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Evaluation of *Melaleuca cajuputi* Powell (Family: Myrtaceae) Extract in Aerosol Can against Dengue Vectors in the Laboratory

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Abstract

The essential oil of *Melaleuca cajuputi* Powell as a botanical insecticide, packaged in aerosol spray cans, was evaluated against dengue vector mosquitoes in the laboratory. Four different concentrations were used in the experiment *viz*: 1, 2, 5, and 10%. A total of 25 adult female *Aedes aegypti* were transferred into 5 cylindrical net cages and hung in a spray testing room. The aerosol cans were weighed before and after spraying. Each aerosol was sprayed for 5 and 10 seconds, respectively, and the test mosquitoes were observed for knockdown and mortality at 1, 5, 10, 15, 20, and 60 minutes. The mosquitoes were then transferred into a paper cup and fed with sugar cubes. After 24 hours, the dead mosquitoes were counted. The experiment was repeated using *Ae. albopictus* females. The results were then compared with the Malaysia SIRIM standard (MS standard) aerosol. High mortality, of 60.2-61.4% and 60.8-64.0%, were observed in both *Aedes* species when sprayed with 5% and 10% *M. cajuputi* aerosol, after exposure for 5 and 10 seconds, respectively. MS standard aerosol exhibited faster and higher mortality, at 97.2-97.6%. There was no significant difference ($p > 0.05$) between *Ae. aegypti* and *Ae. albopictus*. However, *Ae. aegypti* showed higher susceptibility than *Ae. albopictus*. The KT values for the *Aedes* species that had been sprayed for 10 seconds were lower than for 5 seconds. However, the SIRIM standard indicated lower KT values when sprayed for 10 seconds (KT_{50} 0.16 min for *Ae. aegypti* and 2.24 min for *Ae. albopictus*) than essential oil (KT_{50} 168.84 min for *Ae. aegypti* at 10% concentration and 123.71 min for *Ae. albopictus* at 10% concentration). Thus, although the MS standard aerosol (containing 0.07% prallethrin + 0.05% d-phenothrin) was more effective than canned *M. cajuputi* aerosol, aerosol cans containing the essential oil could be utilized for mosquito control, especially since the plant is widely available in Malaysia and elsewhere in the region.

Keywords: *M. cajuputi,* essential oil, aerosol can, *Aedes aegypti*, *Ae. Albopictus*

Introduction

All over the world, mosquito control is becoming increasingly difficult, because several species are becoming physiologically resistant to many of the conventional insecticides [1]. In addition, the extensive and widespread use of synthetic insecticides during the past half century, since the discovery of DDT during

World War II, for the control of household, agricultural, and sylvan pests, and human disease vectors, has prompted concern about the toxicity and environmental impact of some of these agents [2]. Because of these problems and concerns, interest in insecticides derived from natural products, such as plants, has grown worldwide. Therefore, efforts are being made to isolate, screen, and develop phytochemicals with pesticidal activity [2]. Phytochemicals derived from various botanical sources have provided numerous beneficial uses, ranging from pharmaceuticals to insecticides. Several phytochemicals, extracted from various botanical sources, have been reported to have detrimental effects on mosquitoes [3]. *Piper aduncum* (Family Piperaceae), *Litsea elliptica* (Family Lauraceae), and some other plant extracts, reportedly have potential larvicidal activity in the field [4]. The potential of *Melaleuca cajuputi* Powell (Family Myrtaceae) has also been evaluated in the field against *Aedes* spp dengue vectors [5-7].

The prevention of mosquito bites and mosquito-borne diseases through the use of insect repellents is one component in an overall mosquito management strategy [8]. Extracts of 40 essential oils from some plants have been reported effective against mosquitoes, march flies, and sand flies, and of these, the most effective were extracts from *Dacrydium franklini*, *Bachhousia myrtifolia*, *Melaleuca bracteata,* and *Zieria smithii* [9]. Many insect repellent products are currently available commercially in a variety of formulations; some contain active ingredient(s) that are botanical, while some are synthetic organic products. The vast majority are sprays, because of their ease of use, both indoors and outdoors [8]. In Malaysia, some leaf oils have been studied as potentially environmentally friendly insect repellents [10]. This paper reports the insecticidal activities of *M. cajuputi* essential oil extracts in aerosol cans, compared with Malaysia SIRIM standard (MS standard) aerosol cans as control, in the laboratory. The MS standard was used as a standard reference or guideline for the bio-efficacy of household-insecticide testing.

