	0.0	0	0.0

Liver enzyme abnormalities occurred in 74.4% of patients, mainly with elevated AST levels between 40 - 399 U/L (70.0%). This increase was prominent in the Dengue group (68.4%). A warning-level elevation was observed in 2.7% of monitored Dengue patients, and acute liver failure was documented in 1.7% of severe Dengue cases.

Table 3. 8. Clinical and paraclinical characteristics of patients with severe Dengue hemorrhagic fever

(from days 3-7 of illness, multivariable logistic regression analysis)

Signs/indicators	Severe Dengue (n=10) (TL%)	Non-severe Dengue (n=170) (TL%)	p	OR (95% CI)
Nausea/vomiting	4 (40.0)	35 (20.6)	0.39	1.85 (0.45-7.52)
Stomach-ache	4 (40.0)	21 (12.4)	0.07	0.26 (0.06-1.12)
Mucosal bleeding	9 (90.0)	57 (33.5)	0.008	16.87 (2.1-136.9)

Peritoneal/pulmonary effusion	2 (20.0)	19 (11.2)	0.71	1.38 (0.26-7.47)
Hematocrit increased	2 (20.0)	67 (39.4)	0.19	0.3 (0.05-1.84)
BC decreased	9 (90.0)	129 (75.9)	0.45	0.4 (0.41-4.05)
Platelets ≤ 50 G/L	6 (60.0)	106 (62.4)	0.97	0.8 (0.23-4.12)
AST ≥ 400 U/L	3 (30.0)	5 (2.9)	0.06	12.6 (0.92-173.2
ALT ≥ 400 U/L	2 (20.0)	2 (1,2)	0.64	2.2 (0.08 – 58.9

Between days 3 and 7 of the disease, the logistic regression model revealed that mucosal bleeding was the sole independent factor influencing the severity of Dengue patients, with an odds ratio (OR) of 16.87 (95% confidence interval: 2.1-136.9) and a p-value of 0.008.

3.1.4. Characteristics of the serotype causing the disease

DENV type distribution varied over different years. In 2017, DENV1 was predominant at 68.2%, decreasing to 50% in 2018 and 43.7% in 2019. In 2018, only DENV1 and DENV2 circulated, each at 50%. In 2019, three types were observed: DENV1 (43.7%), DENV2 (50.7%), and DENV4 (0.06%).

Table 3. 9. Distribution of DENV types according to study subjects (n=270)

DENV type / Characteristic		DENV1		Other DENV		p
		Quantity	Ratio %	Quantity	Ratio %	
Age group	< 18 (1)	29	72.5	11	27.5	$P_{1-2} > 0.05$
	≥ 18 (2)	133	57.8	97	42.2	
Sex	Female (3)	89	60.5	58	39.5	$P_{3-4} > 0.05$
	Male (4)	73	59.3	50	40.7	
Total		162	60	108	40	

The incidence of DENV1 infection among children (< 18 years old) is 72.5%, which is higher than in adults (≥ 18 years old) at 57.8%. The distribution between the two genders is approximately equal, with a p-value greater than 0.05.

Table 3. 10. Distribution of DENV types according to epidemic status (n=270)

Year / DENV type	Year of epidemic (1)		Year without translation (2)		p
	Quantity	Ratio %	Quantity	Ratio %	
DENV 1 (n=162)	118	68.2	44	45.4	P_{1-2} <0.01
Other DENV (n=108	55	31.8	53	54.6	
Total	173		97		

In the epidemic year (2017), the prevalence of DENV1 was higher at 68.2%, compared to the non-epidemic year (45.4%), with a significant p-value of less than 0.01. The prevalence of DENV type 1 in urban areas is lower than in rural areas, $p > 0.05$.

3.2. Species composition and distribution of *Aedes aegypti* and *Aedes albopictus mosquitoes* in some districts of Hanoi

3.2.1. *Aedes* mosquito species composition at the study site

At the research site, 491 out of 646 Aedes mosquitoes were captured, revealing two species: *Aedes aegypti* and *Aedes albopictus*. In urban areas, *Aedes aegypti* dominated (59.04%), while in rural areas, *Aedes albopictus* prevailed (81.52%).

A*edes* mosquitoes display varied resting heights. *Ae. aegypti* prefers 0.5 - 1m (56.5%) and rarely rests below 0.5 m (12.2%) or above 2 m (4.1%). *Ae. albopictus* mostly rests at 0.5 - 1m (54.6%) and rarely at 1 - 2 m (18.6%) or above 2 m.

