Scientific Reports

https://doi.org/10.1038/s41598-026-61769-z

Article in Press

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Received: 5 March 2026

Accepted: 7 July 2026

Published online: 14 July 2026

Cite this article as: Sutthanont N., Leanpolchareanchai J., Sriwichai P. *et al.* Topical mosquito repellency of a *Litsea cubeba* essential oil spray against *Aedes aegypti*, *Anopheles dirus*, and *Culex quinquefasciatus*. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-61769-z

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Topical mosquito repellency of a *Litsea cubeba* essential oil spray against *Aedes aegypti*, *Anopheles dirus*, and *Culex quinquefasciatus*

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Abstract

Mosquito-borne diseases remain an important global public health concern, and increasing insecticide resistance has strengthened interest in complementary personal protection tools. This study evaluated the short-term dermal tolerability and laboratory repellency of MosShield, a ready-to-use topical spray formulated with 6% w/w *Litsea cubeba* essential oil. Non-confidential formulation quality-control parameters, including appearance, homogeneity, pH, density, and apparent viscosity, were recorded to support formulation consistency before testing. Short-term dermal tolerability was assessed using a human skin-patch test in 48 healthy volunteers, with no visible skin reactions observed up to 96 h after application. Repellency assays were conducted following the World Health Organization arm-in-cage protocol against three medically important mosquito species: *Aedes aegypti, Anopheles dirus*, and *Culex quinquefasciatus*. The formulation

showed species-dependent repellency, achieving median complete protection times of 225 min against *Cx. quinquefasciatus* and 120 min against both *Ae. aegypti* and *An. dirus.* These findings provide product-relevant laboratory evidence supporting further evaluation of the 6% *L. cubeba* essential oil spray as a plant-based topical mosquito repellent under semi-field and field conditions.

Keywords: Arm-in-cage assay; Botanical repellent; Dermal tolerability; Essential oil; *Litsea cubeba*; Topical repellent; Vector control

1. Introduction

Mosquito-borne pathogens remain an important global public health concern, causing substantial morbidity and mortality worldwide each year. Among mosquito vectors, three species are of particular epidemiological importance: *Aedes aegypti*, a major vector of arboviruses including dengue, chikungunya, Zika, and yellow fever; *Anopheles dirus*, an important malaria vector in forested regions of Southeast Asia; and *Culex quinquefasciatus*, a widespread vector associated with lymphatic filariasis and arboviruses such as St. Louis encephalitis and West Nile virus (Obsomer et al. 2007; Lupi et al. 2013). Accurate evaluation of vector control interventions is important for strengthening mosquito-borne disease control programs (Overgaard et al. 2018; Wilson et al. 2020). However, the effectiveness of conventional insecticide-based approaches is increasingly challenged by physiological and behavioral resistance in mosquito populations (Achee et al. 2019). Topical repellents are widely used as personal protection tools to reduce human–vector contact, particularly against outdoor-biting or diurnally active species that may avoid indoor residual spraying and insecticide-treated bed nets (Russell et al. 2011; Lwetoijera et al. 2014; Gryseels et al. 2015). Although synthetic repellents such as *N,N*-diethyl-3-methylbenzamide (DEET) are effective, concerns related to odor acceptability, skin sensitivity, and consumer preference have encouraged interest in repellents derived from natural sources

(Robbins and Cherniack 1986). In this context, plant-based repellents formulated from essential oils have been investigated as complementary tools for personal protection.

Essential oils from several plant species have shown repellent or deterrent activity against mosquitoes (Gryseels et al. 2015; Mapossa et al. 2021). Their activity has been attributed partly to mixtures of volatile plant-derived compounds that may interfere with mosquito host-seeking and olfactory responses (Maia and Moore 2011). Among these botanical candidates, *Litsea cubeba* (Lauraceae), commonly known as May Chang, has attracted attention as a potential mosquito-control and personal-protection agent. Previous studies have reported insecticidal, ovicidal, excito-repellent, and repellent activity of *L. cubeba* essential oil against multiple mosquito species (Amer and Mehlhorn 2006; Phukerd and Soonwera 2014; Kamle et al. 2019). Reliable assessment of botanical repellents requires standardized evaluation methods. The arm-in-cage (AIC) assay is commonly used to determine complete protection time (CPT), defined as the duration during which a repellent prevents mosquito landings or probing under controlled laboratory conditions. This method is described in World Health Organization (WHO) guidelines and enables comparative evaluation of repellent performance (WHO 2009). However, limited information is available on the product-relevant evaluation of *L. cubeba* essential oil as a complete ready-to-use topical spray formulation, including its formulation consistency, short-term dermal tolerability, and repellency performance against multiple medically important mosquito species under standardized laboratory conditions. In the present study, we evaluated the short-term dermal tolerability and laboratory repellency of a proprietary *L. cubeba*–based spray formulation (MosShield) using WHO-standardized AIC protocols. Repellent performance was assessed against three medically important mosquito species: *Ae. aegypti*, *An. dirus*, and *Cx. quinquefasciatus*. Non-confidential formulation quality-control parameters were also recorded to support

interpretation of the biological findings. The findings provide product-relevant laboratory evidence to support further evaluation of *L. cubeba*–derived spray formulations as plant-based topical repellents for personal protection against mosquito bites.

