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SMALL SCALE FIELD EVALUATION OF A NOVEL PALM-BASED INSECTICIDE FORMULATION USING COLD AND THERMAL FOGGING AGAINST *Aedes aegypti*

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ABSTRACT

A new insecticide formulation with deltamethrin as an active ingredient (F1) has been developed in order to suppress the worldwide spread of mosquito-borne diseases. The formulation is naphtha-free and utilises locally sourced palm-based materials to obtain a cost-effective product. The objective of this study is to assess the performance of the formulation, in terms of droplet size and efficacy, in comparison to a commercial naphtha-based insecticide (F2). Open-field bioassay of the formulations against adult Aedes aegypti *was conducted using cold and thermal fogging methods and measured at distance points 25, 50, 75 and 100 m. Overall, droplet size and efficacy significantly decreased with increasing distance from the spray path. No significant differences in volume median diameter (VMD) were observed between fogging methods and formulations. In general, both formulations showed significantly higher efficacy as cold fogs than thermal fogs. Significant differences in knockdown effect were observed between methods, time and formulations. Nevertheless, the definite effect of insecticidal formulations,* i.e. *mortality, was not significantly different between F1 and F2. In conclusion, our results show that the palm-based formulation was able to achieve comparable performance with the commercial formulation without the use of naphtha.*

Keywords: Deltamethrin, droplet size, efficacy, space spray, vector control.

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INTRODUCTION

Dengue, an arboviral disease, has spread rapidly across the globe and is becoming endemic in more than 100 countries (Selvarajoo *et al.*, 2020). Regular dengue outbreaks have occurred in Malaysia since the 1960s, and the case numbers continue to increase alongside Malaysia's rapid population and infrastructure growth. Malaysia recorded the biggest spike in dengue cases in four years in 2019 (AbuBakar *et al.*, 2022). Dengue vector control activities in Malaysia rely heavily on human resources (Yazan *et al.*, 2021). Thus, lockdowns or movement control order (MCO) to contain the spread of Coronavirus disease 2019 (COVID-19) have significantly curtailed regular dengue control operations (Rahim *et al.*, 2021). At present, there are no effective treatments available for dengue. Furthermore, the current vaccine exhibits moderate efficacy and fails to provide balanced protection against all four dengue serotypes (Stanaway *et al.*, 2016). Hence, control of the dengue vector population remains the mainstay strategy for containing the spread of the disease.

Space spraying using thermal and cold foggers is an often-used intervention strategy for controlling mosquito-borne diseases (Pryce *et al.*, 2018). However, space sprays are only effective when the droplets remain airborne. Thus, multiple applications are necessary for effective control of the dengue vector population [World Health Organization (WHO), 2009]. With the ever-increasing fuel cost and concerns for adverse environmental impacts from frequent space spray interventions, the use of water-dilutable water-based formulation is turning into an appealing and trendy alternative to oil-

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based formulation. Questions have been raised about the efficacy of water-based formulations as the vapour pressure of water causes droplet evaporation and consequently size reduction, creating insecticide droplets that are prone to drift (Farajollahi & Williams, 2013). Nevertheless, efficacy studies using water-based formulations have demonstrated that they are equally or more effective than formulations using diesel fuel or mineral oil as a diluent (Farajollahi & Williams, 2013; Harburguer *et al.,* 2012a).

In recent years, bio-based formulations incorporating plant and microbial bioactives have been explored to address environmental impacts and insecticide resistance in mosquito control. Bioformulations of plant extracts from *Carica papaya* and *Parthenium hysterophorous* have shown promising larvicidal activity against *Aedes aegypti* (Jayaraman *et al*., 2023). Entomopathogenic fungi, *Beauveria bassiana* JN5R1W1, when formulated as inverted emulsions with soybean oil demonstrate high mortality rates against adult mosquitoes through both direct and indirect contact methods (Yong-Lee *et al*., 2023). Under the field-simulated trial, *Ocimum kilimandscharicum* oil formulation achieved 98% larvicidal mortality against *Anopheles gambiae* larvae after 24 hr, as opposed to 54% mortality achieved by *Bacillus thuringiensis* subsp. *israelensis* granules (Ochola *et al*., 2022). While these bioformulations comprising bioactives show promise, they often lack the robustness and field applicability of synthetic chemicals. Thus, while bioactives provide potential supplemental strategies, synthetic chemicals remain the cornerstone of effective vector control in the field.

