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Dengue's climate conundrum: how vegetation and temperature shape mosquito populations and disease outbreaks

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Abstract

Introduction Dengue, a prevalent mosquito-borne viral disease in tropical regions, is influenced by environmental factors such as rainfall, temperature, and urbanization. This study aims to assess the effects of microclimate, vegetation, and *Aedes* species distribution on dengue transmission in distinct hotspot and non-hotspot locations.

Methods This cohort study was conducted in two sites within Selangor, Malaysia: a recurrent dengue hotspot and a non-dengue hotspot. Microclimatic variables (temperature, humidity, and rainfall) were monitored over six months using data loggers, and vegetation cover was assessed through visual estimation and GIS mapping. Adult *Aedes* mosquitoes were collected using Gravid Oviposition Sticky (GOS) traps and identified to species level. Dengue virus presence was detected using ProDetect® Dengue NS1 Ag Rapid Test. Weekly indices for mosquito abundance and dengue risk were calculated, and statistical analyses were performed to explore correlations between microclimate, vegetation, and mosquito indices.

Results In the non-dengue hotspot, *Aedes albopictus* was the predominant species, while both *Aedes aegypti* and *Ae. albopictus* coexisted in the dengue hotspot. No dengue virus was detected in *Ae. albopictus*, while intermittent virus presence was noted in *Ae. aegypti* within the dengue hotspot. Significant microclimatic differences were observed: non-dengue hotspot had higher mean humidity and lower minimum temperatures, influenced by greater vegetation cover. In contrast, dengue hotspot showed lower humidity and higher minimum temperatures. Correlation analyses indicated positive associations between temperature and mosquito abundance, with variations in vegetation cover impacting local microclimatic conditions.

Conclusion This study demonstrates how vegetation and microclimatic conditions shape *Aedes* mosquito distribution and dengue risk. Findings highlight the need for targeted urban planning and community interventions that reduce mosquito breeding habitats, with special attention to vegetation management and environmental modifications to control dengue transmission.

Keywords Microclimate, Vegetation cover, *Aedes albopictus*, *Aedes aegypti*, Abundance

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Introduction

Dengue is a prevalent mosquito-borne viral disease commonly found in tropical regions, with varying risk levels influenced by factors such as rainfall, temperature, relative humidity, and rapid, unplanned urbanisation. This disease is transmitted through the bite of an infected *Aedes* mosquito and is commonly caused by four closely related serotypes of the dengue virus which are DENV-1, DENV-2, DENV-3, and DENV-4. The fifth serotype of dengue, DENV-5, has been detected in Sarawak, Malaysia during screening of viral samples taken from a 37 year-old farmer admitted in hospital in the year 2007 [1]. Initially, the infection in the farmer was thought to be a typical case of DENV-4 until virus isolation and complete genetic sequencing were performed [2]. This virus was phylogenetically distinct from the three previous forms of sylvatic DENV-4 and bore some similarity with DENV-2 [2]. Although the exact cause of DENV-5 transmission remains unknown, several factors may contribute to its emergence, including genetic changes from sylvatic strains to human strains [3], the high mutation rate of DENV [4], and extensive deforestation [5]. The emergence of DENV-5 serotype indicates the potential for additional viral variants to emerge in the future. Additionally, dengue can present with a wide spectrum of clinical symptoms, from mild fever to severe haemorrhagic manifestations, which can be fatal if not treated properly.

Controlling the mosquito population is widely regarded as one of the most effective methods to prevent the transmission of arboviruses to humans, especially given the limited availability of effective antiviral treatments or vaccines for many of these viruses [6, 7].

The temporal variation and spatial distribution of dengue incidence are strongly linked to the distribution of *Ae. aegypti*, the primary vector responsible for dengue transmission in Malaysia [8]. While *Ae. albopictus* is implicated in dengue outbreaks in specific regions such as China, Seychelles, Hawaii, and parts of Europe, its role in Malaysia is more limited and primarily associated with other arboviruses, such as Ross River virus (RRV) [9–12]. Meanwhile, multiple studies have linked dengue transmission to climate variables, such as humidity, rainfall, and temperature [13, 14] and vegetation [15, 16]. On the other hand, the local population of *Aedes* mosquitoes is affected by the availability of larval habitats, vector control efforts, and microclimate [17, 18]. Consequently, the relationship between abiotic and biotic factors and mosquito density at local geographical sites is well documented in Malaysia [19–22].

Majority of prior studies are based on the assumption that the mosquito density affects the distribution of dengue cases, and vice versa. Moreover, the potential risk of dengue transmission has been measured based on the immature life indices [23, 24]. However, indicators related to the immature stages of mosquitoes were not directly linked to the risk of DENV infection [25]. Factors such as high larval mortality, the short lifespan of larvae and pupae, and the limited duration of data collection have led to measurements of immature populations that do not always correlate with the biologically relevant adult measures for adult mosquitoes [26]. To address the limitations of previous studies, this cohort study was specifically designed to investigate and compare the microclimatic conditions, vegetation cover, and distribution of adult female *Aedes* species between two distinct site categories: a non-dengue hotspot, referred to as the control site, representing areas with minimal or no reported dengue cases, and a dengue hotspot, referred to as the exposed site, characterized by a high incidence of dengue cases. This classification allows for a targeted examination of environmental and ecological differences that may influence *Aedes* species distribution and dengue transmission dynamics. The result of this study provides information on how the interplay of biotic and abiotic factors influence the dengue virus transmission cycle. By elucidating the environmental drivers of mosquito abundance and virus prevalence, our findings contribute valuable knowledge that can inform targeted urban planning and community-based interventions aimed at reducing dengue transmission risk. Specifically, these insights can aid in identifying and modifying environmental conditions conducive to mosquito breeding and habitat formation, thereby supporting local efforts to mitigate dengue outbreaks effectively.

Methods

Study sites and population

This cohort study was conducted in two distinct sites within Selangor, Malaysia, chosen to represent contrasting dengue transmission profiles: a non-dengue hotspot in Jeram, Kuala Selangor, and a recurrent dengue hotspot in Bukit Raja, Petaling (Fig. 1). The map displays the geographic location of Selangor state within Peninsular Malaysia, highlighted in yellow, to indicate the specific region of interest. The inset zooms into Selangor, showing detailed locations of the study sites. Site A (marked in red) represents the dengue hotspot, while Site B (marked in black) represents the non-dengue hotspot. Both sites are located in central Selangor, selected for their contrasting dengue transmission histories. These sites were selected for their starkly different dengue outbreak histories, enabling an insightful comparative analysis of transmission dynamics and intervention effectiveness in varied settings.