Primary resting substrates include dark fabric (23.1% for *Ae. aegypti*, 21.1% for *Ae. albopictus*), dark walls (12.2% for *Ae. aegypti*, 11.6% for *Ae. albopictus*), light-colored walls (8.8% for *Ae. aegypti*, 11.6% for *Ae. albopictus*), and light-colored fabric (6.8% for *Ae. aegypti*, 4.2% for *Ae. albopictus*).

Urban structures, like water tanks, drums, buckets, crates, pots, flower vases, and ornamental pots, serve as breeding containers. Some lacked both larvae species, mostly containing only one. No container had both Aedes larvae.

In rural areas, there are 808 water containers, 430 with larvae. Among these, 19 containers have *Ae. aegypti* larvae, 394 contain *Ae. albopictus* larvae, and 17 have both larva types.

3.3. Effective in repelling adult *Aedes aegypti* and *Aedes albopictus* mosquitoes Laboratory strains and field strains of *Lemongrass* essential oil and *Melaleuca* essential oil

3.3.1. Effectiveness of *Lemongrass* essential oil in repelling adult *Aedes aegypti* and *Aedes albopictus* mosquitoes

Table 3. 11. Dosage of *Lemongrass* essential oil to repel *Aedes aegypti* mosquitoes

Dosage of *Lemongrass* essential oil (µl/cm^2)	**Laboratory strains**		**Field strain**	
	Number of times mosquitoes land/bite Mean ± SD	Mosquito bite reduction rate (%)	Number of times mosquitoes land/bite Mean ± SD	Mosquito bite reduction rate (%)
C0 - 0	3.8± 1.6		1.9 ± 1.0	
C1 – 0.005	2.9 ± 1.9	23.7	1.2 ± 0.9	36.8
C2 – 0.01	2.2 ± 1.6	42.1	0.8 ± 0.6	57.9
C3 – 0.02	1.7 ± 1.1	55.3	0.7 ± 0.7	63.2
C4 – 0.05	0	100	0	100
C5 – 0.1	0	100	0	100
p	$p_{C0\text{-}C1,2,3} > 0.05$ $p_{C0\text{-}C4.5} < 0.05$		$p_{C0\text{-}C1,2,3} > 0.05$ $p_{C0\text{-}C4.5} < 0.05$	

The mosquito bite reduction rate at concentrations C4 and C5 was 100% for field and laboratory strains. Therefore, 0.05 µl of *Lemongrass* essential oil per 1cm^2 is effective in repelling *Aedes aegypti* mosquitoes.

In Table 3.35, the mosquito bite reduction rate at doses C1, C2, and C3 is below 70%, while doses C4 and C5 in both field and laboratory strains demonstrated a 100% reduction. Consequently, 0.05 µl of *Lemongrass* essential oil per 1cm^2 repels *Ae. albopictus* mosquitoes.

Table 3. 12. Effective duration to repel mosquitoes *Ae. aegypti* and *Ae. albopictus* laboratory strain of *Lemongrass* essential oil diluted in ethanol

Time (minutes)		Average number of times mosquitoes land/bite in 3 trials	
		Ethanol	Ethanol + *Lemongrass* essential oil
Ae. aegypti	0+3 minutes	4.9	0
	30+3 minutes	-	4,6
Ae. albopictus	0+3 minutes	3.6	0
	30+3 minutes	-	4.1

Note: "-" means to stop testing

Tables 3.36 and 3.37 show that *Lemongrass* essential oil diluted in ethanol is effective in repelling *Ae. aegypti* and *Ae. albopictus* in laboratory and field strains for a maximum of 33 minutes.

Table 3. 13. Effective time to repel mosquitoes *Aedes aegypti* and *Aedes albopictus* laboratory strains of *Lemongrass* essential oil diluted in *Coconut* oil

Time (minutes)	Average number of times mosquitoes land/bite in 3 trials			
	Aedes aegypti		*Aedes albopictus*	
	Coconut oil	*Coconut* oil + *Lemongrass* essential oil	*Coconut* oil	*Coconut* oil + *Lemongrass* essential oil
0+3 m	2.9	0	4.9	0
30+3 m	-	0	-	0
60+3 m	-	0	-	0
90+3 m	-	0	-	0
120+3 m	-	0	-	0
150+3 m	-	0	-	1,3
180+3 m	-	1,3	-	2.7
210+3 m	-	2,1	-	4.1
240+3 m	-	2.7	-	-

Note: "-" means to stop testing

Tables 3.38 and 3.39 show the effective time to repel mosquitoes. *Ae. aegypti* was 213 minutes in the laboratory and field strains; *Ae. albopictus* was 183 minutes in the laboratory strain and 153 minutes in the field strain.