In response to these challenges, a novel water-based insecticidal formulation (F1) comprising palm-based solvents and surfactants has been developed to restrain dengue outbreaks and minimise the environmental impact of vector control (Mohsin *et al.,* 2019). The formulation contains deltamethrin, a larvicide and adulticide that causes an instant knockdown by paralysing the nervous system of insects (Kumar *et al*., 2011). F1 is free from highly volatile and flammable naphtha solvent and incorporates palm-based methyl esters (PME) as a substitute. PME is less volatile and possesses higher flash point compared to petroleum-based solvents (Mohsin *et al*., 2017). Additionally, F1 comprises non-toxic and readily biodegradable palm-based surfactants which are instrumental in improving the stability of pesticide emulsions (Ghazali & Ahmad, 2004; Ismail *et al*., 2014; Shaari *et al*., 2022; Siti Afida *et al*., 2016). Malaysia's export revenue of palm-based oleochemicals in 2023 declined by 30.1% from the previous year despite the marginal improvement in export volume (4.8%) due to lower imports by the major buyers of Malaysian palm oil (Parveez *et al.,* 2024). Given the recent decline, leveraging these locally sourced bio-based components offers both economic and strategic benefits by reducing dependency on imported hydrocarbons.

This study addresses the novel application of F1 as an environmentally friendly and cost-effective solution for dengue vector control. By comparing F1 to a commercially available deltamethrin formulation (F2) in an open-field setting, we aim to evaluate their respective efficacies in terms of droplet size distribution, knockdown and mortality of the primary dengue virus vector *Ae. aegypti* using cold and thermal fogging methods. This investigation is critical in determining whether a palm-based water-dilutable insecticide can meet or exceed the performance of conventional formulations, providing a new, sustainable avenue for managing dengue outbreaks in Malaysia and other endemic regions.

MATERIALS AND METHODS

Biological Material

Sucrose-fed adult *Ae. aegypti* females (aged 2-5 days) from the Vector Control Research Unit, Universiti Sains Malaysia (USM), Penang, Malaysia, were used for the adulticidal assessments.

Chemicals

PME comprising a mixture of methyl caproate, methyl octanoate, methyl decanoate and methyl laurate at 3.2 wt.%, 50.8 wt.%, 44.0 wt.% and 2.0 wt.%, respectively, was provided by Emery Oleochemicals Sdn. Bhd. (Selangor, Malaysia). Deltamethrin (98.0%) was donated by Hextar Chemicals Sdn. Bhd. (Selangor, Malaysia). Xanthan gum derived from *Xanthomonas campestris* was purchased from Sigma-Aldrich Sdn. Bhd. (Selangor, Malaysia). Bio-based surfactants, C12-C14 fatty alcohol ethoxylate and polyoxyethylene (20) sorbitan monooleate, are used in the study. The former was obtained from Huntsman Singapore Pte Ltd (Gateway West, Singapore), while the latter was purchased from Chemweb Sdn. Bhd. (Selangor, Malaysia).

Insecticide Formulations

F1 was prepared using the method in a previous study by Mohsin *et al.,* (2019). In brief, the formulation was developed using 2.0 wt.% deltamethrin, 5.0 wt.% mixture of the previously mentioned bio-based surfactants and 13.0 wt.% PME as the oil phase and distilled water and 0.4 wt.% xanthan gum as the aqueous phase. The hydrophilic-lipophilic balance of the surfactant was kept constant at 10. The oil phase was prepared by stirring for 10 min at 55°C, while the aqueous phase was prepared by dispersing xanthan gum in distilled water at 55°C. Thereon, the oil phase was gradually incorporated into the aqueous phase using a Kinematica Polytron® PT3100 homogeniser (Malters, Switzerland) operated at 7,000 rpm for 10 min. The commercial insecticide (F2) is an oil-in-water (o/w) emulsion formulation containing 2.0 wt.% deltamethrin and petroleum-based naphtha from Bayer Environmental Science (Valencia, Spain). The blank formulation (F3) was similarly produced according to the previously mentioned process without deltamethrin to examine the possible insecticidal activity of the emulsion system.