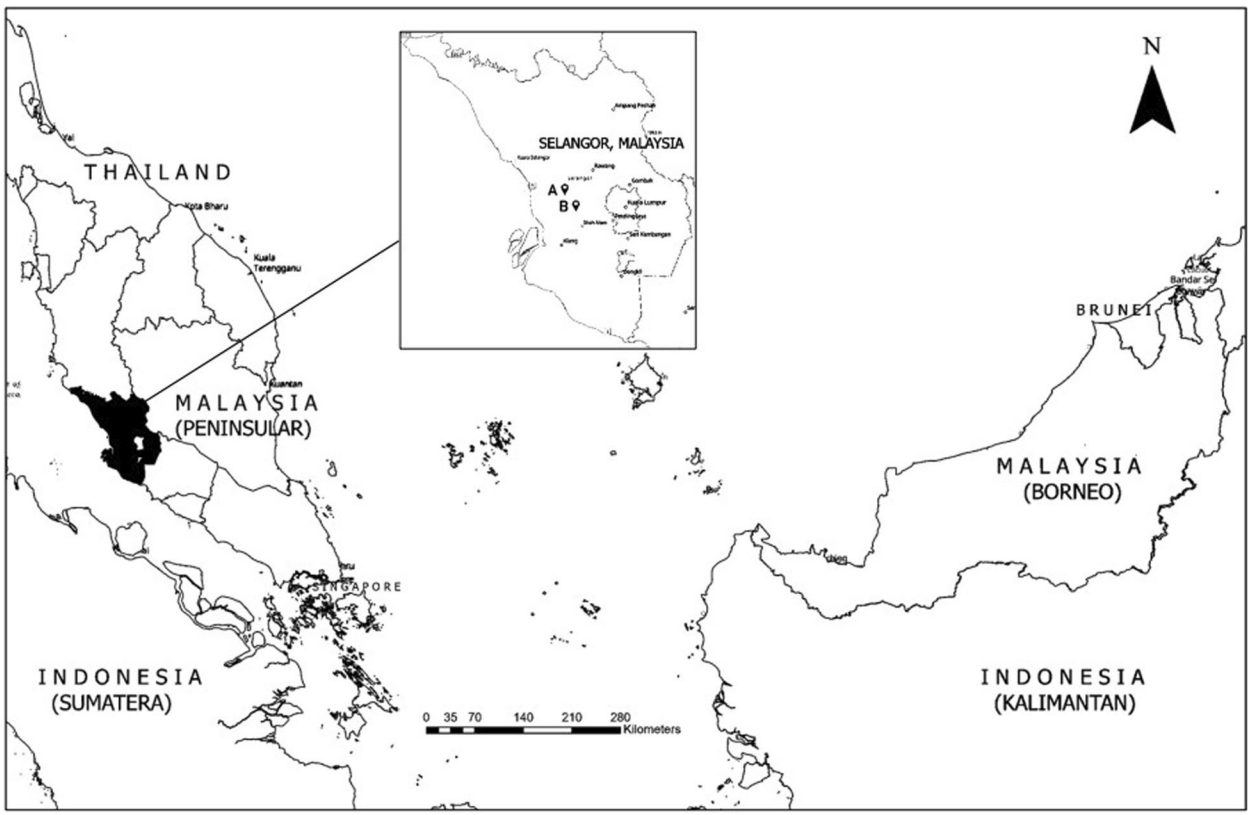

Fig. 1 Map of study locations in Selangor, Malaysia. *Sources: Esri, TomTom, Garmin, FAO, NOAA, USGS, Esri, CGIAR, USGS, Texas Parks & Wildlife, Esri, TomTom, Garmin, SafeGraph, FAO, METI/NASA, USGS, EPA, NPS, USFWS*

The non-dengue hotspot, locatuala Selangor, is a sub-district predominantly comprising a low-density residential population with minimal commercial activity. Geographically, Jeram is situated at approximately 3.2148° N latitude and 101.3204° E longitude. According to epidemiological records from the Ministry of Health, no dengue outbreaks were reported in Jeram from 2017 to 2021. This area includes four ten-storey apartment blocks and is characterized by a mix of rural and suburban settings, where population density is notably lower compared to urban centers. The residential nature and limited commercial zones reduce daily movement and commuting, thereby minimizing the risk of external dengue transmission. A proactive dengue prevention program, involving regular larviciding and targeted source reduction campaigns, has been in place here, though dengue incidence has remained consistently low. These preventive measures were maintained during the study to ensure continuity in intervention efforts, even in the absence of recorded outbreaks.

Conversely, the recurrent dengue hotspot, located in Bukit Raja, Petaling, has been identified as a high-risk area for dengue transmission, experiencing repeated outbreaks in 2017, 2018, and 2020. Bukit Raja is situated at approximately 3.0864° N latitude and 101.4333° E longitude. This sub-district displays a much higher population density, encompassing a more complex blend of residential, commercial, and industrial sectors. The selected study area in Bukit Raja consists of five four-storey apartment blocks surrounded by a mix of small businesses, retail spaces, and industrial facilities, creating a setting where dense population clusters and frequent movement foster an environment conducive to dengue transmission. Ministry of Health data highlights substantial case clusters, indicating the regular occurrence of dengue transmission events within this locale. Although dengue prevention measures such as monthly fogging, larval source reduction, and routine public health campaigns are consistently applied, the high density and mixed land use contribute to persistent transmission. Furthermore, the population of Bukit Raja exhibits limited commuting to workplaces outside the sub-district, suggesting that most dengue cases in the area are attributed to local transmission rather than importation from external sources.

Sample size and data collection

For this study, a sample size calculation was performed using Cochran's formula to ensure sufficient statistical power due to unknown *Aedes* mosquito population at both sites. The formula incorporates the Z-score for a desired confidence level, the estimated population proportion (p), its complement ($q = 1 - p$), and the margin of error (e). The calculation assumes that $p = 0.5$, $q = 0.5$, $e = 5\%$, and the confidence level set to 95% which gives the Z values of 1.95. This indicated a minimum sample size of 385 female *Aedes* mosquitoes to capture meaningful differences in mosquito populations across the study sites.

Data collection at both sites was conducted over a six-month period, from 6th February to 6th August 2023, with approval from local authorities. This timeframe was chosen to encompass the peak monsoon season, a period known to influence *Aedes* mosquito breeding and increase dengue incidence in Malaysia [25–27]. The study population included residents who had lived within the designated study areas for at least one year prior to the study, ensuring a stable population and reducing confounding factors from recent migration or temporary residency. To ensure comparability between the study sites, the selected households were subject to similar environmental conditions, such as weather patterns, sanitation levels, and mosquito control measures**.** This approach facilitated a standardized comparison of vector dynamics and dengue transmission between the non-dengue and dengue hotspot areas.

Microclimate data collection

To capture the microclimatic conditions at the study sites, relative humidity and temperature were monitored on an hourly basis using Tinytag Plus 2 data loggers (Gemini Data Loggers, UK). These loggers are known for their high accuracy in environmental monitoring and were selected due to their robustness and reliability in field conditions. The data loggers were strategically positioned in well-ventilated areas to minimize environmental biases, and were mounted at a height of 2 m above ground level to ensure consistency in data collection across the study sites. This height was chosen to avoid ground-level temperature fluctuations and ensure that the recorded data represented ambient atmospheric conditions. The relative humidity and temperature data were recorded continuously for the entire study period across both the non-dengue and dengue hotspot sites. After data collection, the hourly microclimate data were aggregated and processed into weekly averages using Microsoft® Excel™ Open XML Spreadsheet (XLSX) format. Weekly aggregation was chosen to capture broader trends in temperature and humidity fluctuations, while still maintaining temporal resolution adequate for analysing the impact of microclimate on mosquito behaviour and dengue transmission.

Additionally, rainfall data were obtained from the Info Banjir JPS Selangor website, which provided rainfall readings from 11 rain gauge stations in the Kuala Selangor district and 15 rain gauge stations in the Petaling district [28]. The rainfall data were retrieved in daily intervals, and a point shapefile of the rain gauge locations was generated using ArcGIS Desktop Version 10.8. The shapefile served as the foundation for spatial interpolation across the study sites. To estimate the rainfall at each study site, Inverse Distance Weighting (IDW) interpolation was employed. This geostatistical method assumes that the measured rainfall at each gauge has a local influence that diminishes with distance. The IDW function in ArcGIS was executed with its default settings, where the power parameter was set to 2, ensuring that closer rain gauge stations had a more significant influence on the interpolated values. This choice of power value was based on existing literature suggesting that it optimally balances local variability in rainfall while reducing interpolation bias from distant gauges. The final interpolated rainfall data were extracted at each study site, providing estimates of site-specific rainfall that were integrated with temperature and humidity data for subsequent analyses.