2. Materials and methods

2.1 Mosquito colonies and rearing conditions

Laboratory colonies of pathogen-free *Ae. aegypti* (Bora Bora strain), *Cx. quinquefasciatus* (Nonthaburi strain), and *An. dirus* (Kao Mai Kaew strain) were maintained at the Insecticide Research Unit, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Thailand. These laboratory strains were used to standardize mosquito age, physiological status, and rearing conditions for repellency testing. Eggs were hatched in plastic trays containing dechlorinated water, and larvae were reared at a density of approximately 200 larvae per 1,000 mL of water. Larvae were fed daily with powdered fish food (Optimum, Perfect Companion Group Co., Ltd., Bangkok, Thailand). Pupae were collected daily and transferred to plastic cups placed inside adult emergence cages (20 × 20 × 30 cm). Adult mosquitoes were maintained on 5% sucrose solution provided on cotton pads, which were replaced every 2–3 days. All developmental stages were maintained under controlled insectary conditions at 25 ± 2 °C, 65 ± 10% relative humidity, and a 12:12 h light–dark photoperiod. For repellency bioassays, fresh cohorts of 250 non-blood-fed female mosquitoes aged 5–7 days were prepared for each test cage. Prior to testing, mosquitoes were sugar-starved for 8–12 h to enhance host-seeking behavior.

2.2 Essential oil procurement

A 100% pure *L. cubeba* essential oil was obtained from a certified commercial supplier (Aromahub Group Co., Ltd., Nonthaburi, Thailand; product code 20021). The oil was purchased as a commercially supplied material and was not distilled in-house. Supplier-provided quality

documentation, including the certificate of analysis and safety data sheet, was retained in laboratory records for traceability. The same purchased oil batch was used as the active ingredient for preparation of the MosShield formulation evaluated in this study.

2.3 Test repellent formulation

The mosquito repellent evaluated in this study, designated MosShield, is a proprietary plant-based topical spray formulation developed by the Insecticide Research Unit, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, in collaboration with the Department of Pharmacy, Faculty of Pharmacy, Mahidol University. The formulation contains *L. cubeba* essential oil (6% w/w) as the active ingredient and is designed as a ready-to-use topical spray for personal protection. The detailed formulation and exact excipient ratios are protected under the Thai Trade Secret Act B.E. 2545 (2002), as amended by B.E. 2558 (2015), and therefore cannot be disclosed. However, the formulation incorporates cosmetic-grade ingredients commonly used to support physical stability, dermal tolerability, and user acceptability. A summary of the functional components is provided in Table 1.

Table 1. Functional components of the proprietary MosShield repellent spray

Component type	Ingredient	Function in formulation
Active ingredient	*Litsea cubeba* essential oil	Provides mosquito-repellent activity
Solvent	Alcohol-based carrier	Dissolves and disperses the active ingredient
Solubilizer	PEG-40 hydrogenated castor oil	Enhances miscibility and clarity
Stabilizer	Dipropylene glycol	Improves formulation stability
Fixative	Vanillin	Reduces volatility and prolongs scent retention

Note: Specific excipient concentrations and ratios are protected under trade-secret registration and are therefore not disclosed.

To improve reproducibility without disclosing protected formulation details, non-confidential formulation characteristics and internal quality-control criteria were recorded. A single laboratory-scale preparation of the MosShield formulation was prepared for experimental use according to the established formulation procedure and active ingredient concentration. This preparation was identified by preparation date in laboratory records and aliquoted into three separate vials for testing against the three mosquito species. Formal industrial batch numbers were not assigned because the formulation was prepared at laboratory scale. Before aliquoting and biological testing, the formulation was assessed for appearance, homogeneity, visible phase separation, pH, density, and apparent viscosity. The formulation was used for testing only when it appeared as a homogeneous pale-yellow liquid with no visible phase separation. Quantitative parameters were measured in triplicate and recorded as non-confidential pre-testing quality-control characteristics to document formulation consistency (Table 2). The formulation was stored in tightly closed spray containers at room temperature, protected from direct light, until use.

The pH was measured using a pH meter (LAQUAtwin pH-22, HORIBA, Ltd., Kyoto, Japan) at 25 ± 1 °C. Density was determined gravimetrically by weighing 1.00 mL of the formulation at 25 ± 1 °C. Apparent viscosity was measured using a rotational rheometer equipped with a cylindrical stainless-steel measurement system (ϕ 34 mm ISO 3219 Z34 DIN, HAAKE RotoVisco 1, Thermo Fisher Scientific GmbH, Dreieich, Germany) at a shear rate of 100 s^{-1} and 30.0 ± 0.5 °C. All measurements were performed in triplicate. For comparative efficacy evaluation, a commercially available botanical repellent, Soffell Spray Natural (11% w/w citronella oil; PT Herlina Indah, Indonesia), was used as a practical positive control.

Table 2. Non-confidential pre-testing quality-control assessment of the MosShield spray formulation

Characteristic	Pre-testing assessment	Observed result
Appearance	Visual assessment before testing	Pale-yellow liquid
Homogeneity	No visible heterogeneity	Homogeneous
Visible phase separation	No visible phase separation	Absent
pH	Measured before testing	6.15 ± 0.01
Density	Measured before testing	0.87 ± 0.01 g/mL
Apparent viscosity	Measured before testing	3.23 ± 0.21 mPa·s

Note: Quantitative values are presented as mean ± SD of triplicate measurements. These non-confidential parameters were recorded before aliquoting and biological testing to document formulation consistency at laboratory scale.

