https://doi.org/10.59459/1859-1655/JMM.523

ENTOMOLOGICAL SURVEY ON THE SPECIES COMPOSITION AND BEHAVIORAL CHARACTERISTICS OF MALARIA VECTORS IN KRONG NA COMMUNE, BUON DON DISTRICT, DAK LAK PROVINCE, VIET NAM

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ABSTRACT

Objectives: *This study aimed to determine the species composition and behavioral characteristics of malaria vectors in the forest habitat of Krong Na commune, Buon Don district, Dak Lak province.*

Subjects and methods: *A cross-sectional descriptive study was conducted in October 2023, targeting adult female Anopheles mosquitoes collected from forest habitat around border guard stations in Krong Na commune.*

Results: *A total of 12 Anopheles species were identified, including two primary malaria vectors-Anopheles dirus and Anopheles minimus-along with six secondary vectors. An. dirus were observed to display strong anthropophilic tendencies, biting both indoors and outdoors, while An. minimus were observed to show predominantly zoophilic behavior with occasional human biting. Both species exhibited peak biting activity between 19:00 and 21:00 and were active throughout the 18:00-24:00 period. The most dominant species were An. philippinensis (37.49%), An. minimus (32.83%), and An. maculatus (18.16%).*

Conclusions: *It is necessary to develop adaptive and context-specific vector control strategies to mitigate malaria transmission in forested border regions.*

Keywords: Krong Na commune, malaria vectors, *Anopheles.*

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Receipt date: 29/8/2024; scientific review: 11/2024; accepted: 21/5/2025.

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1. INTRODUCTIONS

Malaria remains a significant vector-borne disease capable of causing outbreaks and high mortality. Transmission occurs primarily through the bites of female *Anopheles* mosquitoes infected with *Plasmodium* parasites. In Vietnam, a total of 64 *Anopheles* species have been identified, of which *An. dirus*, *An. minimus*, and *An. epiroticus* are recognized as the primary malaria vectors [5]. The distribution and abundance of *Anopheles* species vary across regions, driven by ecological and environmental factors. In recent years, climate change has altered vector distribution, species composition, and vectorial capacity, thereby complicating malaria transmission dynamics [6]. Thus, entomological investigations of *Anopheles* species composition and behavior are essential for guiding region-specific, evidence-based vector control strategies.

Dak Lak province, located in Vietnam's Central Highlands, is recognized as one of the country's major malaria-endemic regions. Within this province,

Krong Na Commune in Buon Don District (Dak Lak Province) has consistently reported high malaria incidence in recent years. Situated in the core zone of Yok Don National Park, this area provides optimal environmental conditions for *Anopheles* mosquitoes to thrive. Additionally, the commune borders Cambodia, where Border Guard units are stationed. These factors collectively elevate the risk of malaria transmission. To date, studies on the distribution and behaviors of *Anopheles* species in this region remain scarce.

To inform malaria prevention and control strategies for border guard troops and local residents, this study was conducted to identify the species composition and behavioral characteristics of malaria vectors in the forest habitat of Krong Na Commune, Buon Don District, Dak Lak Province, Vietnam.

2. SUBJECTS AND METHODS

2.1. Subjects

An entomological survey targeting adult female *Anopheles* mosquitoes was conducted in forest habitats around border guard stations over four consecutive nights at the end of the rainy season in October 2023.

2.2. Methods

- Study design: cross-sectional descriptive study.

- Sampling methods:

+ Human double net (HDN) method: Two HDN traps were deployed each night, one indoor and one outdoor, at least 100 m apart. Each trap was monitored by a collector from 18:00 to 00:00.

+ US-CDC light trap (LT) method: Four LTs were deployed each night, two indoors and two outdoors (at least 100 m apart) from from 18:00 to 06:00 the following day.

+ Cattle shed (CS) collection: Each night, mosquitoes were collected around cattle sheds using a mouth aspirator by a collector, from 18:00 to 00:00 the following day.

- Specimen processing method: Each collected specimen was preserved in a 1.5 mL Eppendorf tube with silica gel for desiccation. Specimens were transported to the Military Institute of Preventive Medicine and identified using morphological characters based on standard taxonomic keys [4].