These detailed microclimate datasets, consisting of temperature, humidity, and rainfall, allowed for comprehensive spatiotemporal analysis of environmental conditions in both the non-dengue and dengue hotspots. This approach enabled us to investigate potential correlations between local microclimatic variations and dengue transmission patterns over the study period.

Vegetation assessment and classification

The assessment of *onsite* vegetation cover was conducted using a visual estimation method, adapted from the protocol described by Walker et al. [29]. This method was selected for its practicality in field settings, allowing for a rapid evaluation of vegetation density around mosquito traps, which was critical for understanding the potential influence of vegetative environments on mosquito abundance and behaviour. Visual estimation was chosen over more objective methods to accommodate the study's time and resource constraints, enabling efficient data collection across multiple sites. Vegetation cover was assessed within a 3-m radius around each trap location, with all assessments conducted by a single trained observer to ensure consistency and reduce observer bias.

Each site was assigned to one of four vegetation cover categories, based on the estimated percentage of green vegetation within the defined radius. The categories were as follows: Category 1: Less than 10% green vegetation

cover; Category 2: Between 10 and 25% green vegetation cover; Category 3: Between 25 and 50% green vegetation cover and Category 4: More than 50% green vegetation cover. This categorical approach allowed for a simplified and standardized classification of vegetation density, facilitating statistical analysis of its relationship with mosquito abundance and microclimatic conditions. Each visual assessment involved a thorough scan of the entire 3-m radius, during which the observer estimated the percentage of area covered by any form of vegetation, including grass, shrubs, and trees. The vegetation cover data were recorded at each trap placement event, ensuring that temporal changes in vegetation were accounted for throughout the study period. This was particularly important in tropical environments where rapid vegetation growth or decay could significantly influence local microclimates and, by extension, vector ecology.

In addition to manual observation, photographic documentation of each trap site was taken at the time of assessment to provide a visual record of the vegetation cover. These photographs were archived and could be reviewed for quality control purposes, ensuring that the visual estimates were accurate and consistent over time. This supplementary documentation was also valuable in detecting any major changes in vegetation that could influence mosquito population dynamics, providing a qualitative complement to the quantitative data. The use of this well-established visual estimation method, combined with consistent observer training and photographic verification, allowed for reliable classification of vegetation cover across all study sites. This methodological approach facilitated the integration of vegetation data with other environmental variables such as temperature, humidity, and rainfall, supporting a comprehensive analysis of habitat factors influencing mosquito ecology.

Mosquito collection, identification, and virus detection

Gravid female adult mosquitoes were collected using Gravid Oviposition Sticky (GOS) traps, chosen for their effectiveness in targeting ovipositing *Aedes* females, which are critical for understanding dengue transmission dynamics. This makes the sample more relevant to potential dengue risk, as female mosquitoes must take a blood meal before laying eggs. However, we acknowledge that the sole use of GOS traps may limit the capture of host-seeking mosquitoes, and incorporating additional trap types, such as BG-Sentinel or CDC light traps, could provide a broader understanding of the mosquito population, including host-seeking individuals.

In this study, each GOS trap was baited with 300 mL of 10% hay infusion water, prepared from week-old fermented hay, following the attractant preparation protocol described by Liew et al. [30]. This attractant was chosen for its proven efficacy in luring gravid female mosquitoes, particularly *Ae. aegypti* and *Ae. albopictus*, the primary vectors of the dengue virus. A total of 20 GOS traps were deployed at each study site, positioned 20 m apart to ensure comprehensive coverage (Fig. 2). This spacing was based on established guidelines to optimize capture rates while minimizing trap interference, as detailed by James et al. [31]. The uniform distribution of traps across both non-dengue and dengue hotspots allowed for representative mosquito density estimates for each site. Trap contents were collected weekly to monitor temporal changes in mosquito populations, with careful attention to trap integrity and replacement of any damaged or missing traps to maintain sampling consistency throughout the study. The collected mosquitoes were subsequently transported to the laboratory for species identification and further processing.

In the laboratory, trapped adult female *Aedes* mosquitoes were sorted and identified to the species level based on morphological characteristics using standardized taxonomic keys. This identification process was essential for accurately determining species composition at each site, with a particular focus on *Ae. aegypti* and *Ae. albopictus*, the primary dengue vectors in the region. To ensure accuracy, trained entomologists conducted species identification. Following identification, adult female mosquitoes were pooled by trap location for dengue virus detection.

Dengue virus detection was performed using the ProDetect® Dengue NS1 Ag Rapid Test, a diagnostic tool that identifies the presence of the dengue virus NS1 antigen in mosquito samples. Cheng et al. [32] reported that the modified rapid NS1 test successfully detected 84–89% of infected mosquitoes between 5 to 21 days post-infection (PI) and as early as three days PI, with approximately 60% of infected mosquitoes being detected. The modification to pool mosquitoes rather than testing individual samples was based on Cheng et al.'s findings that individual mosquito homogenates might yield undetectable NS1 levels due to low secretion or antigen concentration, hence pooling to increase NS1 detection sensitivity. For reproducibility, we followed standardized procedures adapted from Cheng et al. [32], which included specific steps for sample preparation, pooling, and testing using the NS1 rapid test. The homogenized lysate from each pool was centrifuged at 8000 rpm for 1 min at room temperature and subjected to the ProDetect® Dengue NS1 Ag Rapid Test to determine the presence of dengue virus, aligning with our study's objective to assess infection rates in local mosquito populations. This approach was chosen for its sensitivity, ease of use, and suitability for large-scale mosquito testing in the field setting.

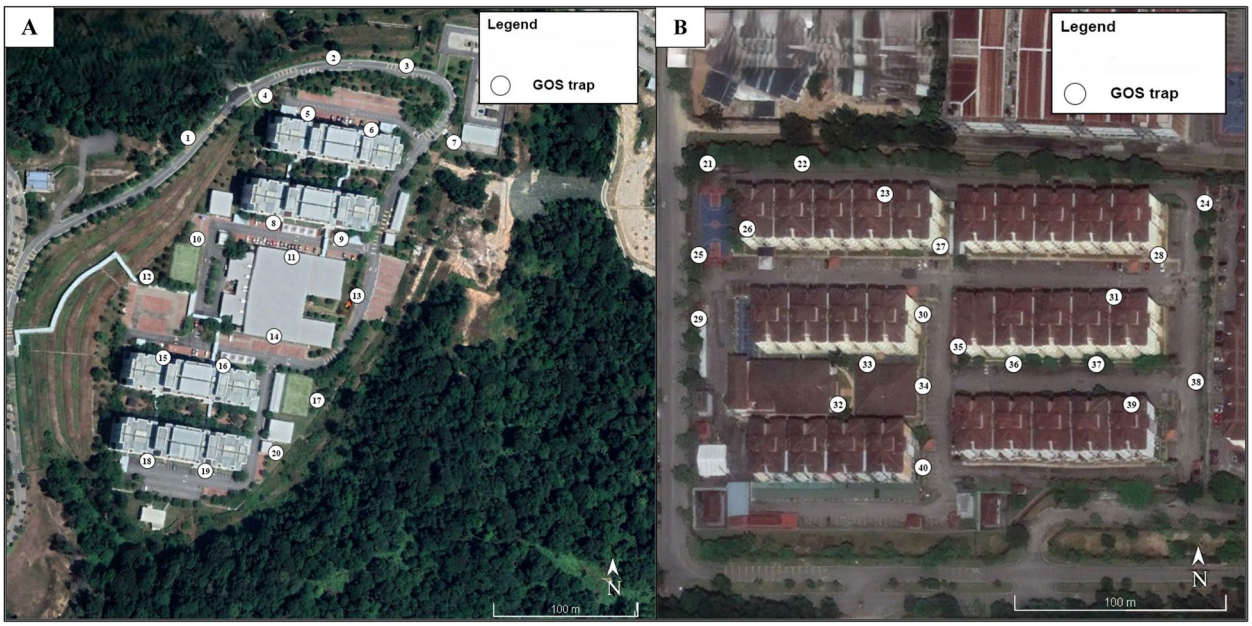

Fig. 2 Aerial view of non-dengue and dengue hotspots with GOS trap placement. The image presents an aerial view of the designated non-dengue hotspot (**A**) and dengue hotspot (**B**) within the study area. GOS (Gravid Oviposition Sticky) traps are strategically positioned across both sites to monitor *Aedes* mosquito populations. The spatial arrangement of traps provides comprehensive coverage, facilitating the study of mosquito activity and distribution patterns relative to environmental characteristics. This layout supports comparisons of *Aedes* species distribution and dengue virus prevalence between the two distinct ecological settings

This study focused exclusively on detecting the presence of the dengue virus rather than identifying specific serotypes, as serotyping was beyond the scope of this study. However, the methodology employed provides a reliable and reproducible framework for assessing dengue virus presence in mosquito populations, supporting the study's aim to evaluate local infection rates and dengue transmission potential.