3.3.2. Effectiveness of *Melaleuca* essential oil in repelling adult *Aedes aegypti* and *Aedes albopictus* mosquitoes

The mosquito-repelling effect of *Melaleuca* essential oil protects the person using it from mosquito bites. Thus, the repelling effect concerns the protective effect of the essential oil.

Table 3. 14. Dosage of *Melaleuca* essential oil with the effect of repelling *Aedes aegypti* mosquitoes

Dosage of *Melaleuca* essential oil (µl/cm^2)	Laboratory strains		Field strain	
	Number of times mosquitoes land/bite Mean ± SD	Mosquito bite reduction rate (%)	Number of times mosquitoes land/bite Mean ± SD	Mosquito bite reduction rate (%)
C0 - 0	7.2 ± 2.5		8.4 ± 2.5	
C1 – 0.005	6.7 ± 0.6	6.9	8.2 ± 5.1	2,4
C2 – 0.01	6.7 ± 2.7	6.9	7.0 ± 3.5	16.7
C3 – 0.02	2.3 ± 0.6	68.1	4.4 ± 2.1	47.6
C4 – 0.05	0.0 ± 0.0	100	0.0 ± 0.0	100.0
C5 – 0.1	0.0 ± 0.0	100	0.0 ± 0.0	100.0
p	$p_{C0\text{-}C1,2} > 0.05$ $p_{C0\text{-}C3,4,5} < 0.05$		$p_{C0\text{-}C1,2,3} > 0.05$ $p_{C0\text{-}C4.5} < 0.05$	

The mosquito bite reduction rate at C4 and C5 concentrations was 100% in field and laboratory strains. Thus, 0.05µl of *Melaleuca* essential oil per 1cm^2 is effective in repelling *Aedes aegypti* mosquitoes.

In Table 3.41, the mosquito bite reduction rate at doses C1, C2, and C3 is below 70%; C4 and C5 doses in both field and laboratory strains were >90%. Thus, 0.05 µl of *Lemongrass* essential oil per 1cm^2 repels *Ae. albopictus* mosquitoes

Table 3.42. Duration of protection against mosquitoes *Ae. aegypti* and *Ae. albopictus* laboratory strain of *Melaleuca* essential oil diluted in ethanol

Time (minutes)		Average number of times mosquitoes land/bite in 3 trials	
		Ethanol	Ethanol + *Lemongrass* oil
Ae. aegypti	0+3 minutes	4.6	0
	30+3 minutes	-	5.5
Ae. albopictus	0+3 minutes	3.6	0
	30+3 minutes	-	4.5

Note: "-" means to stop testing

Tables 3.42 and 3.43 *Melaleuca* essential oil diluted in ethanol has a protective effect against *Ae. aegypti* mosquitoes and *Ae. albopictus* maximum 33 minutes.

Table 3. 15. Duration of protection from laboratory strains of *Aedes aegypti* and *Aedes albopictus* mosquitoes by *Melaleuca* essential oil diluted in *Coconut* oil

Time (minutes)	Average number of times mosquitoes land/bite in 3 trials			
	Aedes aegypti		*Aedes albopictus*	
	Coconut oil	*Coconut* oil + *Melaleuca* essential oil	*Coconut* oil	*Coconut* oil + *Melaleuca* essential oil
0+3 minutes	3.3	0	2.9	0
30+3 minutes	-	0	-	0
60+3 minutes	-	0	-	0
90+3 minutes	-	0	-	1,3
120+3 minutes	-	0.7	-	0.7
150+3 minutes	-	1,3	-	1.7
180+3 minutes	-	1.7	-	2,3
210+3 minutes	-	2,3	-	3.3

Note: "-" means to stop testing

Tables 3.44 and 3.45 show that the time to protect against mosquitoes of *Melaleuca* essential oil + *Coconut* oil for *Aedes aegypti* laboratory strain and field strain is 213 minutes. For *Ae. albopictus* mosquitoes is 183 minutes of laboratory strain and 153 minutes of field strain.

CHAPTER 4: DISCUSSION

4.1. Characteristics of Dengue hemorrhagic fever in some districts of Hanoi (2017 - 2019).

4.1.1. Characteristics of Dengue hemorrhagic fever patients

Patients in our study share characteristics with other Dengue epidemics in Vietnam, aligning with studies by Nguyen Van Tai, Ha Van Phuc, and Dhungana in terms of age, gender, occupation, and place of residence.

The outbreak in 2017 saw an early increase in cases (May to June). In non-outbreak years, cases increased later (August to September), in line with findings from Truong Thi Thuy Dung and Nguyen Thi Lien Huong's research.