- Ethics approval: All collectors were provided with personal protective equipment (mosquito-proof head coverings, long-sleeved clothing, gloves, etc…) to avoid mosquito bites and malaria exposure.

- Data analysis: processed and analyzed using Microsoft Excel 2016.

3. RESULTS

3.1. Species composition of *Anopheles* mosquitoes

Table 1. Species composition of *Anopheles* mosquitoes in the forest habitat of Krong Na

No.	Species	Quantity	Rate %
1	*Anopheles aconitus**	9	1.05
2	*Anopheles barbirostris**	23	2.68
3	*Anopheles dirus***	7	0.81
4	*Anopheles jamesi*	17	1.98
5	*Anopheles karwari*	6	0.70
6	*Anopheles kochi*	10	1.16
7	*Anopheles maculatus**	156	18.16
8	*Anopheles minimus***	282	32.83
9	*Anopheles peditaeniatus*	5	0.
10	*Anopheles philippinensis**	322	37.49
11	*Anopheles sinensis**	9	1.05
12	*Anopheles vagus**	13	1.51
Total		859	100
*Note: **: primary vector; *: secondary vector [10].*			

A total of 859 adult female *Anopheles* mosquitoes were collected belong to 12 species. Among there, there were two primary vectors (*An. dirus* and *An. minimus*) and six secondary vectors (*An. aconitus, An. barbirostris, An. maculatus, An. philippinensis, An. sinensis* and *An. vagus). An. philippinensis* was the most dominant species (37.49%) followed by *An. minimus* (32.83%), and *An. maculatus* (18.16%). Other species were accounted for less than 3% of the total, including *An. dirus* (0.81%, n = 7).

3.2. Behavioral characteristics *of* malaria vectors

- Host preference: The majority of specimens were collected using CS method (88.01%),

followed by the LT method (9.20%) and the HDN method (2.79%). Eleven species were collected using the CS method, including one primary vector and six secondary vectors. The LT method captured 10 species (including 1 primary and 6 secondary malaria vectors), whereas the HDN method collected 5 species (including 2 primary vectors and 3 secondary vectors).

An. dirus was exclusively collected using HDN. In contrast, *An. minimus* was collected by all three methods, in which the CS method was the most effective method, accounting for 83.33% of the specimens, followed by the LT method (13.48%) and the HDN method (3.19%). Secondary vectors were dominantly collected by the CS method, accounting for 55.56% to 94.23% of the collected specimens of each species, with lower proportions were obtained using the LT and HDN methods (0-33.33%).

Table 2. Malaria vectors across sampling methods

No.	Vector	Sampling methods			Total
		HDN	LT	CS	
1	*An. aconitus**	-	3 (33.33%)	6 (66.67%)	9
2	*An. barbirostris**	-	3 (13.04%)	20 (86.96%)	23
3	*An. dirus***	7 (100%)	-	-	7
4	*An. maculatus **	-	9 (5.77%)	147 (94.23%)	156
5	*An. minimus***	9 (3.19%)	38 (13.48%)	235 (83.33%)	283
6	*An. philippinensis**	5 (1.55%)	15 (4.66%)	302 (93.79%)	322
7	*An. sinensis**	1 (11.11%)	3 (33.33%)	5 (55.56%)	9
8	*An. vagus**	2 (15.38%)	2 (15.38%)	9 (69.23%)	13
Total		24 (2.79%)	79 (9.20%)	756 (88.01%)	859

- Indoor and outdoor activity behavior:

Table 3. Malaria vectors distribution: indoor vs. outdoor collections

No.	Vector	HDN			LT		
		Indoor	Outdoor	Total	Indoor	Outdoor	Total
1	*An. dirus***	3 (42.86%)	4 (57.14%)	7	-	-	-
2	*An. minimus***	3 (33.33%)	6 (66.67%)	9	16 (42.11%)	22 (57.89%)	38
3	*An. philippinensis**	2 (40.00%)	3 (60.00%)	5	4 (26.67%)	11 (73.33%)	15
4	*An. sinensis**	-	1 (100%)	1	-	3 (100%)	3
5	*An. vagus**	-	2 (100%)	2	1 (50.00%)	1 (50.00%)	2
6	*An. aconitus**	-	-	-	-	3 (100%)	3
7	*An. barbirostris**	-	-	-	1 (33.33%)	2 (66.67%)	3
8	*An. maculatus **	-	-	-	2 (22.22%)	7 (77.78%)	9
Total		8 (33.33%)	16 (66.67%)	24	24 (32.88%)	49 (67.12%)	73