Fogging Method

All insecticide formulations were water-diluted at a dilution ratio of 1:100 v/v. Then, thermal fogging was applied at 5,000 mL ha^{-1} using a hand carried Agrofog Hand Held Thermal Fogger (Sing Industrial Complex, Singapore). On the other hand, cold fogging was performed at 500 mL ha^{-1} using a truck mounted LECO ULV Fog Generator Model 1800 (St. Joseph, MI, USA) with its sprayer head nozzle angled 30° upwards to the horizontal plane. The truck then travelled 6-9 km hr^{-1} at a distance of 200 m perpendicular to the spray angle.

Droplet Size Measurement and Efficacy Assessment

Field tests were performed in accordance with the WHO guidelines WHO (2009) in an open field in USM, with no tall grasses that should hinder fog movement. The study was conducted during fair weather. The temperature during treatment was 27°C-30°C, the relative humidity was 70%-80% and the wind speed was 1.5-3.0 m s^{-1}. A total of 20 mosquitoes were held in nylon mesh-screened cylindrical cages (1.2 mm mesh size) with wire frame support (10 cm diameter × 15 cm height × 10 cm tapping cover). The cages were suspended at 1.5 m from the ground on poles distanced at 25, 50, 75 and 100 m from the spray nozzle. Knockdown was recorded after 15 min, and the mosquitoes were transferred into mesh covered cups using battery operated aspirator and returned to the laboratory. The cups were provided with a cotton pad soaked with 10% sucrose solution. Then, knockdown was recorded again at 30 min and 60 min post-treatment while adult mortality was recorded at 24 hr post-treatment. Three replicates were conducted for each formulation (including water control *i.e.* water without insecticide). Results from the water control treatment were excluded from reporting as knockdown and mortality activities were not observed. In each test, one set of adult cages was kept in the laboratory as a control. If mortality was above 20% in the concurrent controls, the whole test would be rejected. Results from the treated samples will be corrected using Abbott's formula if mortality in the controls was above 5% (Abbott, 1925). Abbott's formula for calculating corrected mortality is as Equation (1):

$$\frac{\%\ \text{Mortality in treated} - \%\ \text{Mortality in control}}{100\% - \%\ \text{Mortality in control}} \times 100 \qquad (1)$$

To record fog droplet sizes at varied pole distances, magnesium oxide (MgO)-coated slides were placed approximately 1.5 m above the ground on a slide rotator at each pole. The droplets were examined under a microscope and their volume medium diameter (VMD) in mm was calculated using Microsoft Excel developed by Clarke Engineering Technologies (St. Charles, IL, USA).

Statistical Analysis

Data were analysed using IBM SPSS Statistics 25 (IBM, Armonk, NY, USA). Two-way analysis of variance (ANOVA) followed by Tukey's Honestly-Significant-Difference (Tukey HSD) post-hoc test was carried out to determine significant differences in fog droplet sizes between formulations (F1, F2 and F3) and distance (25, 50, 75 and 100 m). Similarly, two-way ANOVA and Tukey HSD were carried out to determine significant differences in the efficacy results (knockdown and mortality) between distance and knockdown time (15, 30 and 60 min). Differences between means were considered statistically significant at $p<0.05$. In addition, t-tests were carried out to compare the means of droplet sizes between fogging methods and means of efficacy results between formulations (F1 and F2) and fogging methods.

RESULTS AND DISCUSSION

Droplet Size

Fog droplet size is an important factor influencing droplet deposition and the efficacy of insecticides. During space spraying, large droplets may fail to stay airborne or penetrate obstacles, while very small droplets may fail to land on target insects due to aerodynamics and convection currents (Farajollahi & Williams, 2013). VMD is a commonly used parameter to describe the droplet diameter, *i.e.* where 50% of the spray liquid volume consists of droplets smaller than this value

(He *et al.*, 2022). Accordingly, the optimum droplet size for efficacious ground adulticiding was determined to be between 12 and 20 µm VMD (Bonds, 2012). The VMD of droplets produced in this study are within said range until up to 50 m but decline below the optimal size at further distances (*Table 1*).