Mosquito indices calculation

The outcomes of the mosquito collection were quantified using weekly mosquito indices, which were crucial for evaluating the vector population dynamics at each study site. These indices were disaggregated by species, focusing on *Ae. aegypti* and *Ae. albopictus*. The indices calculated included: (i) Adult Sticky Trap Index (ASTI): The proportion of GOS traps that captured adult *Aedes* mosquitoes out of the total number of traps deployed; (ii) Adult Index (AI): The average number of adult *Aedes* mosquitoes captured per GOS trap, providing a direct measure of mosquito abundance and (iii) Dengue-Positive Trap Index (DPTI): The proportion of GOS traps that yielded dengue virus-positive mosquito pools, indicating the level of active dengue virus circulation within the mosquito population. These indices were modified from the methodology proposed by Liew et al. [30], with adjustments made to suit the specific context of this study. The ASTI and AI provided insights into mosquito density and distribution, while the DPTI offered a direct measure of dengue transmission risk in the study areas.

Data analysis

All statistical analyses were performed using IBM SPSS Statistics, Version 21.0 (IBM Corp., Armonk, NY). The choice of statistical tests was determined based on the distribution of the data, which was assessed using the Shapiro–Wilk test for normality. Depending on the outcome of this test, appropriate parametric or non-parametric tests were selected. To compare weekly microclimate variables (i.e., temperature, relative humidity, and rainfall) between the non-dengue and dengue hotspots, two different tests were utilized. If the data met the assumptions of normality, an independent t-test was conducted to determine any significant differences between the two hotspots. For non-normally distributed data, the Mann–Whitney U-test was employed as a non-parametric alternative. These tests provided a comparison of the central tendency of microclimate variables between the two distinct locations, allowing for an evaluation of environmental differences that could influence mosquito population dynamics and dengue virus transmission.

The relationship between microclimate variables was further explored using correlation analysis. Pearson's

correlation was applied to normally distributed variables, while Spearman's rank correlation was used for non-normally distributed variables. These tests assessed the strength and direction of the relationship between the various microclimate factors (temperature, humidity, and rainfall), helping to identify potential interdependencies that could affect mosquito behaviour and virus activity. The correlation coefficients and significance levels were reported for each pair of variables to provide a clear understanding of the environmental interrelationships within the study sites. To assess differences in mosquito indices (i.e., Adult Sticky Trap Index [ASTI], Adult Index [AI], and Dengue-Positive Trap Index [DPTI]) between species (*Ae. aegypti* and *Ae. albopictus*) and across the study locations (non-dengue vs. dengue hotspots), the Kruskal–Wallis test was conducted. This non-parametric test was chosen due to the non-normal distribution of the mosquito indices. When significant differences were identified by the Kruskal–Wallis test, Dunn's post-hoc test with Bonferroni correction was applied to conduct pairwise comparisons. The Bonferroni correction adjusted the p-values to account for the multiple comparisons made in the post-hoc analysis, reducing the risk of Type I errors. This allowed for the identification of specific differences in mosquito indices between species and study sites while controlling for the increased likelihood of false positives due to the multiple comparisons. All statistical tests were two-sided, and *p-values* less than 0.05 were considered statistically significant. Results were presented with corresponding effect sizes where applicable to provide context for the magnitude of observed differences and correlations. By applying these rigorous statistical methods, the analysis ensured robust and reliable conclusions regarding the relationships between microclimate variables and mosquito indices, as well as differences between non-dengue and dengue hotspots.

Results

Analysis of microclimate conditions in dengue hotspot and non-dengue hotspot areas

The weekly mean temperature at the non-dengue hotspot remained stable throughout the study period, averaging 28.90 °C. The lowest weekly mean temperature of 26.28 °C occurred in week 4, while the highest of 30.38 °C was observed in week 14. Minimum temperatures showed slight early fluctuations, with a low of 23.45 °C in week 4 and a high of 26.25 °C in week 14. Maximum temperatures varied more, averaging 37.39 °C, with the lowest at 31.18 °C in week 4 and the highest at 35.34 °C in week 17. Relative humidity at the non-dengue hotspot had a weekly mean of 80.33%, ranging from a low of 73.64% in week 6 to a high of 88.63% in week 13. Minimum relative humidity varied during the early weeks, peaking at 63.07% in week 4 but dropping to 23.73% by week 22. Maximum relative humidity remained consistently high, averaging 97.75%, with values reaching 100% in weeks 12, 13, 16, and 21. Rainfall levels at this site were low, with a mean weekly total of 5.37 mm. The highest recorded rainfall occurred in week 12 (21.09 mm), and the lowest in week 20 (0.03 mm).

At the dengue hotspot, weekly mean temperature stability was similar, averaging 28.83 °C. The highest weekly mean temperature was 30.28 °C in week 14, while the lowest was 26.37 °C in week 4. Minimum temperatures ranged from 24.11 °C (week 4) to 27.59 °C (week 26), while maximum temperatures ranged between 36.46 °C (week 14) and 30.93 °C (week 4), with an average of 34.28 °C. The weekly mean relative humidity at the dengue hotspot showed a significant decline over time, especially between weeks 7 and 11. The mean relative humidity was 55.13%, with a high of 81.18% in week 14 and a low of 36.10% in week 11. Minimum relative humidity followed a similar trend, dropping sharply between weeks 7 and 9, with the lowest minimum relative humidity at 0% in multiple weeks (9, 10, 11, and 13) and the highest at 60.64% in week 4. Maximum relative humidity fluctuated, reaching 100% in weeks 9, 10, 11, and 13, with a low of 85.15% in week 2. Rainfall at the dengue hotspot was comparable to the non-dengue site, with a weekly mean total of 5.44 mm, ranging from 19.75 mm in week 4 to 0.10 mm in week 20.

Overall, microclimate patterns differed notably between the two sites, particularly in relative humidity. The non-dengue hotspot experienced higher average humidity levels, while the dengue hotspot showed larger fluctuations and significantly lower minimum humidity levels, especially between weeks 9 and 13. Temperature patterns were similar across both sites. These microclimate variations could influence mosquito breeding conditions and dengue transmission dynamics. Figure 3 illustrates weekly microclimate variations, including temperature, relative humidity, and rainfall, at both sites.

To assess the differences in microclimatic conditions between non-dengue and dengue hotspots, further statistical analyses were performed. The normality of the data was first evaluated using the Shapiro–Wilk test. The results indicated that both the mean and minimum temperature data followed a normal distribution ($p > 0.05$), while the maximum temperature data deviated from normality ($p < 0.001$). Subsequently, an independent t-test was conducted to compare the mean temperatures between the two study sites. The analysis revealed no statistically significant difference in the mean temperature between non-dengue and dengue hotspots ($t(25) = 1.504$, $p = 0.145$), suggesting that the overall temperature patterns were comparable across the two locations.