Materials and methods

Plant collection

M. cajuputi leaves were obtained from Port Dickson, Negeri Sembilan (2”31’22.07N 101”48’00.01E) in Peninsular Malaysia, about 120 km from Kuala Lumpur (3”09’00.76”N 101”42’27.73”E). Botanists at the Forest Research Institute of Malaysia (FRIM) identified and confirmed the species. Voucher specimens were deposited at the FRIM Herbarium.

Extraction of essential oils

The essential oils were produced/isolated by hydro-distillation and clevenger apparatus, which comprised a thermal pot, a 500-ml to 1,000-ml round-bottomed distillation flask, an oil separation tube, and a condenser. *M. cajuputi* leaves were left to dry at room temperature, then collected and ground into small particles. The samples were transferred into distillation flasks and their weight recorded; the flasks were immersed in distilled water and heated at 65-70°C. The samples were allowed to boil slowly for 8 hours until distillation was complete. The mixture of distillate oil and water was allowed to settle for 24 hours to facilitate oil collection, by ensuring a clear separation of water and oil layers. The water was slowly drawn off and the remaining oil layer was slowly collected into a glass beaker and dried over anhydrous sodium sulfate, which absorbed the remaining water molecules. Using a small spatula, powdered anhydrous sodium sulfate was mixed with the collected oil until the oil was water-free. The pure oil was pipetted into an amber-colored bottle, the final volume recorded, and then kept at 4-5°C. Extraction was repeated to make sufficient stock. Five hundred milliliters of essential oil was needed for the aerosol study. Extraction took place at the Forest Research Institute of Malaysia [FRIM] and the essential oils were collected and sent to Homesafe Products (M) Sdn Bhd Malaysia, to be placed into aerosol spray cans. Four different aerosol concentrations were produced–1, 2, 5, and 10%. Each can contained 41% kerosene + 58% Liquid Petroleum Gas (LPG) for the 1% concentration; 40% kerosene + 58%

LPG for 2%; 37% kerosene + 58% LPG for 5%; and 32% kerosene + 58% LPG for 10%.

Specimen preparation

Ae. aegypti and *Ae. albopictus* were used in the experiment. Both species were colonized and maintained continuously at 25-30°C in the insectarium of the Department of Biomedical Science, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia. Larvae were fed with a finely powdered mixture of dried liver, ground cat biscuits, milk powder, and ground oatmeal. The adult colony was fed with 10% sucrose and periodically blood-fed on restrained guinea pigs for breeding purposes. Adult females aged 3-5 days, fed only with 10% sucrose, were used in the study. A total of 125 adult mosquitoes of each *Aedes* spp was used for each spraying replication in the study.

Bioassay

The biological efficacy of the insecticidal aerosol spray against adult mosquitoes was assayed following a version of the SIRIM Malaysian standard method (MS 1221:1991 UDC 632.982.2). A spraying room (4.08 m x 3.42 m x 2.18 m) was used for testing the insecticidal aerosol. Twenty-five adult female *Ae. aegypti* and a sugar cube were transferred into each of 5 cylindrical net cages hung from the ceiling. The aerosol cans were weighed before and after spraying, to determine the discharge rate. The aerosols were sprayed vertically and evenly through the spraying opening for 5 and 10 seconds for each concentration of *M. cajuputi* essential oils. Knockdown was observed at 1, 2, 5, 10, 15, and 20 minutes after spraying. of Knockdown was determined as the mosquitoes dropping while still conscious, but unable to fly and unable to move the body for the first hour. Mortality was defined as a mosquito lying unconscious within 24 hours, with no detectable movement within the 24 hours. All dead mosquitoes were discarded after recording, while those still alive were transferred into 250 ml paper cups (2.5 cm base diameter; 4.0 cm lip diameter). The cups were covered with netting and the mosquitoes were fed with 10% sucrose in soaked cotton, placed on the net. Percentage mortality after 24 hours was calculated and recorded. The spraying room was then cleaned with alcohol containing 10% acetone or detergent to remove any residual toxic substances. The experiment was replicated 4 times and repeated with both species of mosquitoes. Results obtained were then compared with the MS Standard aerosol as the positive control, which contained 0.07% prallethrin + 0.05% d-phenothrin and odorless 40% kerosene, and 60% LPG. The results were analysed statistically for knockdown values (KT_{50} and KT_{95}) and regression slope using probit analysis (SPSS 11.5 determination computer program). Mean and least significant difference test were also used for knockdown and mortality.