The HDN method collected five species of malaria vectors. Among them, three species were recorded indoors, while all five species were recorded outdoors. For the two primary vectors, *An. dirus* and *An. minimus*, a higher proportion of specimens was collected outdoors (57.14% and 66.67%) than indoors (42.86% and 33.33%).

Meanwhile, five species were collected indoors and seven species were collected outdoors using the LT method. *An. minimus* was more frequently collected outdoors (57.89%) than indoors (42.11%). Similarly, all other vectors, except for *An. vagus*, were also collected in higher proportions outdoors, ranging from 66.67% to 100%.

Overall, malaria vectors were observed to favor outdoor activity rather than indoor activity in both HDN and LT methods

- Biting time of malaria vectors using HDN: *An. dirus* was observed to exhibit early evening activity from 19:00-20:00, peaking at 20:00-21:00 (0.38 mosquitoes/collector/hour), activity persisting until 00:00.

An. minimus was observed to begin biting from 18:00-19:00, peaked at 20:00-21:00 (0.38 mosquitoes/collector/hour), and remained active until 00:00.

An. sinensis observed to be active from 20:00-21:00, with a biting rate of 0.13 mosquitoes/collector/hour. *An. vagus* was detected as active at 18:00-19:00 and 20:00-21:00, with a biting rate of 0.13 mosquitoes/collector/hour. *An. philippinensis* peaked at 20:00-21:00, with a biting rate of 0.25 mosquitoes/collector/hour, the highest recorded, and remained active until 23:00-00:00.