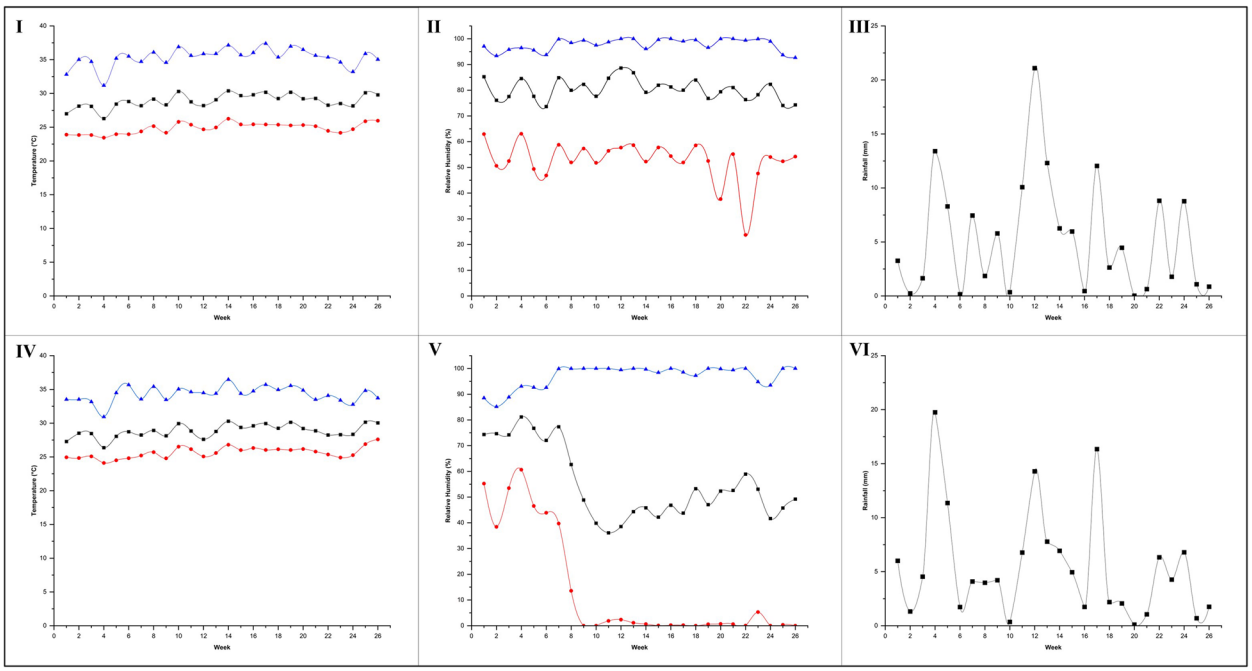

Fig. 3 Weekly mean microclimate conditions at non-dengue (I to III) and Dengue Hotspots (IV to VI).This figure illustrates the weekly variations in key microclimatic parameters, including temperature (°C), relative humidity (%), and rainfall (mm), observed at the non-dengue hotspot (panels I to III) and dengue hotspot (panels IV to VI) over a 26-week study period.Note: Blue line = maximum microclimate, black line = mean microclimate, red line = minimum microclimate

However, a significant difference was detected in the minimum temperature between the two sites. The minimum temperature at the non-dengue hotspot (24.85 °C) was significantly lower than at the dengue hotspot (25.64 °C), as confirmed by the t-test ($t(25) = -15.493$, $p < 0.001$). For the maximum temperature, the Mann–Whitney U test was employed due to the non-normal distribution of the data. The analysis indicated that the maximum temperature at the non-dengue hotspot (35.34 °C) was significantly higher than at the dengue hotspot (34.28 °C), with $U = 158.00$, $p = 0.001$. These findings highlight significant variations in the microclimatic conditions between the two hotspots, particularly in the minimum and maximum temperatures, which may influence mosquito behaviour and dengue transmission. The results of the statistical tests are visualized in Fig. 4.

The Shapiro–Wilk test indicated that relative humidity data did not follow a normal distribution ($p < 0.05$). Consequently, the Mann–Whitney U test was used to compare relative humidity between the non-dengue and dengue hotspots. The analysis revealed that both the mean ($U = 34.00$, $p < 0.001$) and minimum ($U = 62.00$, $p < 0.001$) relative humidity levels were significantly higher at the non-dengue hotspot compared to the dengue hotspot. These findings suggest that the higher relative humidity at the non-dengue hotspot could create microclimatic conditions less favourable for dengue virus transmission. In contrast, no significant difference was observed in maximum relative humidity between the two sites ($U = 371.50$, $p = 0.537$). Additionally, the total rainfall data also did not follow a normal distribution ($p < 0.05$), and the Mann–Whitney U test showed no significant difference in total rainfall between the non-dengue and dengue hotspots ($U = 353.50$, $p = 0.777$). Overall, the significant disparities in relative humidity levels between the two sites could influence mosquito behaviour and dengue transmission potential, whereas total rainfall was consistent across sites. By focusing on these significant findings, the results underscore the role of relative humidity in shaping environmental conditions that may impact vector activity and disease dynamics. The summary of these findings is illustrated in Fig. 5.

Vegetation cover patterns in dengue and non-dengue hotspots

The analysis of vegetation cover data provided critical insights into the spatial distribution of vegetation around GOS trap locations at both dengue and non-dengue hotspots. GIS software was employed to map and quantify vegetation coverage within a 10-m radius of each trap, offering a precise visualization of green space in relation to urban structures. In the non-dengue hotspot,

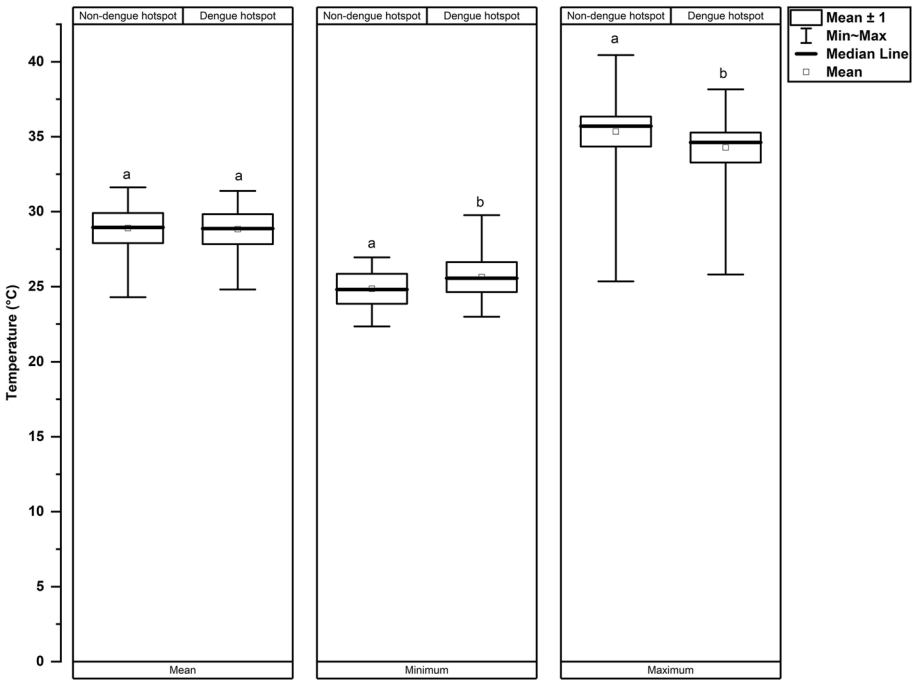

Fig. 4 Temperature comparisons at non-dengue and dengue hotspots. Note: Statistical comparisons are indicated by letters; box plots with the same letter are not significantly different ($p < 0.05$). These temperature profiles highlight environmental differences that may influence *Aedes* mosquito distribution and dengue transmission dynamics, with warmer conditions in the dengue hotspot potentially supporting *Ae. aegypti*, the primary dengue vector