2.4 Human volunteers

A total of 48 healthy adult volunteers (24 males and 24 females), aged 20–50 years, were recruited for this study. The sample size was based on the WHO arm-in-cage repellent testing guideline and previous repellency studies using human volunteers, which recommend multiple participants to account for inter-individual variation in mosquito attractiveness and skin response. Participants were allocated into three mosquito-species groups while maintaining equal sex distribution, with 16 volunteers per species group (8 males and 8 females). This allocation was used for the evaluation of the MosShield formulation against *Ae. aegypti*, *An. dirus*, and *Cx. quinquefasciatus*. Within each mosquito-species group, the same 16 volunteers were also tested with Soffell Spray Natural under the same arm-in-cage procedure as the commercial positive control. Thus, comparisons between MosShield and Soffell Spray Natural within each mosquito species were based on paired observations from the same volunteer group, whereas comparisons of MosShield CPT among mosquito species involved different volunteer groups. Volunteers were screened to confirm the absence of known allergies to mosquito bites or essential oils. Exclusion criteria

included pregnancy, known hypersensitivity to topical formulations, and the use of medications that could affect skin sensitivity. To minimize potential confounding factors, participants were instructed to refrain from using perfumes, scented cosmetics, or other mosquito repellents for at least 12 h before and during the study period. Tobacco users were required to abstain from smoking for at least 12 h before testing. All participants received detailed information regarding the study objectives, procedures, and potential risks, and provided written informed consent before participation.

2.5 Skin patch testing

Prior to repellency testing, a dermal irritation assessment was conducted to evaluate the short-term skin tolerability of the complete final MosShield formulation. A circular filter paper disc (0.6 cm diameter; Finn Chamber, SmartPractice Dermatology, Phoenix, AZ, USA) was impregnated with 10 µL of the MosShield formulation and applied to the outer upper arm of each volunteer. The test site was covered with a waterproof occlusive dressing (3M Tegaderm, 3M, St. Paul, MN, USA) and maintained for 48 h. Skin reactions were evaluated at 30 min after application, immediately after patch removal at 48 h, and at 96 h. The test site was visually examined for erythema, pruritus, edema, vesiculation, or other signs of irritation. Reactions were graded according to the International Contact Dermatitis Research Group (ICDRG) criteria (Fregert 1981; Bruze and Svedman 2025), ranging from negative reaction (–, score 0) to strong positive reaction (+++, score 3) (Supplementary Table S1). A vehicle-only control patch was not included because the objective was to evaluate short-term dermal tolerability of the complete ready-to-use formulation. Evaluators were not blinded to treatment allocation. These methodological constraints are acknowledged in the Discussion. Any participant showing an adverse reaction was withdrawn and provided appropriate medical care.

2.6 Repellent activity test

Repellent efficacy was evaluated using the WHO-standardized AIC assay (WHO 2009) against *Ae. aegypti*, *An. dirus*, and *Cx. quinquefasciatus* under controlled laboratory conditions at 25 ± 2 °C and 60 ± 10% RH. For each mosquito species, each volunteer was tested using a separate standard cage (30 × 30 × 30 cm) containing a fresh cohort of 250 non-blood-fed female mosquitoes aged 5–7 days. Mosquitoes were acclimated for 1–2 h before testing and were not reused between volunteers, test days, mosquito species, or product comparisons. A cage was considered valid only if at least ten mosquitoes attempted to land or probe on the untreated control arm within 3 min; otherwise, a new cage was used. All volunteers within the same test session began exposure at the same scheduled time to minimize variation related to testing time and mosquito activity period. Before exposure, volunteers washed their forearms with unscented soap and water. A 3 × 10 cm area on the inner forearm was marked, corresponding to a treated surface area of 30 cm², and hands were protected using plastic sleeves and rubber gloves. A fixed volume of 100 µL of the MosShield formulation was applied to the marked area using a calibrated micropipette, corresponding to 3.33 µL/cm². The formulation was spread evenly by a trained operator wearing clean gloves and allowed to dry for 1–2 min before testing. The same application procedure was used for all participants.

The contralateral forearm served as an untreated control and was exposed first to confirm mosquito responsiveness. The treated arm was then exposed for 3 min at 30-min intervals, beginning 30 min after application, until protection failure occurred or until the predefined maximum observation period of 360 min was reached. To minimize cross-contamination, treated and untreated arms were not introduced into the cage simultaneously, gloves and sleeves were replaced or cleaned as appropriate between tests, and contact between treated skin and untreated

surfaces was avoided. CPT was defined as the elapsed time from repellent application to the second confirmed mosquito landing or probing event on the treated area. A landing was defined as physical contact with the exposed skin surface, with or without probing. The second landing/probing event could occur within the same exposure period or across consecutive intervals. Events were observed and confirmed by trained study personnel. Individual mosquitoes were not marked; therefore, each observed landing/probing event was counted separately. Observations reaching 360 min without protection failure were treated as right-censored observations in the Kaplan–Meier analysis. This endpoint was adapted from WHO guidance and previous repellency studies (WHO 2009; TISI 2010; Sutthanont et al. 2022; 2026).