Results and Discussion

This study evaluated the effectiveness of *M. cajuputi* essential oil in aerosol cans, at concentrations of 1, 2, 5, and 10%. MS Standard aerosol was used as a positive control, for comparative bio-efficacy. Higher doses and longer exposure times yielded higher knockdown and mortality rates. Table 1 shows the average total discharge, and discharge rate, for each concentration at 5 and 10 seconds' spraying. Total average discharge for 5 seconds spraying of *Ae. aegypti* ranged between 11.4-14.7 g, and for *Ae. albopictus* 12.7-15.4 g. Spraying *Ae. aegypti* for 10 seconds, the aerosol discharged 19.1-25.3 g, and spraying *Ae. albopictus* 22.9-24.6 g. Overall, the average discharge rate ranged between 1.9-3.1 g/sec.

Table 2 shows percentage mean knockdown and mortality for each test group. The differences in response between *Ae. aegypti* and *Ae. albopictus* were analyzed statistically. The 5% and 10% concentrations performed better than 1% and 2% against time exposure, respectively. At 1% concentration, 5 seconds spraying produced mortality values of 2.0% ± 0.83 and 1.4% ± 1.40 for *Ae. aegypti* and *Ae. albopictus*, respectively, with no knockdown response. Exposure for 10 seconds gave knockdown of 10.2% ± 1.83 and 5.8% ± 0.68

for *Ae. aegypti* and *Ae. albopictus*, respectively, with mortality values for *Ae. aegypti* of 27.6% ± 4.81, and *Ae. albopictus* 16.0% ± 1.82. There was no significant difference ($p > 0.05$) between *A. aegypti* and *Ae. albopictus*. At 2% concentration, 5 and 10 seconds' spraying showed no significant difference ($p > 0.05$) between *Ae. aegypti* and *Ae. albopictus* for knockdown or mortality. Mean percentage knockdown was between 6.0% ± 1.16 and 14.0% ± 3.09, while mortality was 23.8% ± 2.59 to 38.4% ± 4.01. At 5% concentration, knockdown and mortality values for 5 and 10 seconds' spraying, for both species, showed no significant difference ($p > 0.05$). The values for 5 seconds for *Ae. aegypti* were 9.2% ± 2.56, and for *Ae. albopictus* 20.8% ± 5.57. At 10 seconds, the values were 14.0% ± 3.09 for *Ae. aegypti* and 23.4% ± 3.42 for *Ae. albopictus*. The mortality rates at 5 seconds were 32.6% ± 6.16 for *Ae. aegypti* and 47.8% ± 3.22 for *Ae. albopictus*. Meanwhile, mortality rates for 10 seconds were 51.4% ± 1.74 for *Ae. aegypti* and 56.6% ± 3.63 for *Ae. albopictus*. At 10% concentration, knockdown and mortality rates were not significantly different ($p > 0.05$) between the 2 species for 5 and 10 seconds' spraying. However, of the 4 concentrations, 10% gave higher knockdown and mortality rates in 5 and 10 seconds for both species. Knockdown rates ranged between 26.0% ± 2.61 to 36.6% ± 1.61 for *Ae. aegypti*, and 28.6% ± 3.02 to 37.5% ± 1.33 for *Ae. albopictus*. Mortality rates were 60.2% ± 6.54 to 64.0% ± 5.72 for *Ae. aegypti* and 60.8% ± 3.22 to 61.4% ± 2.22 for *Ae. albopictus*. There was a significant difference ($p < 0.05$) between 10% concentration of *M. cajuputi* aerosol and MS Standard for knockdown and mortality. In a previous study [6], *M. cajuputi* extracts of essential oil caused high rates of mortality with dengue vectors when sprayed together with Resigen® in a field trial using ultra-low-volume (ULV) spraying. Therefore, the present study supported previous findings and also indicated that *M. cajuputi* essential oil should be developed as a household insecticide for the control of dengue vector species.