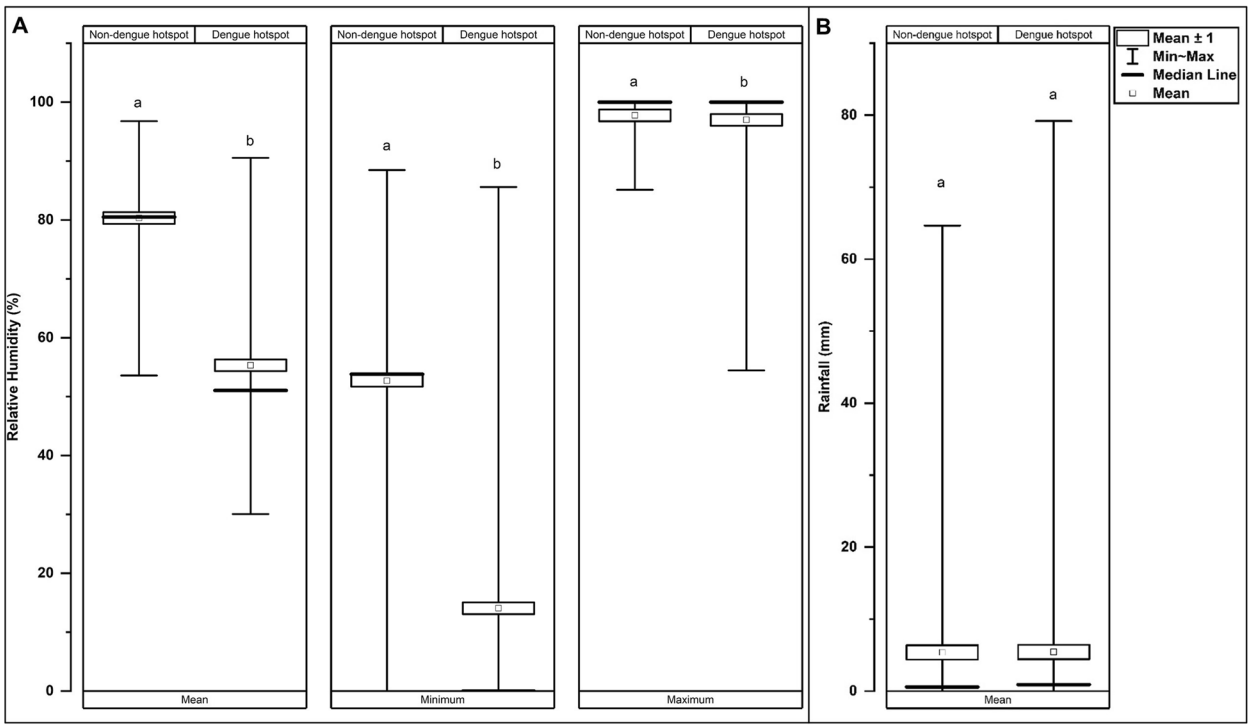

Fig. 5 Relative humidity and rainfall comparison at non-dengue and dengue hotspots. This figure presents box plots of mean, minimum, and maximum relative humidity (**A**) and mean rainfall (**B**) at the non-dengue and dengue hotspots. Statistical comparisons are indicated by letters; box plots with the same letter are not significantly different ($p < 0.05$). These humidity and rainfall measurements highlight microclimatic differences that could impact *Aedes* mosquito activity and dengue transmission potential between the two sites

vegetation cover showed a diverse range of levels across the 20 GOS traps. The majority of traps, 14 out of 20 (70%), were located in areas with less than 10% vegetation cover. These traps were predominantly situated in close proximity to buildings, where vegetation was sparse due to urban infrastructure. Five traps (25%) were found in areas with moderate vegetation cover (10–25%), often at the periphery of built-up zones or adjacent to small patches of green space. Only one trap (5%) recorded a relatively higher vegetation coverage of 25–50%, located in a semi-urban zone with more substantial green areas, potentially due to residential gardens or undeveloped land. Conversely, the dengue hotspot exhibited a markedly different vegetation profile. All 20 traps in this location were surrounded by less than 10% vegetation cover, reflecting the highly urbanized nature of the area. The dense concentration of buildings and extensive asphalted surfaces left minimal space for vegetation, confirming the limited green coverage within the dengue hotspot. This urban landscape, characterized by limited vegetation, presents an environment that could influence mosquito breeding patterns and potential dengue transmission. The spatial analysis revealed a clear correlation between vegetation cover and urbanisation, with vegetation levels decreasing significantly in areas closer to buildings and infrastructure. These patterns suggest that reduced vegetation cover may contribute to different microclimatic conditions, which, in turn, may influence mosquito habitat suitability and breeding activity. The distinct difference in vegetation cover between the non-dengue and dengue hotspots supports the hypothesis that urban density and lack of vegetation could be key factors influencing mosquito ecology and dengue transmission dynamics.

Comparative analysis of mosquito indices in dengue and non-dengue hotspots

The analysis of weekly mosquito indices across the dengue and non-dengue hotspots revealed distinct patterns in *Aedes* mosquito activity and dengue virus transmission potential. Specifically, the Adult Stage Trap Index (ASTI) and Adult Index (AI) showed higher mosquito abundance at the non-dengue hotspot, primarily due to the prevalence of *Ae. albopictus*, while the dengue hotspot exhibited lower overall mosquito counts but a higher presence of *Ae. aegypti*, the primary dengue vector. The Dengue Positive Trap Index (DPTI) further highlighted the dengue hotspot's elevated infection risk, with intermittent dengue virus detections in *Ae. aegypti* and *Ae. albopictus* throughout the study period. These findings underscore the influence of species composition and environmental factors on dengue transmission dynamics in each area.

At the non-dengue hotspot, the only species observed was *Ae. albopictus* with a total of 529 adult female *Ae. albopictus* mosquitoes were trapped. The weekly mean ASTI of *Ae. albopictus* was 21.39%, showing some fluctuation during the study period. The lowest weekly ASTI was recorded in week 3 at 14.82%, while the highest weekly ASTI peaked at 31.19% in week 20. These variations suggest a moderate level of *Ae. albopictus* presence throughout the study, with occasional surges possibly linked to environmental factors conducive to mosquito breeding. The AI, reflecting the average number of adult mosquitoes per trap, further emphasized this trend. The overall weekly mean AI was 0.66 mosquitoes per trap. The lowest AI was observed in week 26, with 0.40 mosquitoes per trap, while the highest AI occurred in week 4, with 1.05 mosquitoes per trap. These figures suggest that, although mosquito density was relatively low, there were periodic increases in *Ae. albopictus* abundance. Additionally, no virus was detected in trapped *Ae. albopictus* using NS1 test kit.

In contrast, the dengue hotspot exhibited different dynamics, with the 258 adult female *Ae. albopictus* and 156 adult female *Ae. aegypti* captured at the site. The weekly mean ASTI for *Ae. aegypti* was 7.36%, significantly lower than the non-dengue site. The highest ASTI was 17.55% in week 13, while the lowest was 1.85% in week 8, indicating more variability in mosquito activity over time. The weekly mean AI of *Ae. aegypti* was also lower than that of *Ae. albopictus* at the non-dengue hotspot, averaging 0.24 mosquitoes per trap. The highest AI of 0.54 mosquitoes per trap was recorded in week 13, while the lowest AI of 0.05 mosquitoes per trap occurred in week 4. This lower density of *Ae. aegypti* compared to *Ae. albopictus* reflects the urbanized nature of the dengue hotspot, where environmental factors such as less vegetation and higher levels of infrastructure may inhibit mosquito proliferation.