Soffell Spray Natural was tested as a commercial positive control under the same AIC procedure and laboratory conditions. Within each mosquito-species group, the same 16 volunteers were tested with both MosShield and Soffell Spray Natural. Because the two products differed in active ingredient identity, concentration, and overall formulation composition, comparisons were interpreted as benchmark performance rather than ingredient-equivalent efficacy testing. According to Thai Food and Drug Administration (Thai FDA) criteria, topical mosquito repellents must provide a minimum of 120 min protection against *Ae. aegypti* to be considered effective (TISI 2010). Exposure periods were aligned with mosquito activity patterns: *Ae. aegypti* and *An. dirus* from 08:00 to 15:00 h, and *Cx. quinquefasciatus* from 15:00 to 22:00 h.

2.7 Data analysis

CPT values were recorded for each valid paired test observation, and CPT observation was used as the analytical unit for repellency comparisons. Sample-size annotations in the figures and supplementary tables therefore indicate paired CPT observations rather than the number of unique recruited volunteers. All statistical analyses were performed using R version 4.5.1 for Windows in

RStudio version 1.4.1717. Data normality was assessed using the Shapiro–Wilk test. As the data did not follow a normal distribution, non-parametric statistical methods were applied. CPT was analyzed as time-to-event data, with protection failure defined as the second confirmed mosquito landing or probing event on the treated area. Observations reaching the predefined maximum observation period of 360 min without protection failure were treated as right-censored. Median CPT values were estimated using Kaplan–Meier survival analysis with the survival package and reported with 95% confidence intervals where estimable. Descriptive CPT values are presented as median with interquartile range (IQR).

Differences in CPT among mosquito species for MosShield were analyzed using the Kruskal–Wallis test with the rstatix package, followed by Dunn's post-hoc test with Bonferroni adjustment using the FSA package, because different volunteer groups were used for the three mosquito species. Comparisons between MosShield and Soffell Spray Natural within each mosquito species were performed using the Wilcoxon signed-rank test, because the same volunteers within each mosquito-species group were tested with both formulations. Figures were generated using the survminer, ggplot2, and ggprism packages. Statistical significance was defined as $p < 0.05$.

3. Results

3.1 Skin patch testing

Dermal irritation testing was completed in all 48 volunteers prior to repellency evaluation. All participants showed negative ICDRG reactions (–, score 0) at all assessment time points, including 30 min, 48 h, and 96 h after application. No visible erythema, pruritus, pain, edema, vesicle formation, bullae, or other signs of irritation were observed. These results indicate that the complete MosShield formulation was well tolerated under the short-term test conditions.

3.2 Repellent activity

The MosShield spray formulation containing 6% w/w L. cubeba essential oil showed species-dependent CPT values under laboratory conditions (Table 3; Figure 1). The longest median CPT was observed against *Cx. quinquefasciatus* (225 min; IQR 150–307.5 min), followed by *Ae. aegypti* (120 min; IQR 120–180 min) and *An. dirus* (120 min; IQR 90–120 min). CPT distributions differed significantly among mosquito species for MosShield (Kruskal–Wallis test, $H = 45.231$, df = 2, $p < 0.001$; Supplementary Table S3). Dunn's post-hoc test with Bonferroni adjustment showed significant pairwise differences among all three species: *Ae. aegypti* vs *An. dirus* ($p = 0.0135$), *Ae. aegypti* vs *Cx. quinquefasciatus* ($p = 0.00034$), and *An. dirus* vs *Cx. quinquefasciatus* ($p < 0.0001$; Supplementary Table S4).

For the commercial positive control, Soffell Spray Natural (11% w/w citronella oil), median CPT values were 360 min for *Cx. quinquefasciatus*, 300 min for *Ae. aegypti*, and 150 min for *An. dirus*. Soffell Spray Natural showed significantly longer CPT values than MosShield for all three mosquito species (Wilcoxon signed-rank test, $p < 0.001$; Table 3; Supplementary Table S5). Values of 360 min indicate the predefined maximum observation period and were treated as right-censored observations in the Kaplan–Meier analysis. Kaplan–Meier median CPT estimates, 95% confidence intervals, and censoring details are provided in Supplementary Table S6. The median CPT of MosShield reached the Thai FDA efficacy threshold of ≥120 min under the tested laboratory conditions.

Table 3. Median complete protection time (CPT) of MosShield spray and Soffell Spray Natural against mosquitoes.

Mosquito species	MosShield spray, median CPT (IQR), min	Soffell Spray Natural, median CPT (IQR), min	*p*-value

Ae. aegypti	120 (120–180)	300 (300–330)	<0.001
An. dirus	120 (90–120)	150 (120–180)	<0.001
Cx. quinquefasciatus	225 (150–307.5)	360 (360–360)	<0.001

Note: Data are presented as median CPT (IQR). *p*-values compare paired CPT observations between MosShield spray and Soffell Spray Natural within each mosquito species using the Wilcoxon signed-rank test. A value of 360 min indicates the predefined maximum observation period. Kaplan–Meier estimates, 95% CIs, and censoring information are provided in Supplementary Table S6.