Two-way ANOVA analysis showed no significant difference in VMD between formulations (*Table 2*), indicating that droplets produced using palm-based formulations F1 and F3 were comparable to the commercial product, F2. The water-based formulations exhibited significant droplet size reduction with increasing distance. The finding is in line with previous studies by Bengoa *et al.* (2014) and Farajollahi and Williams (2013), which attributed the droplet size reduction to water evaporation in the outdoor environment as they travelled further away from the spray nozzle. Additionally, t-test analysis revealed that there is no significant difference in the droplet sizes produced by cold and thermal foggers (*Table 2*), which is in agreement with the findings obtained by Harburguer *et al.* (2012b) and Megat Nabil Mohsin *et al.* (2023). There was a statistically significant interaction between method and distance. In contrast, the interaction between formulation and distance and the interaction between formulation and method were non-significant (*Table 2*).

Knockdown of Adult Mosquitoes

Quick knockdown from exposure to insecticidal formulation ensures a reduction in feeding and insect reproduction and diminishes the chances for insects to escape (Georgia *et al.*, 2018). In the absence of deltamethrin, F3 showed negligible knockdown with the maximum being only 3.33% at all checkpoints via cold fogger and 1.67% at the 25 m checkpoint via thermal fogger after 60 min (*Figure 1*). The results showed that the palm-based emulsion system alone had no knockdown activity and thus is excluded from statistical analysis.

A significant decrease in knockdown was observed as the distance increased, similar to the trend reported for droplet size. However, unlike droplet size, knockdown was significantly different between methods and between formulations F1 and F2 (*Table 3*). With cold fogging, knockdown by F1 was slightly lower than the commercial insecticide at the 15 min interval (*Figure 1a*). However, the opposite result was observed with thermal fogging (*Figure 1b*). In general, it was observed that knockdown significantly increased with time. At the 30- and 60-min intervals, cold fogging using F1 and F2 had achieved 100% knockdown at all checkpoints intervals, indicative of a low recovery rate for the knocked-down mosquitoes. Nevertheless, lower knockdown percentages were observed with thermal fogging compared to cold fogging as both F1 and F2 had failed to achieve complete knockdown even at 60 min (*Figure 1b*).

Two-way ANOVA revealed significant interactions between method and distance, method and formulation, method and time, time and distance, as well as time and formulation. However, there was no significant difference in the interaction between formulation and distance (*Table 3*). A note

TABLE 1. DROPLET MEASUREMENT AT SPECIFIED DISTANCES USING DIFFERENT FOGGING METHODS

Fogging method	Formulation	VMD (µm) (mean ± S.E.)			
		25 m	50 m	75 m	100 m
Cold	F1	13.40 ± 0.12	12.60 ± 0.10	11.57 ± 0.06	10.20 ± 0.06
	F2	13.40 ± 0.06	12.73 ± 0.03	11.50 ± 0.06	10.23 ± 0.09
	F3	13.33 ± 0.12	12.63 ± 0.03	11.97 ± 0.18	10.10 ± 0.00
Thermal	F1	12.83 ± 0.12	12.30 ± 0.12	11.87 ± 0.20	10.30 ± 0.06
	F2	12.70 ± 0.10	12.27 ± 0.03	12.07 ± 0.09	10.30 ± 0.12
	F3	12.87 ± 0.07	12.23 ± 0.07	11.90 ± 0.06	10.30 ± 0.06

Note: F1 - novel water-based insecticidal formulation; F2 - commercial insecticide; F3 - blank formulation; VMD - volume median diameter; S.E. - standard error.