Notably, clusters of adult female *Ae. aegypti* positive for dengue virus were detected intermittently throughout the study period. The first cluster appeared between weeks 19 and 22, while sporadic detections occurred between weeks 3 and 13. This highlights the persistent risk of dengue transmission, even in weeks with lower mosquito indices. The DPTI, representing the percentage of traps with dengue-positive mosquitoes, averaged 2.14% at the dengue hotspot. The highest weekly DPTI was 8.23% in week 20, while the lowest weekly DPTI was 0%, which occurred during several weeks (weeks 1, 3, 4, 5, 8, 9, 11, 12, 14–19, and week 24). These findings underline the sporadic yet significant presence of dengue-infected mosquitoes, with periods of heightened risk corresponding to increases in DPTI.

Overall, the mosquito indices highlight key differences in mosquito species composition, abundance, and dengue virus detection between the two sites. While *Ae. albopictus* was more prevalent at the non-dengue hotspot, *Ae. aegypti* and dengue-positive mosquitoes posed a greater threat at the dengue hotspot, particularly during specific periods. These data provide crucial information for targeted mosquito control efforts and dengue prevention strategies in urban and semi-urban environments. Figure 6 summarizes the weekly mosquito indices for both sites.

The statistical analyses revealed significant differences in mosquito indices between the dengue and non-dengue hotspots. The Shapiro–Wilk test showed that most mean *Aedes* species trap indices (ASTI) followed a normal distribution, except for *Ae. aegypti* at the dengue hotspot, which displayed a non-normal distribution ($p=0.04$). This variability likely reflects fluctuations in mosquito abundance specific to the dengue hotspot. The Kruskal–Wallis test, used due to non-normal distributions in some data, indicated a significant difference in mean ASTI among the *Aedes* species across both sites ($H(2)=50.10$, $p<0.001$). Pairwise comparisons using Dunn's test confirmed that each species differed significantly from the others ($p<0.05$). Notably, *Ae. albopictus* at the non-dengue hotspot had the highest mean ASTI (57.74%), followed by *Ae. aegypti* (46.22%) and *Ae. albopictus* at the dengue hotspot (36.30%). This pattern suggests a notably higher mosquito abundance at the non-dengue hotspot, which may impact the epidemiological dynamics in this area.

For the adult index (AI), the Shapiro–Wilk test indicated a non-normal distribution for *Ae. albopictus* at the dengue hotspot ($p=0.04$). Similar to ASTI, the Kruskal–Wallis test revealed significant differences in mean AI across the *Aedes* species ($H(2)=48.98$, $p<0.001$). Dunn's pairwise comparisons indicated significant differences between all species pairs ($p<0.05$). The highest weekly mean AI was observed at the non-dengue hotspot (1.79 mosquitoes per trap), followed by *Ae. albopictus* (1.05 mosquitoes per trap) and *Ae. aegypti* (0.64 mosquitoes per trap) at the dengue hotspot. These findings emphasize the higher abundance of mosquitoes, particularly *Ae. albopictus*, at the non-dengue site. Figure 7 further illustrates these trends, showing weekly variations in mosquito capture rates and the dengue-positive trap index (DPTI) across both sites, highlighting distinct spatial and temporal patterns in mosquito populations and their potential implications for dengue transmission dynamics.

Correlation analysis of microclimate variables in dengue and non-dengue hotspots

In non-dengue hotspot, Spearman's correlation analysis showed strong positive correlations among temperature and humidity variables. The mean temperature had a strong positive correlation with both minimum temperature ($rs=0.883$) and maximum temperature ($rs=0.834$), indicating that higher mean temperatures are associated with higher minimum and maximum temperatures. Minimum temperature also had a moderate to strong positive correlation with maximum temperature ($rs=0.714$). For humidity, mean relative humidity was strongly positively correlated with minimum relative humidity ($rs=0.775$) and moderately positively correlated with maximum relative humidity ($rs=0.634$) and mean rainfall ($rs=0.588$). Minimum relative humidity showed a weak to moderate positive correlation with mean rainfall ($rs=0.430$).

In dengue hotspot, the correlations were more varied. Mean temperature had a strong positive correlation with minimum temperature ($rs=0.876$) and a moderate positive correlation with maximum temperature ($rs=0.673$). It also showed a weak to moderate positive correlation with maximum relative humidity ($rs=0.453$), but a weak to moderate negative correlation with mean relative

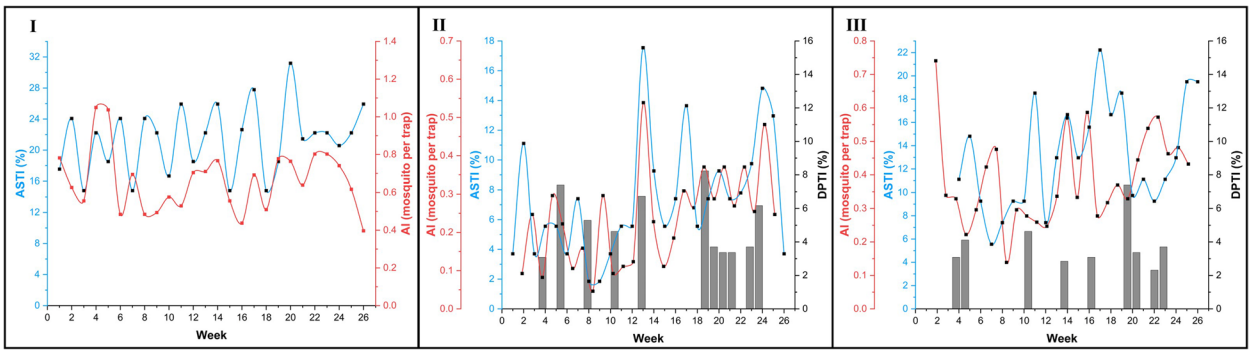

Fig. 6 Weekly mosquito indices at non-dengue and dengue hotspots. The weekly trends of three mosquito indices; Adult Stage Trap Index (ASTI), Adult Index (AI), and Dengue Positive Trap Index (DPTI)—at the non-dengue and dengue hotspots. Note: **I** display ASTI and AI at the non-dengue hotspot; **II** shows ASTI, AI, and DPTI for *Ae. aegypti* at the dengue hotspot and **III** presents ASTI, AI, and DPTI for *Ae. albopictus* at the dengue hotspot