4.1.2. Clinical characteristics of Dengue hemorrhagic fever patients

Patients with typical Dengue show less severe Dengue. Universal fever persists for 4-7 days, echoing Bui Dai and Nguyen Van Tai's 100% fever manifestation. Common symptoms across all stages, like fever, body aches, skin congestion, headache, and subcutaneous bleeding, exceed 50%. Less common symptoms range from 13.9% to 36.7%, including mucosal bleeding, nausea/vomiting, eye socket pain, diarrhea, and abdominal pain, aligning with documented Dengue manifestations globally. Notably, subcutaneous bleeding frequency (57.8%) is lower than Nguyen Van Tai's (100%), while mucosal bleeding (36.7%) surpasses Cao The Hung's (25.6%). Abdominal pain signs (15.6%) exceed Dhungana's (3.17%). This variation highlights Dengue's diverse presentations influenced by factors like geographic location, patient demographics, or circulating Dengue virus serotypes.

4.1.3. Paraclinical characteristics of Dengue hemorrhagic fever

The dangerous level thrombocytopenia rate is high (62.2%), aligning with Naik's study (67.5%). The increased hematocrit rate indirectly indicates plasma leakage in 38.3% of patients, surpassing Kuo's study (0.4%). Warning-significant increased liver enzymes were observed in 4.4%. In severe patients, mucosal bleeding signs independently impact disease severity.

4.1.4. Characteristics of the serotype causing the disease

From 2017 to 2019, three Dengue virus types (DENV 1, 2, 4) circulated, with DENV 4 having a low prevalence of 5.6%. The distribution of DENV types showed no significant variations by age, gender, or ecological region. However, a temporal difference was observed. In the epidemic year of 2017, DENV1 played a predominant pathogenic role. This aligns with studies, including Dang Thi Thuy's highlighting DENV1 dominance in 2017 and Trinh Cong Thuc's noting DENV types 1, 2, and 4 circulations in Da Nang. Cao et al.'s study in China similarly identified DENV 1 as the predominant type.

4.2. Species composition and distribution of *Aedes aegypti* and *Aedes albopictus mosquitoes* in some districts of Hanoi

At the research site, two Dengue disease vectors, *Aedes aegypti* and *Aedes albopictus*, were identified. In the inner-city area, *Ae. aegypti* prevailed, showing the highest values for DI, AHI, HI, and BI indices. Conversely, in rural areas, *Ae. albopictus* dominated, with elevated mosquito and larval indexes. Both species preferred resting at a height of 0.5-1m (>50%), with dark fabric as the favored substrate (21-23%). Larvae nests primarily originated from scrap tools and pots, characteristic of the landscape in all three ecological regions. These findings align with prior research by Tran Thanh Duong, Pham Van Minh, and Muhammad.

4.3. Effective in repelling adult *Aedes aegypti* and *Aedes albopictus mosquitoes* Laboratory strains and field strains of *Lemongrass* essential oil and *Melaleuca* essential oil

4.3.1. Effective in repelling adult *Aedes aegypti* and *Aedes albopictus* mosquitoes of *Lemongrass* essential oil

Lemongrass essential oil effectively repels *Aedes* mosquitoes in both laboratory and field strains at a concentration of 0.05 µl/cm^2. Mixed with ethanol at a 2.5% concentration, it provides mosquito bite protection for 33 minutes. When combined with coconut oil, the repellent time increases to 213 minutes for *Ae. aegypti* and 183 minutes for *Ae. albopictus* in the laboratory strain. In the field strain, the duration is 153 minutes for *Ae. albopictus*, significantly surpassing the 87-minute duration reported by Sritabutra and Soonwera (2013) in Thailand, using a 10% concentration of *Lemongrass* essential oil with *Coconut* oil for *Ae. aegypti*. Moreover, Hazarika et al.'s research showed that a blend of *Lemongrass* essential oil and clove essential oil achieved a mosquito-repellent time of up to 228 minutes.

4.3.2. Effectiveness of *Melaleuca* essential oil in repelling adult *Aedes aegypti* and *Aedes albopictus mosquitoes*

Melaleuca essential oil, at an effective dose of 0.05 µl/cm^2, exhibits varying protection times against *Ae. aegypti* and *Ae. albopictus*. The protective duration for *Aedes aegypti* mosquitoes is 210 minutes in both field and laboratory strains. In the field strain of *Aedes albopictus*,

the protection time is 150 minutes, whereas in the laboratory strain, it is 180 minutes. This suggests that the field strain displays superior resistance compared to the laboratory strain. In Amer et al.'s study (2006), a 20% concentration of *Melaleuca* essential oil combined with solvent compounds (genapol, ethanol, PEG) achieved a repellent time of 360 minutes for *Ae. aegypti*. Tawatsin et al. (2006) attained a repellent time of 126 minutes for *Ae. aegypti* by blending 10% *Melaleuca* essential oil with vanillin and ethanol solvent.