Tables 3 and 4 show knockdown-time values (KT_{50} and KT_{95}) for 50% and 95% of the population sample size. At 1% and 2% concentrations, knockdown response was < 5% to produce a normal distribution; therefore, no KT_{50} or KT_{95} values were obtained.

The KT_{50} values for *Ae. aegypti* and *Ae. albopictus* at 5% concentration were > 7,000 minutes, when sprayed for 5 seconds. However, for 10 seconds' spraying *Ae. albopictus* showed KT_{50} of 737.12 minutes. Generally, *Ae. albopictus* showed lower KT_{50} than *Ae. aegypti*. KT_{50} values at 10%, for 10 seconds' spraying, were 168.84 minutes for *Ae. aegypti* and 123.71 for *Ae. albopictus*. KT_{50} values for MS Standard were 0.16 minutes for *Ae. aegypti* and 2.24 for *Ae. albopictus*. *Ae. albopictus* also displayed greater sensitivity to *M. cajuputi* essential oil than *Ae. aegypti*. This finding disagreed with a previous study [4], where *Ae. aegypti* larvae showed greater larvicidal sensitivity to most methanol extracts of selected Malaysian plants than *Ae. albopictus*. Since *Ae. albopictus* plays a significant role in dengue and chikungunya transmission [11], this study could provide useful information on adulticidal effects. The results can also be used to develop and improve the production of essential oil extract, for greater effectiveness as a household insecticide. The differential responses induced by phytochemicals by various species of mosquitoes were influenced by extrinsic and intrinsic factors, such as plant species, parts of plant used, solvents used in extraction, geographical location where the plants were grown, and evaluation methods [3].

The bio-efficacy of natural products in aerosol household insecticide depends on many factors. These include the type of active ingredients and formulations, the mode of application, and local conditions (temperature and humidity). Many highly volatile plant extracts and essential oils commonly act on mosquitoes in the vapor phase [12]. This is supported by many research studies of repellency. Plant-derived topical repellent products improved repellency after formulation with some bases or fixative materials, such as liquid paraffin, vanillin [13], and mustard and coconut oils. In conclusion, this study indicated that *M. cajuputi* essential oil extract has potential

Table 1 Discharge rate for each concentration after spraying *Aedes aegypti* and *Ae. albopictus* for 5 and 10 seconds.

Discharge	*Aedes aegypti*								*Aedes albopictus*							
	5 seconds				10 seconds				5 seconds				10 seconds			
	1%	2%	5%	10%	1%	2%	5%	10%	1%	2%	5%	10%	1%	2%	5%	10%
Average total discharge (g)	14.7	14.5	11.4	13.1	25.3	22.1	19.1	23.3	15.4	15.2	12.7	13.2	24.1	24.6	22.9	24.1
Average discharge rate (g/sec)	2.94	2.90	2.28	2.62	2.53	2.21	1.91	2.33	3.08	3.04	2.54	2.64	2.41	2.46	2.29	2.41

Table 2 Mean percentage (%) knockdown and mortality response of aerosol *Melaleuca cajuputi* extract essential oil spraying against female *Aedes aegypti* and *Ae. albopictus* in the laboratory.