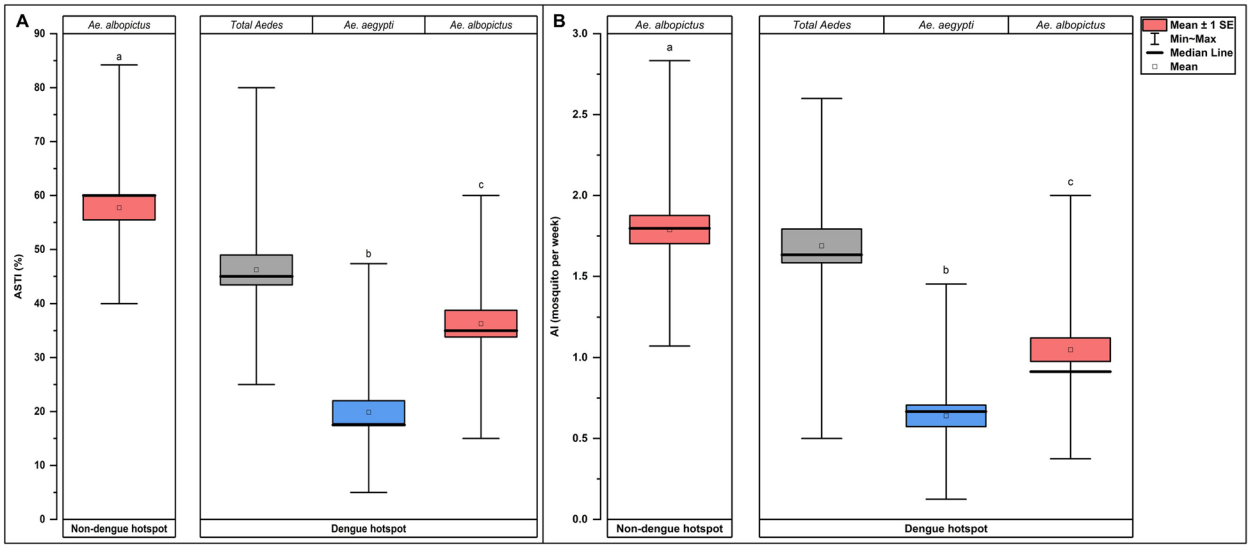

Fig. 7 Comparison of mosquito indices (ASTI and AI) at non-dengue and dengue hotspots. Note: ASTI (**A**): Indicates higher *Ae. albopictus* abundance in the non-dengue hotspot and moderate *Ae. aegypti* presence in the dengue hotspot. AI (**B**): Reflects greater mosquito density for *Ae. albopictus* in the non-dengue hotspot and *Ae. aegypti* in the dengue hotspot. Statistical significance is marked by different letters ($p < 0.05$). This comparison reveals species distribution patterns related to dengue transmission risk

humidity ($rs = -0.475$), minimum relative humidity ($rs = -0.524$), and mean rainfall ($rs = -0.440$). Minimum temperature was positively correlated with maximum temperature ($rs = 0.544$) and maximum relative humidity ($rs = 0.606$), but negatively correlated with mean relative humidity ($rs = -0.569$) and minimum relative humidity ($rs = -0.591$). Mean relative humidity had a moderate positive correlation with minimum relative humidity ($rs = 0.611$) but a moderate negative correlation with maximum relative humidity ($rs = -0.514$).

Discussion

This study provides detailed insights into the distribution patterns of *Aedes* species and their association with environmental factors in dengue and non-dengue hotspots. We found that *Ae. albopictus* was the predominant species in non-dengue hotspot, while both *Ae. aegypti* and *Ae. albopictus* coexisted in dengue hotspot. The higher detection of dengue virus in *Ae. aegypti* compared to in *Ae. albopictus* at dengue hotspot highlights the distinct epidemiological roles these species play in dengue transmission. As the primary dengue vector in Malaysia, *Ae. aegypti* demonstrated a higher virus detection rate, more consistent with its established role as a competent vector for dengue virus transmission, despite its lower capture rate [33, 34]. Non-dengue hotspot has lower minimum temperature, but significantly higher maximum temperatures compared to dengue hotspot. Higher mean and minimum relative humidity levels were also observed in non-dengue hotspot. These environmental differences support our correlation analysis, which demonstrated a strong positive relationship between mean temperature and both minimum and maximum temperatures in non-dengue area. Conversely, dengue hotspot exhibited a positive correlation between mean and minimum temperatures and maximum temperatures, but a negative correlation with relative humidity. This contrast suggests that, *Ae. aegypti* thrives under conditions of lower humidity and higher temperatures found in urban residential areas [35]. This finding is critical, as it underscores the influence of microclimatic conditions on vector competence and the spatial dynamics of dengue transmission.

The observed microclimatic variations between the dengue and non-dengue hotspots can be attributed to differences in vegetation cover. Abundant vegetation in non-dengue hotspot likely contributed to increased evapotranspiration, helping to moderate minimum temperatures and raise humidity levels. This effect is consistent with the findings of Giyasoya et al., [36] which demonstrated that urban vegetation could reduce ambient temperatures through shading and increase humidity through transpiration, thus influencing the local microclimate. Higher vegetation on non-dengue hotspot could thus support *Ae. albopictus*, which is known to tolerate higher humidity [37]. In contrast, low vegetation cover at dengue hotspot led to lower humidity and higher minimum temperature. This finding aligns with Meili et al., [38] who observed that

urban vegetation can reduce thermal stress but also raise humidity, which may indirectly impact mosquito species distribution and disease transmission potential.

These microclimatic and variation between dengue and non-dengue hotspots have important implications for vector control and dengue transmission dynamics. In non-dengue hotspot, higher humidity and moderate temperatures create favourable conditions for mosquito breeding, however, the dengue virus was absent in the sample of *Ae. albopictus* collected from this area. This may be due to *Ae. albopictus* mosquitoes having lower competence for dengue virus transmission compared to *Ae. aegypti*, as the virus survives longer in the latter species [39]. Additionally, the vector control programme at non-dengue hotspot may efficiently lower mosquito populations and interrupt transmission cycles [40]. In contrast, the coexistence of both *Ae. aegypti* and *Ae. albopictus* in dengue hotspot suggests a complex interaction where species-specific traits and environmental factors jointly influence vector competence and disease dynamics.

A limitation of this study is the six-month data collection period, which, while covering the peak monsoon season, may not fully capture seasonal variations across an entire year. Although short-term data collection during high-incidence periods has been shown to capture significant seasonal trends in Malaysia, a year-round study covering different seasons and climate variations could provide a more comprehensive understanding of the environmental influences on dengue transmission patterns. This is particularly important in light of potential changes due to climate variability. Another limitation is the exclusive use of Gravid Oviposition Sticky (GOS) traps, which target ovipositing females, to estimate mosquito abundance. Although effective in studying dengue transmission dynamics, GOS traps may under-represent host-seeking mosquito populations. Future studies could incorporate additional trap types, such as BG-Sentinel or CDC light traps, to capture a broader spectrum of mosquito behaviours and stages, including host-seeking individuals.

For future research, further investigation into the interaction between environmental variables and mosquito populations is needed to deepen our understanding of dengue transmission dynamics. Studies examining how vegetation regulates microclimatic conditions and influences mosquito life cycles could provide critical insights. Additionally, exploring how different *Aedes* species interact with these variables in varying environments will enhance our understanding of their vector competence. Such studies would support the development of targeted and context-specific vector control strategies, taking into account microclimatic and ecological differences across urban and rural settings. In summary, our study underscores the critical role of microclimatic factors, such as temperature, humidity, and vegetation, in shaping *Aedes* mosquito distribution and dengue virus dynamics. Understanding these relationships is essential for designing effective vector management and disease prevention strategies tailored to specific environmental conditions.

Conclusion

This study reveals significant differences in *Aedes* species distribution and dengue virus prevalence across distinct environmental settings. *Ae. albopictus* predominated in non-dengue hotspot, while *Ae. aegypti*, the primary dengue vector, was present in dengue hotspot with a higher virus detection rate. Environmental factors, particularly temperature, humidity, and vegetation cover, were found to influence mosquito ecology and dengue transmission dynamics. The findings underscore the importance of considering microclimatic and ecological variables in dengue control efforts, providing a foundation for targeted vector management strategies. This research contributes valuable insights into the environmental determinants of dengue risk, highlighting the need for integrated, eco-sensitive approaches in dengue prevention and control.

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Authors' contributions

Conceptualisation and Methodology: Nur Athen Mohd Hardy Abdullah, Nazri Che Dom, Siti Aekball Salleh, Rahmat Dapari, Nopadol Precha Writing – original draft, review & editing Nur Athen Mohd Hardy Abdullah, Nazri Che Dom, Siti Aekball Salleh, Rahmat Dapari, Nopadol Precha.

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Data availability

All relevant data are within the manuscript.

Declarations

Ethics approval and consent to participate

Ethics review exemption was obtained from UiTM Research Ethics Committee (REC/01/2023 (PG/EX/2) on 16 January 2023.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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