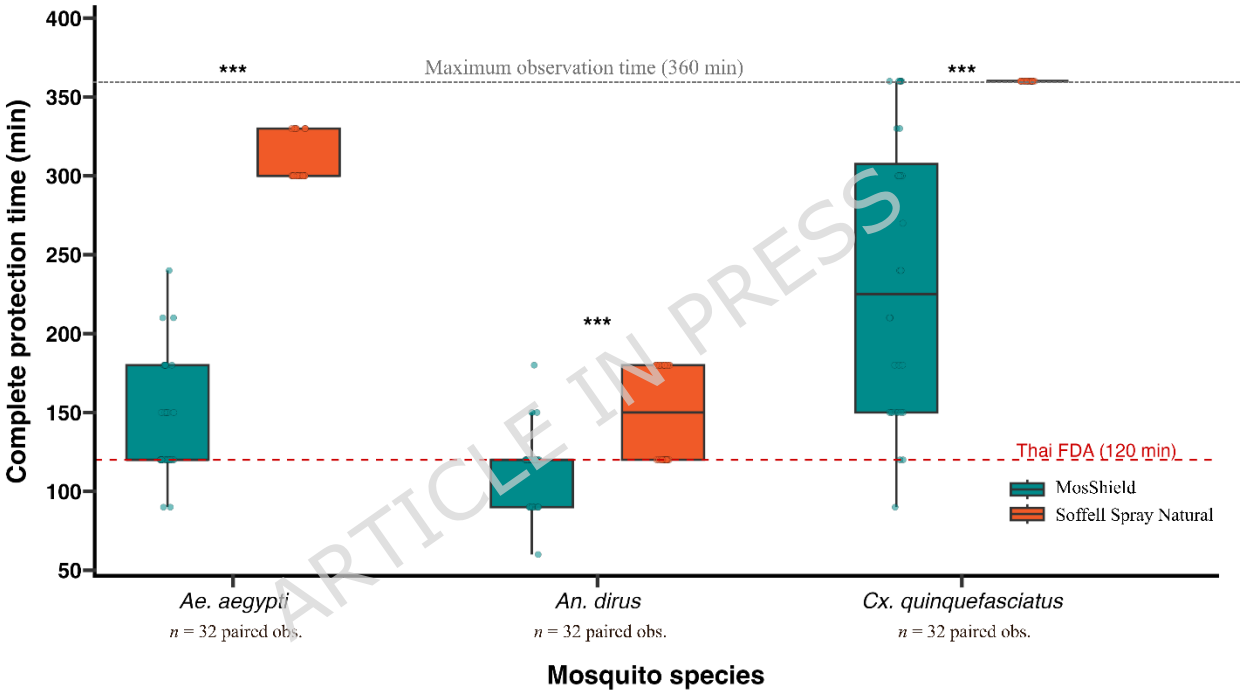

Fig 1. Distribution of complete protection time (CPT) for MosShield spray and Soffell Spray Natural against three mosquito species. Boxes show IQR, horizontal lines indicate medians, whiskers indicate ranges, and points represent individual CPT observations. Sample-size annotations indicate paired CPT observations, not unique recruited volunteers. The red dashed line indicates the Thai FDA efficacy threshold of 120 min, and the grey dashed line indicates the maximum observation time of 360 min; observations reaching 360 min without protection failure

were treated as right-censored. Asterisks (***) indicate significant differences between formulations within each mosquito species (Wilcoxon signed-rank test, $p < 0.001$).

4. Discussion

The present study provides product-relevant laboratory evidence supporting MosShield, a ready-to-use 6% w/w *L. cubeba* essential oil–based spray formulation, as a plant-based topical mosquito repellent candidate. Under standardized arm-in-cage conditions, the formulation provided measurable repellency against three medically important mosquito species and achieved median CPT values of 225 min against *Cx. quinquefasciatus* and 120 min against both *Ae. aegypti* and *An. dirus*. These median CPT values reached the Thai FDA efficacy criterion of ≥120 min under the tested laboratory conditions. Importantly, this study evaluated the complete final formulation rather than the essential oil alone, providing evidence that is more directly relevant to product development. In addition, no visible dermal irritation was observed in the short-term skin-patch test, supporting the preliminary human tolerability of the ready-to-use formulation. The repellent activity observed in the present study is consistent with previous reports showing that plant-derived essential oils, including *L. cubeba* essential oil, can reduce mosquito host-seeking or biting responses (Amer and Mehlhorn 2006; Noosidum et al. 2008; Maia and Moore 2011). Botanical repellents are generally considered to act, at least in part, by interfering with mosquito orientation to host-associated cues such as skin-derived odors, carbon dioxide, heat, and moisture (Gibson and Torr 1999; Bohbot and Dickens 2010; Takken and Verhulst 2013).