TABLE 2. THE EFFECTS OF FOGGING METHOD, FORMULATION AND DISTANCE ON FOG DROPLET SIZE

Variable	df	F-value	*P*-value
Fogging method	70	4.417	0.580 (2-tailed)
Formulation	2	0.219	0.804
Distance	3	885.136	<0.001
Fogging method × distance	3	24.004	<0.001
Fogging method × formulation	2	0.237	0.790
Formulation × distance	6	0.972	0.453

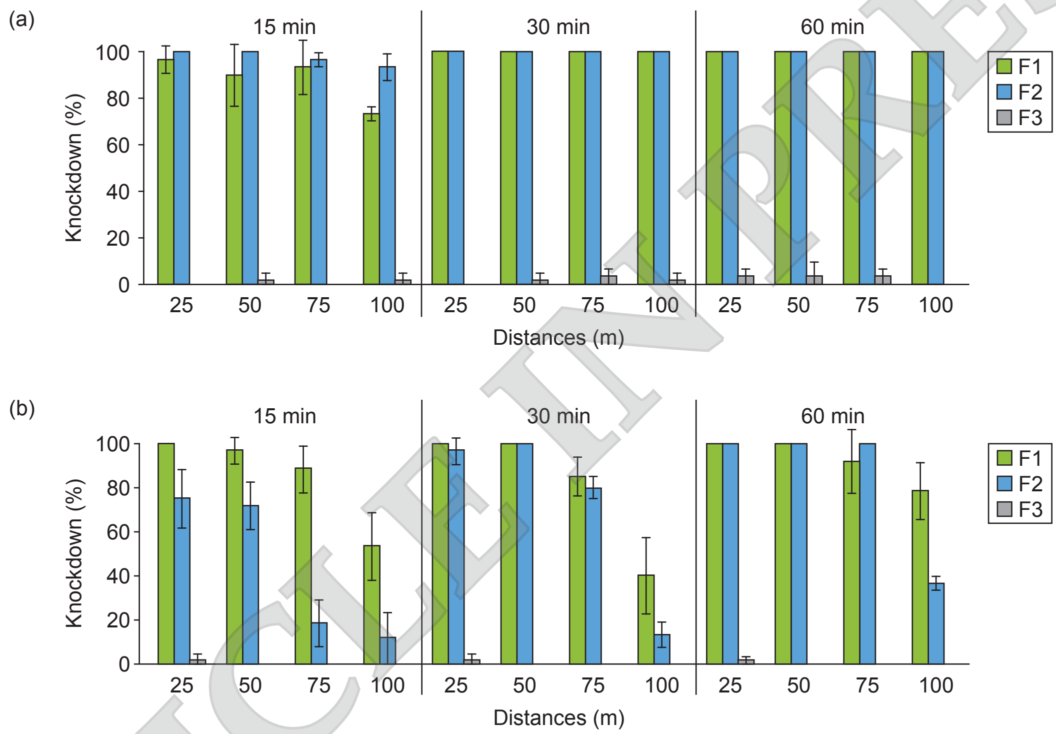
Note: df - degree of freedom.

of caution is due here since the knockdown effect, *i.e.* the paralysis of insects due to insecticides is potentially reversible (Tsaganou *et al.*, 2021). Therefore, we must also look into the delayed but definite effect of insecticidal formulations, *i.e.*, mortality.

Adult Mortality

The objective of chemical space spraying is to minimise disease transmission by reducing the target infectious adult mosquitoes and their populations (Zuharah *et al.*, 2019). As shown in *Figure 2*, negligible adult mortality was observed with F3, ranging from 0%-3% for cold fogging and 0%-5% for thermal fogging. This result further reinforces that the palm-based emulsion system used in the formulation provided no insecticidal activity.

The statistical analysis demonstrated no significant differences in adult mortality between F1 and F2 (*Table 4*). Despite the absence of highly volatile and flammable naphtha solvents, the palm-based formulation was able to provide comparable adulticidal efficacy with the commercial formulation. In this study, mortality



Note: F1 - novel water-based insecticidal formulation; F2 - commercial insecticide; F3 - blank formulation.

Figure 1. Percentage knockdown (mean ± S.E.) of adult Ae. aegypti *after exposure to fogging formulations using (a) cold and (b) thermal fogger.*

TABLE 3. THE EFFECTS OF FOGGING METHOD, FORMULATION, KNOCKDOWN TIME AND DISTANCE ON PERCENTAGE KNOCKDOWN

Variable	df	F-value	*P*-value
Fogging method	142	97.787	<0.001 (2-tailed)
Formulation	142	16.819	0.049 (2-tailed)
Knockdown time	2	24.530	<0.001
Distance	3	60.943	<0.001
Fogging method × distance	3	44.297	<0.001
Fogging method × formulation	1	36.872	<0.001
Fogging method × time	2	7.390	0.001
Knockdown time × distance	6	2.378	0.033
Knockdown time × formulation	2	4.279	0.016
Distance × formulation	3	2.500	0.063