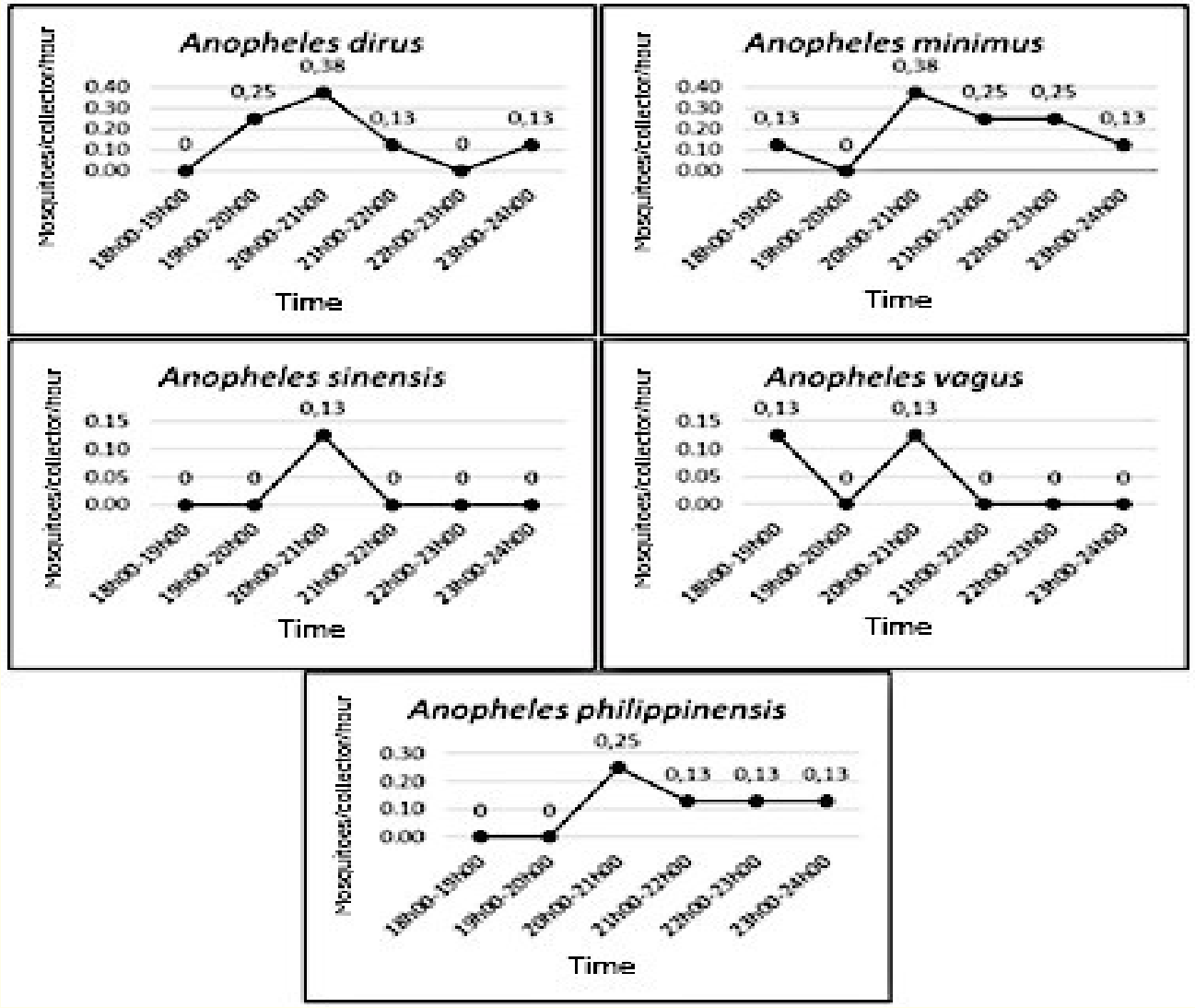

Figure 1. Biting time of malaria vectors by HDN.

- Biting time of malaria vectors by CS:

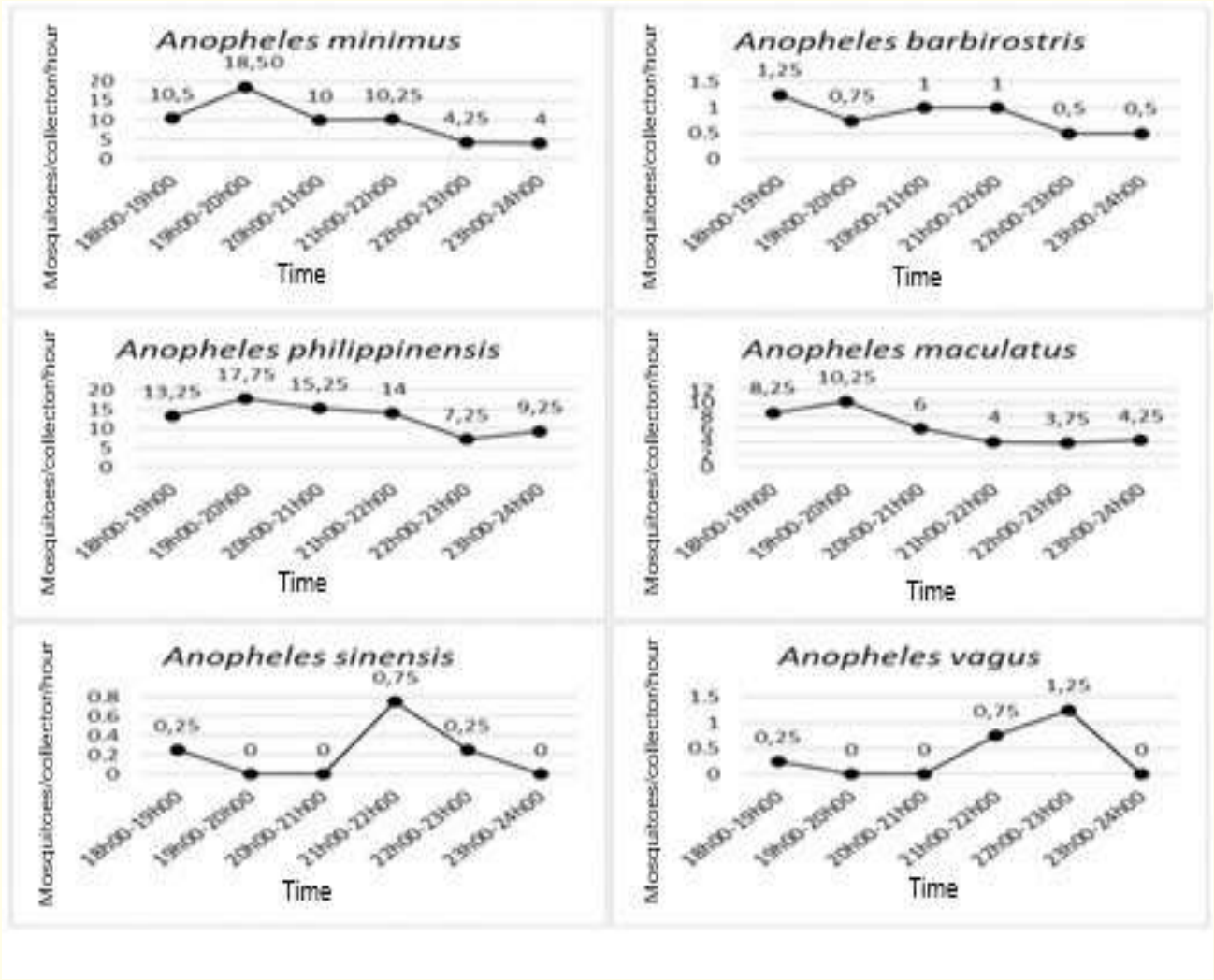

Figure 2. Biting time of malaria vectors by CS.

At the CS collection, *An. minimus* exhibited host-seeking behavior throughout the surveyed period, starting at 18:00-19:00, peaking at 19:00-20:00 with a biting rate of 18.50 mosquitoes/collector/hour, and gradually declining thereafter, though it remained present until 00:00. *An. barbirostris* peaked at 18:00-19:00 with a density of 1.25 mosquitoes/collector/hour, followed by a decreasing trend, yet persisted until 23:00-00:00. *An. maculatus* and *An. philippinensis* initiated host-seeking at 18:00-19:00, peaking at 19:00-20:00, with biting rate of 10.25 and 17.25 mosquitoes/collector/hour, respectively. Both species remained active until 23:00-00:00. *An. sinensis* and *An. vagus* were observed to be active at 18:00-19:00, absent during the following two hours (19:00-21:00), and reappeared from 21:00-23:00.

4. DISCUSSIONS

4.1 Species composition of *Anopheles*

This survey identified 12 *Anopheles* in the forest habitat of Krong Na Commune, Buon Don District, Dak Lak province. This species diversity exceeds that reported by N.V Dung (2023) at Ia Dom commune, Duc Co district, Gia Lai province, of which 7 *Anopheles* species were recorded including 2 primary malaria vectors (*An. dirus* and *An. minimus*) and 4 secondary malaria vectors [8]. The two study sites, located approximately 70km apart, both consist of deciduous broadleaf forest. Our survey consequently revealed four species not previously noted in N.V Dung's study. However, *An. splendidus*, which was collected in N.V Dung's study, was not recorded in our survey.

According to P.V Quang et al. (2023), a study conducted at the Ea So Nature Reserve, Ea So Commune, Ea Kar District, Dak Lak province - approximately 100 km from our research site - identified 13 *Anopheles* species, including 2 primary malaria vectors and 6 secondary malaria vectors, of which 10 species overlap with those recorded in our survey [3]. Two species, *An. karwari* and *An. kochi*, were documented in our survey, but absent from P.V Quang's research. Conversely, *An. separatus*, *An. jeyporiensis*, and *An. tessellatus* were reported in P.V Quang's study but were not detected in ours. The patterns of *Anopheles* distribution highlight the diversity of *Anopheles* species in our study area and adjacent regions. Notably, the presence of the primary malaria vectors *An. dirus* and *An. minimus* across three distinct sites underscores their widespread distribution in the Central Highlands.