Previous studies have suggested that citral-related compounds and other volatile monoterpenes may influence mosquito host-seeking behavior through olfactory-mediated pathways, including modulation of sensory responses associated with TRPA1-mediated aversion and odorant receptor-related processes in experimental insect systems (Bohbot and Dickens 2010;

Kwon et al. 2010; Melo et al. 2021). Other plant-derived monoterpenes, such as limonene, linalool, eucalyptol, citronellal, α-pinene, and β-pinene, have also been reported in previous studies to show repellent, insecticidal, or sensory-modulating activity against mosquitoes or other arthropods (Hollingsworth 2005; Tambwe et al. 2014; Gadelhaq et al. 2023; Lin et al. 2024; Kumar et al. 2025). In the present study, individual chemical constituents or defined mixtures were not tested separately, and mosquito olfactory receptors, ion channels, odorant-binding proteins, neurophysiological responses, and detoxification pathways were not assessed. Therefore, the observed repellency should be interpreted as the activity of the complete MosShield formulation under the tested laboratory conditions. Potential mechanisms involving olfactory interference, host-cue masking, sensory modulation, or additive effects of volatile constituents should be regarded as literature-supported hypotheses rather than mechanisms directly demonstrated by the current experiments.

A notable finding of the present study was the variation in CPT among the three mosquito species. *Cx. quinquefasciatus* showed the longest median CPT with MosShield, whereas *Ae. aegypti* and *An. dirus* showed shorter median CPT values. This variation may reflect species-specific differences in host-seeking behavior, biting activity, or responsiveness to plant-based repellent formulations. For example, *Cx. quinquefasciatus* is predominantly nocturnal and relies strongly on host-associated cues for host localization, whereas *Ae. aegypti* is diurnal and integrates olfactory, visual, and thermal cues during host seeking (Gibson and Torr 1999; van Breugel et al. 2015). Such behavioral differences may influence responses to volatile plant-derived repellents (Takken and Verhulst 2013). The longer CPT observed against *Cx. quinquefasciatus* compared with *Ae. aegypti* and *An. dirus* therefore suggests species-dependent variation in responsiveness to the MosShield formulation. Previous studies have also shown that mosquito species can differ in

their responses to plant-derived repellents and host-associated cues (Carey et al. 2010; Takken and Verhulst 2013; Uniyal et al. 2016; Afify and Potter 2020). Further studies incorporating sensory, physiological, or field-derived population data would help clarify the biological basis of these species-dependent differences.

Soffell Spray Natural, a commercial citronella-based repellent, was included as a practical positive control and commercial benchmark. Although Soffell Spray Natural showed longer CPT values than MosShield for all three mosquito species, the comparison provides useful context for positioning MosShield against an existing botanical repellent product. Because the two products differed in active ingredient identity, active ingredient concentration, and overall formulation composition, the observed differences in CPT should be interpreted as benchmark performance rather than ingredient-equivalent or concentration-matched efficacy (Maia and Moore 2011). These findings indicate that MosShield achieved measurable protection and met the Thai FDA efficacy criterion despite using a different plant-derived active ingredient and formulation system. Further studies using standardized concentrations and matched formulation bases would be needed to directly compare the repellent performance of *L. cubeba* oil and citronella oil.

Beyond repellency, dermal tolerability is an important consideration for topical repellent formulations. In the present study, short-term skin-patch testing showed no visible signs of erythema, edema, vesiculation, or irritation in any of the 48 volunteers during the 96-h observation period. The formulation also had a pH of 6.15, which is close to the physiological skin-surface pH range reported in previous dermatological studies (Schmid-Wendtner and Korting 2006; Proksch 2018). These findings indicate that the complete MosShield formulation was well tolerated under the specific short-term test conditions (Tisserand and Young 2014). A vehicle-only control patch was not included in this study; therefore, the absence of visible adverse skin reactions should be

interpreted as evidence of short-term tolerability of the complete final formulation only. It cannot distinguish whether the tolerability outcome was related to the active ingredient (*L. cubeba* essential oil), the excipient components, or their combined formulation. Future tolerability studies should include matched vehicle-only patches alongside the complete formulation to better clarify the contribution of individual formulation components. The study also incorporated non-confidential formulation-level information to improve transparency while maintaining trade-secret protection. Although the exact excipient ratios of MosShield could not be disclosed, the manuscript provides functional formulation components and non-confidential pre-testing physicochemical characteristics to support interpretation and reproducibility of the biological findings. This is particularly relevant for product-oriented repellent research, where performance depends not only on the active essential oil but also on the final formulation system. In the present study, a single laboratory-scale formulation preparation was aliquoted into three vials for testing against the three mosquito species, minimizing formulation variation during the laboratory evaluation. Future studies assessing independently prepared batches would further support inter-batch consistency. Because the repellency assays were conducted under controlled arm-in-cage conditions using pathogen-free laboratory mosquito colonies, semi-field and field studies using broader mosquito populations are warranted to confirm product performance under real-use conditions.

Overall, this study supports the applied value of MosShield as a plant-based topical mosquito repellent candidate. Under controlled laboratory conditions, the 6% w/w *L. cubeba* essential oil spray provided measurable protection against *Ae. aegypti*, *An. dirus*, and *Cx. quinquefasciatus*, met the Thai FDA efficacy criterion, and showed no visible irritation in the short-term skin-patch test. By evaluating the complete final formulation together with non-confidential quality-control parameters, this study provides a practical foundation for further

product-oriented development, including semi-field and field validation, dose–response testing, repeated-use dermal safety assessment, vehicle-controlled tolerability testing, formal stability, and post-opening repellency studies.