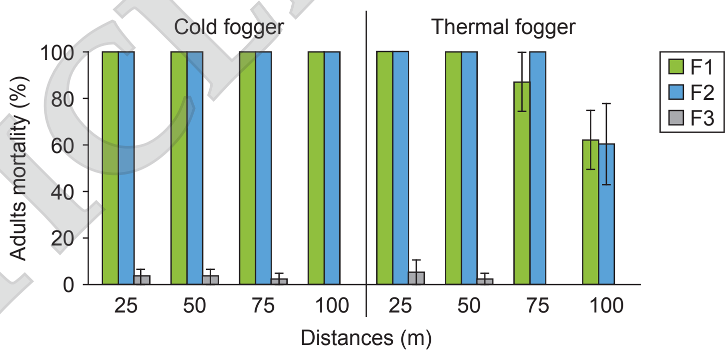
Note: df - degree of freedom.

was seen to decline significantly with increasing distance, a similar pattern was observed with knockdown. This may be attributed to droplet size reduction with increasing travel distance. Larger droplets are able to accomplish higher efficacies compared to smaller droplets because they are harder to transport by air currents because of their larger inertial force (Sugiura *et al.*, 2011). In contrast to knockdown, mortality was significantly different between fogging methods (*Table 4*). It is worth highlighting that the cold fogging method was more effective in controlling adult *Ae. aegypti*, achieving 100% mortalities at all distance points (*Figure 2*). This pattern is in accord with previous studies, which attributed the poorer performance of their water-based products via thermal fogging to excessive heat by the applicator, resulting in reduced efficacy (Fulcher *et al.*, 2016; Megat-Nabil-Mohsin *et al.*, 2023). Nevertheless, additional studies are required to assess the efficacies of this palm-based formulation against immature *Ae. aegypti* stages, pupae and larvae.

Two-way ANOVA revealed that similarly with knockdown, a significant interaction was observed between method and distance, whereas no significant interaction was observed between formulation and distance. Nonetheless, contrary to the trends observed with knockdown, a significant difference was not observed in the interaction between method and formulation (*Table 4*).

CONCLUSION

In conclusion, this study successfully demonstrated that the novel palm-based insecticide formulation (F1) offers comparable efficacy to the commercial naphtha-based formulation (F2) in controlling *Ae. aegypti* mosquitoes, without the environmental and safety concerns associated with petroleum-derived solvents. The field trials using both cold and thermal fogging methods showed no significant differences in droplet sizes between formulations, while the performance of cold fogging was generally superior in terms of knockdown and mortality. Importantly, this research highlights the potential of utilising palm-based ingredients, particularly PME and surfactants, which are biodegradable and locally abundant, reducing dependence on non-renewable resources. The use of F1 also provides a cost-effective and sustainable alternative for dengue vector control, aligning with global trends toward greener, more environmentally responsible pest control solutions. This study is unique in its focus on replacing volatile, flammable naphtha with a bio-based formulation, which is particularly relevant in regions like Malaysia, where palm oil is a major agricultural product. The findings suggest that palm-based insecticidal formulations could play a significant role in future vector control strategies, particularly in tropical and subtropical regions where mosquito-borne diseases are prevalent.



Note: F1 - novel water-based insecticidal formulation; F2 - commercial insecticide; F3 - blank formulation.

Figure 2. Percentage mortality (mean ± S.E.) of adult Ae. aegypti *after exposure to fogging formulations using cold and thermal foggers.*

TABLE 4. THE EFFECTS OF FOGGING METHOD, FORMULATION AND DISTANCE ON ADULT MORTALITY

Variable	df	F-value	*P*-value
Fogging method	46	57.982	0.004 (2-tailed)
Formulation	46	0.239	0.725 (2-tailed)
Distance	3	27.445	<0.001
Fogging method × distance	3	27.445	<0.001
Fogging method × formulation	1	0.665	0.420
Distance × formulation	3	0.955	0.425

Note: df - degree of freedom.

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