Previous studies, such as D.M Cuong et al. (2010) and X.Q Nguyen et al (2019), have identified *An. dirus* as a primary malaria vector in Vietnam, attributed to its strong anthropophilic behavior and capacity to transmit malaria parasites [7, 9]. Given its preference for forest habitats, this malaria vector is closely associated with the concept of "forest malaria" in Vietnam. The presence of *An. dirus* in the forest habitat of Krong Na suggests an elevated malaria risk, particularly for military personnel stationed there who work extensively in the forest. Furthermore, the co-occurrence of *An. minimus*, another primary malaria vector in Vietnam, alongside six secondary vectors in the area, heightens the malaria risk for both soldiers and local residents in the region.

4.2. Behavioral characteristics of malaria vectors

The *Anopheles dirus* was collected exclusively using the HDN method and was not captured with either the LT or CS methods. This finding provides strong evidence of the anthropophilic behavior of *An. dirus*. This result is consistent with studies by T.K Nam and D.M Cuong et al, which indicated that *An. dirus* prefers feeding on humans over livestock [1, 7].

In contrast, *An. minimus* was predominantly collected using the CS method (83.33%), with only 16.67% of specimens captured using the HDN method. These findings indicate a primarily zoophilic feeding pattern, though *An. minimus* retains the capacity to bite humans under opportunistic circumstances. The host-seeking behavior of *An. minimus* observed in the forest habitat of Krong Na commune is aligns with the findings of N.V Dung (2023), who documented greater abundance and biting density of this species using animal-baited traps (n = 39; 1.63 mosquitoes/collector/hour) compared to the HDN method (n = 15; 0.63 mosquitoes/collector/hour) [8]. Similar patterns were reported by D.M Cuong et al (2010) in Truong Xuan Commune, Quang Ninh District, Quang Binh Province, where *An. minimus* was also more frequently captured using animal-baited traps (n = 14) than the HDN method (n = 5) [7].

Both *An. dirus* and *An. minimus* were observed to exhibit higher outdoor biting activity than indoor activity, indicating predominantly exophagic behavior. Several secondary vector species, including *An. philippinensis*, *An. maculatus*, *An. barbirostris*, and *An. vagus*, were also observed to display both indoor and outdoor feeding behaviors, with a greater tendency toward exophagy. This prevalent exophagic behavior (i.e., preference for outdoor biting) may reduce the protective efficacy of key malaria control measures, such as indoor residual spraying (IRS) and insecticide-treated nets (ITNs). Nevertheless, both primary vectors, *An. dirus* and *An. minimus*, retain some degree of endophagic behavior (i.e., indoor biting). Consequently, strict adherence to ITNs use and the periodic application of IRS remain essential for effective malaria prevention and control.

Furthermore, both primary malaria vectors were observed to initiate host-seeking activity early in the evening (18:00-20:00), with peak biting intensity occurring between 20:00 and 21:00. These peak activity periods overlap with times when military personnel and local communities engage in outdoor activities, thus reducing the protective efficacy of IRS and ITNs. The sustained presence of *An. dirus* and *An. minimus* throughout the evening (18:00-24:00) further heightens the risk of malaria transmission, particularly among Border Guard personnel active during these hours. These finding aligns with those of N.X Quang et al (2019), who reported that the peak biting activity of *An. dirus* and *An. minimus* occurs during the early evening, specifically between 21:00-23:00 and 20:00-21:00, respectively [2].

The peak biting density of *An. dirus* in the forest habitat of Krong Na Commune was recorded at 0.38 mosquitoes/collector/hour, exceeding that reported in Ia Dreh Commune, Krong Pa District, Gia Lai Province - another high malaria transmission area - where the density was 0.25 mosquitoes/collector/hour [9]. This elevated biting density underscores the heightened potential for malaria transmission in the forest habitat of Krong Na commune, presenting a significant risk to both military personnel and local communities.

5. CONCLUSIONS

This study documented 12 *Anopheles* species in the forest habitat of Krong Na commune, including two primary malaria vectors (*An. dirus* and *An. minimus*) and six secondary vectors. *An. dirus* was observed to exhibit strong anthropophilic behavior, while *An. minimus* was observed to show a zoophilic tendency, with opportunistic human biting. Both species were predominantly active between 18:00 and 24:00, with peak biting activity occurring between19:00 and 21:00. These findings underscore the necessity for adaptive and context-specific vector control strategies aimed at mitigating outdoor malaria transmission in forested border regions.

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