Doses (%)	*Aedes aegypti*				*Aedes albopictus*			
	5 seconds		10 seconds		5 seconds		10 seconds	
	Knockdown (%) Mean ± SE	Mortality (%) Mean ± SE	Knockdown (%) Mean ± SE	Mortality (%) Mean ± SE	Knockdown (%) Mean ± SE	Mortality (%) Mean ± SE	Knockdown (%) Mean ± SE	Mortality (%) Mean ± SE
1	-	2.0 ± 0.83 [a]	10.2 ± 1.83 [a]	27.6 ± 4.81 [a]	-	1.4 ± 1.40	5.8 ± 0.68	16.0 ± 1.82
2	8.0 ± 1.73 [a]	26.2 ± 5.13 [a]	6.0 ± 1.16 [a]	38.4 ± 0.01 [a]	13.2 ± 4.51	23.8 ± 2.89	14.0 ± 3.09	38.4 ± 4.01
5	9.2 ± 2.56 [a]	32.6 ± 6.16 [a]	14.0 ± 3.09 [a]	51.4 ± 1.74 [a]	20.8 ± 5.57	47.8 ± 3.22	23.4 ± 3.42	56.6 ± 3.63
10	36.6 ± 1.61 [a b]	60.2 ± 6.54 [a b]	26.0 ± 2.61 [a b]	64.0 ± 5.72 [a b]	37.5 ± 1.33 [b]	61.4 ± 2.22	28.6 ± 3.02 [b]	60.8 ± 3.22 [b]
MS Standard	56.0 ± 7.07	97.2 ± 0.83	93.0 ± 1.83	97.6 ± 0.98	56.8 ± 5.23	93.2 ± 1.06	94.0 ± 2.1	97.4 ± 0.89

[a] $P > 0.05$ = non-significant difference compared with *Ae. albopictus*
[b] $P < 0.05$ = significant difference compared with MS Standard

Table 3 KT_{50} and KT_{95} values of ***Melaleuca cajuputi*** essential oil extract for adult female ***Aedes aegypti*** and ***Ae. albopictus*** in the laboratory; spraying time = 5 seconds.

Doses (%)	*Aedes aegypti*			*Aedes albopictus*		
	KT_{50} & Confidence Limit (min)	KT_{95} & Confidence Limit (min)	Regression Coefficient ± SE	KT_{50} & Confidence Limit (min)	KT_{95} & Confidence Limit (min)	Regression Coefficient ± SE
5	676,526.77* (7,536.99 – 5,772,220.0)	582,063,144,441.2* (3,678,780.96 – 9,447,687.0)	0.33 ± 0.14	Not enough cases accepted in this subfile		
10	264.25* (201.76 – 374.88)	10,371.04* (5,169.10 – 25,769.46)	1.03 ± 0.07	177.34* (145.52 – 227.98)	3,095.87* (1,860.78 – 5,951.37)	1.32 ± 0.06
MS Standard	3.21* (2.73 – 3.69)	26.13* (21.79 – 32.56)	1.80 ± 0.04	3.072* (25.19 – 36.33)	43.431* (34.82 – 56.98)	1.43 ± 0.03

* Heterogeneity factor used in calculating confidence limits.

Table 4 KT_{50} and KT_{95} values of ***Melaleuca cajuputi*** essential oil extract for adult female ***Aedes aegypti*** and ***Ae. albopictus*** in the laboratory; spraying time = 10 seconds.

Doses (%)	*Aedes aegypti*			*Aedes albopictus*		
	KT_{50} & Confidence Limit (min)	KT_{95} & Confidence Limit (min)	Regression Coefficient ± SE	KT_{50} & Confidence Limit (min)	KT_{95} & Confidence Limit (min)	Regression Coefficient ± SE
5	98,497.90* (9,751.84 – 16,950,094.3)	1,707,795,715.16* (11,502,389.79 – 1,193,348.0)	0.39 ± 0.08	737.12* (460.83 – 1,394.42)	170,422.07* (51,703.54 – 868,757.63)	0.70 ± 0.06
10	168.84* (137.39 – 216.87)	580.30* (349.80 – 1,352.48)	3.25 ± 0.42	123.71* (105.46 – 150.45)	2,162.11* (1,400.79 – 3,709.81)	1.32 ± 0.07
MS Standard	0.16* (0.06 – 0.33)	10.73* (8.27 – 14.65)	0.91 ± 0.04	2.24* (0.07 – 0.48)	11.29* (8.34 – 16.61)	0.98 ± 0.04

*Heterogeneity factor used in calculating confidence limits.

adulticidal effects against dengue vectors. Extracts of essential oil are worthy of consideration as household products. In addition, natural plant extracts in aerosol cans are environmentally safe, user friendly, and effective.

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