5. Conclusion

This study demonstrates the applied value of MosShield as a ready-to-use, plant-based topical mosquito repellent candidate formulated with 6% w/w *L. cubeba* essential oil. Under standardized laboratory conditions, the formulation provided measurable topical protection against *Cx. quinquefasciatus, Ae. aegypti,* and *An. dirus*, met the Thai FDA efficacy criterion, and showed no visible dermal irritation in the short-term skin-patch test. By evaluating the complete final formulation together with non-confidential quality-control parameters, this study provides product-relevant evidence that bridges laboratory repellent testing and further formulation development. These findings support further development of MosShield through semi-field and field validation, dose–response assessment, repeated-use dermal safety testing, and formal stability and post-opening repellency studies.

Declarations

Funding

Financial support for this study was provided by the Mahidol University Fundamental Fund (fiscal year 2025), funded by the National Science Research and Innovation Fund (NSRF).

Competing interests

The authors declare that the MosShield formulation is protected as a trade secret under the Thai Trade Secret Act B.E. 2545 (2002), as amended by B.E. 2558 (2015). Apart from this legally protected confidential information, the authors declare no competing interests.

Ethical approval and consent

All experimental procedures were conducted in accordance with institutional ethical standards and national regulations. Animal-related procedures were approved by the Institutional Animal Care and Use Committee (FTM-ACUC), Faculty of Tropical Medicine, Mahidol University (Certificate No. FTM-ACUC 020/2024). Human volunteer procedures were reviewed and approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (Certificate No. MUTM 2024-091-01). Written informed consent was obtained from all participants prior to enrolment, and all procedures complied with the Declaration of Helsinki and relevant local guidelines. Participants provided informed consent for the use and publication of anonymized data.

Clinical trial number: Not applicable.

Acknowledgements

This research project has been supported by Mahidol University (Fundamental Fund: fiscal year 2025 by National Science Research and Innovation Fund (NSRF)). The authors gratefully acknowledge Professor Passanesh Sukphopetch, M.D., Ph.D., Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, for valuable supervision and guidance during the allergy patch testing procedures. The authors also thank the staff of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, for their technical assistance and collaboration, which were essential to the successful completion of this study.

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Data availability

All data generated or analyzed during this study are included in this published article and its Supplementary Information. Additional raw repellency datasets are available from the corresponding author upon reasonable request. Proprietary formulation details are not publicly available due to trade-secret protection.

References

Achee NL, Grieco JP, Vatandoost H et al (2019) Alternative strategies for mosquito-borne arbovirus control. PLoS Negl Trop Dis 13:e0006822. https://doi.org/10.1371/journal.pntd.0006822

Afify A, Potter CJ (2020) Insect repellents mediate species-specific olfactory behaviours in mosquitoes. Malar J 19:127. https://doi.org/10.1186/s12936-020-03206-8

Amer A, Mehlhorn H (2006) Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. Parasitol Res 99:478-490. https://doi.org/10.1007/s00436-006-0184-1

Bohbot JD, Dickens JC (2010) Insect repellents: modulators of mosquito odorant receptor activity. PLoS One 5:e12138. https://doi.org/10.1371/journal.pone.0012138

Bruze M, Svedman C (2025) Clarification and modification of the International Contact Dermatitis Research Group classification of patch test reactions on behalf of the International Contact Dermatitis Research Group. Dermatitis 36:440-446. https://doi.org/10.1089/derm.2024.0365

Carey AF, Wang G, Su CY et al (2010) Odorant reception in the malaria mosquito *Anopheles gambiae*. Nature 464:66-71. https://doi.org/10.1038/nature08834

Fregert S (1981) Manual of contact dermatitis, 2nd edn. Munksgaard, Copenhagen, pp 1-139

Gadelhaq SM, Boelhadid SM, Abdel-Baki AS et al (2023) D-limonene nanoemulsion: lousicidal activity, stability, and effect on the cuticle of *Columbicola columbae*. Med Vet Entomol 37:63-75. https://doi.org/10.1111/mve.12607

Gibson G, Torr SJ (1999) Visual and olfactory responses of haematophagous Diptera to host stimuli. Med Vet Entomol 13:2-23. https://doi.org/10.1046/j.1365-2915.1999.00163.x

Gryseels C, Uk S, Sluydts V et al (2015) Factors influencing the use of topical repellents: implications for the effectiveness of malaria elimination strategies. Sci Rep 5:16847. https://doi.org/10.1038/srep16847

Hollingsworth RG (2005) Limonene, a citrus extract, for control of mealybugs and scale insects. J Econ Entomol 98:772-779. https://doi.org/10.1603/0022-0493-98.3.772

Kamle M, Mahato DK, Lee KE et al (2019) Ethnopharmacological properties and medicinal uses of *Litsea cubeba*. Plants (Basel) 8:150. https://doi.org/10.3390/plants8060150

Kumar R, Mittal P, Airi M (2025) Bioactivity of linalool against *Culex quinquefasciatus*: repellent and larvicidal evaluations. Arch Insect Biochem Physiol 120:e70098. https://doi.org/10.1002/arch.70098

Kwon Y, Kim SH, Ronderos DS et al (2010) Drosophila TRPA1 channel is required to avoid the naturally occurring insect repellent citronellal. Curr Biol 20:1672-1678. https://doi.org/10.1016/j.cub.2010.08.016

Lin H, Li ZY, Sun Y et al (2024) D-Limonene: promising and sustainable natural bioactive compound. Appl Sci 14:4605. https://doi.org/10.3390/app14114605