CONCLUSION

1. Characteristics of Dengue hemorrhagic fever patients in some districts of Hanoi city (2017-2019)

The disease affects all age groups, with a concentration in the 18-29 age group (33.9%). Urban areas (82.7%) have the highest patient concentration, mainly students (25.2%) and freelancers (24.9%). In the epidemic year (2017), cases peaked in July and August after a surge in May and June. In non-epidemic years (2018-2019), cases peaked in October and November after a rise in July and August.

Dengue severity distribution shows mild Dengue as the most common form (54%), followed by Dengue with warning signs (40%), and severe Dengue as the least common (6%). Symptoms like fever and body aches were observed in 100% of patients. Subcutaneous bleeding was present in 57.8%, mucosal bleeding in 36.7%, and other symptoms had lower rates (ranging from 21.7% to 2.2%).

A low platelet count (≤ 100 G/L) was found in 91.7%, and leukopenia was observed in 76.7%. AST and ALT enzyme activity increased significantly (>50%), while increased hematocrit was observed in only 38.3%. Red blood cell value showed little variation at 53.3%.

Over the three years (2017-2019), three pathogenic DENV types were identified, with DENV1 having the highest rates of 68.2%, 50.0%, and 43.7%, DENV2 with rates of 31.8%, 50.0%, and 50.7%, and DENV4 appearing only in 2019 at 0.06%. The distribution of DENV types varied over time, with the rate of DENV1 in epidemic years (68.2%) being higher than in non-epidemic years (45.4%). The

distribution of DENV type 1 compared to other DENV types did not differ among subjects and ecological areas.

2. Species composition and distribution of *Aedes aegypti* and *Aedes albopictus mosquitoes* in some districts of Hanoi

Aedes mosquito species, recognized vectors of Dengue disease (*Ae. aegypti* and *Ae. albopictus*), were identified in both urban and rural areas. In urban settings, *Ae. aegypti* prevails (59.04% of mosquitoes, DI = 0.27 mosquitoes/house, AHI = 18.45%, BI = 47.10); in rural areas, *Ae. albopictus* dominates (81.52% of mosquitoes, DI = 0.39 mosquitoes/house, AHI = 24.62%, BI = 80.73).

Both *Ae. aegypti* and *Ae. albopictus* species exhibit a preference for resting at a height of 0.5 - 1 m. Dark fabric emerges as the most favored resting substrate for *Aedes mosquitoes* (23.1% for *Ae. aegypti* and 21.1% for *Ae. albopictus*).

Various water containers serve as breeding grounds for *Aedes* larvae, with larval sources predominantly found in suburban areas, where waste tools account for the highest rate (60.7%). In inner-city areas, ornamental pots emerge as the primary larval habitats, constituting the highest rate at 29.7%. Other water containers, such as flower vases and buckets/containers, exhibit larvae-containing rates below 16%.

3. Effectiveness in repelling mosquitoes *Ae. aegypti* and *Ae. albopictus* mature laboratory strains and field strains of *Lemongrass* and *Melaleuca* essential oils

Lemongrass and *Melaleuca* essential oils, at a concentration of 0.05µl/cm2, exhibit mosquito-repelling effects (protection time from mosquito bites) on both *Aedes aegypti* and *Aedes albopictus*, in both field and laboratory strains.

Using *Lemongrass* and cajeput essential oils at a concentration of 2.5% in ethanol solvent, the mosquito repellent time (protection time from mosquitoes) for *Ae. aegypti* and *Ae. albopictus* in both field and laboratory strains is 33 minutes. However, there are variations in repellent time between the two *Aedes* mosquito species in coconut oil solvent. For *Ae. aegypti*, the repellent time is 213 minutes in both

laboratory and field strains, while for *Ae. albopictus*, it is 183 minutes in the laboratory strain and 153 minutes in the field strain.

RECOMMENDATIONS

Strengthen health education and communication in high-risk areas to prevent Dengue hemorrhagic fever. Prioritize larvae elimination in high-risk water containers (e.g., waste containers, ornamental pots, flower vases, and buckets) during the epidemic season.

Remove preferred resting spots for mosquitoes, especially dark fabrics. Employ *Lemongrass*/*Melaleuca* essential oil in bedrooms and living rooms for effective mosquito repellence.

Maintain research initiatives to evaluate the practical efficacy of repelling *Aedes aegypti* and *Aedes albopictus* mosquitoes in the field.