Lupi E, Hatz C, Schlagenhauf P (2013) The efficacy of repellents against *Aedes*, *Anopheles*, *Culex* and *Ixodes* spp. - a literature review. Travel Med Infect Dis 11:374-411. https://doi.org/10.1016/j.tmaid.2013.10.005

Lwetoijera DW, Harris C, Kiware SS et al (2014) Increasing role of *Anopheles funestus* and *Anopheles arabiensis* in malaria transmission in the Kilombero Valley, Tanzania. Malaria J 13:331. https://doi.org/10.1186/1475-2875-13-331

Maia MF, Moore SJ (2011) Plant-based insect repellents: a review of their efficacy, development and testing. Malaria J 10 (Suppl 1):S11. https://doi.org/10.1186/1475-2875-10-s1-s11

Mapossa AB, Focke WW, Tewo RK et al (2021) Mosquito-repellent controlled-release formulations for fighting infectious diseases. Malaria J 20:165. https://doi.org/10.1186/s12936-021-03681-7

Melo N, Capek M, Arenas OM et al (2021) The irritant receptor TRPA1 mediates the mosquito repellent effect of catnip. Curr Biol 31:1988-1994.e5. https://doi.org/10.1016/j.cub.2021.02.010

Noosidum A, Prabaripai A, Chareonviriyaphap T et al (2008) Excito-repellency properties of essential oils from *Melaleuca leucadendron* L., *Litsea cubeba* (Lour.) Persoon, and *Litsea salicifolia* (Nees) on *Aedes aegypti* (L.) mosquitoes. J Vector Ecol 33:305-312. https://doi.org/10.3376/1081-1710-33.2.305

Obsomer V, Defourny P, Coosemans M (2007) The *Anopheles dirus* complex: spatial distribution and environmental drivers. Malaria J 6:26. https://doi.org/10.1186/1475-2875-6-26

Overgaard HJ, Pientong C, Thaewnongiew K et al (2018) Assessing dengue transmission risk and a vector control intervention using entomological and immunological indices in Thailand: study protocol for a cluster-randomized controlled trial. Trials 19:122. https://doi.org/10.1186/s13063-018-2490-1

Phukerd U, Soonwera M (2014) Repellency of essential oils extracted from Thai native plants against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say). Parasitol Res 113:3333-3340. https://doi.org/10.1007/s00436-014-3996-4

Proksch E (2018) pH in nature, humans and skin. J Dermatol 45:1044–1052. https://doi.org/10.1111/1346-8138.14489

Robbins PJ, Cherniack MG (1986) Review of the biodistribution and toxicity of the insect repellent *N,N*-diethyl-m-toluamide (DEET). J Toxicol Environ Health 18:503-525. https://doi.org/10.1080/15287398609530891

Russell TL, Govella NJ, Azizi S et al (2011) Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. Malaria J 10:80. https://doi.org/10.1186/1475-2875-10-80

Sutthanont N, Sudsawang M, Phanpoowong T et al (2022) Effectiveness of herbal essential oils as single and combined repellents against *Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus* (Diptera: Culicidae). Insects 13:658. https://doi.org/10.3390/insects13070658

Sutthanont N, Sukphopetch P, Rungruang R et al (2026) Formulation and evaluation of topical essential oil lotion as repellent against *Aedes aegypti*. Curr Res Parasitol Vector Borne Dis 9:100354. https://doi.org/10.1016/j.crpvbd.2026.100354

Schmid-Wendtner MH, Korting HC (2006) The pH of the skin surface and its impact on the barrier function. Skin Pharmacol Physiol 19:296–302. https://doi.org/10.1159/000094670

Takken W, Verhulst NO (2013) Host preferences of blood-feeding mosquitoes. Annu Rev Entomol 58:433-453. https://doi.org/10.1146/annurev-ento-120811-153618

Tambwe MM, Mbeyela EM, Massinda BM et al (2014) Experimental hut evaluation of linalool spatial repellent agar gel against *Anopheles gambiae* sensu stricto mosquitoes in a semi-field system in Bagamoyo, Tanzania. Parasit Vectors 7:550. https://doi.org/10.1186/s13071-014-0550-2

Thai Industrial Standards Institute (2010) Mosquito repellents: Thai Industrial Standards. Ministry of Industry, Bangkok

Tisserand R, Young R (2014) Essential oil safety: a guide for health care professionals, 2nd edn. Churchill Livingstone/Elsevier, St. Louis

Uniyal A, Tikar SN, Mendki MJ et al (2016) Behavioral response of *Aedes aegypti* mosquito towards essential oils using olfactometer. J Arthropod Borne Dis 10:370-380

van Breugel F, Riffell J, Fairhall A et al (2015) Mosquitoes use vision to associate odor plumes with thermal targets. Curr Biol 25:2123-2129. https://doi.org/10.1016/j.cub.2015.06.046

Wilson AL, Courtenay O, Kelly-Hope LA et al (2020) The importance of vector control for the control and elimination of vector-borne diseases. PLoS Negl Trop Dis 14:e0007831. https://doi.org/10.1371/journal.pntd.0007831

World Health Organization (2009) Guidelines for efficacy testing of mosquito repellents for human skin. WHO, Geneva