Original Article

Evaluation of *Melaleuca cajuputi* (Family: Myrtaceae) Essential Oil in Aerosol Spray Cans against Dengue Vectors in Low Cost Housing Flats

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Abstract

Background: *Melaleuca cajuputi* essential oil in aerosol spray was evaluated against the dengue vectors *Aedes aegypti* and *Ae. albopictus* at low cost housing flats in Section 10, Setapak, Kuala Lumpur, Malaysia.

Methods: Essential oil in aerosol viz: 5% and 10% of concentrations were sprayed for 5 seconds each towards hung mosquitoes in 5 cylindrical net cages. Aerosol weights were recorded before and after spraying to determine discharge rates. Knockdown and mortality number were observed and compared to MS standard aerosol which contain 0.07% prallethrin and 0.05% d-phenothrin as positive control and aerosol contain 40% kerosene and 60% LPG was used as negative control.

Results: High knockdown and mortality was observed in both species of mosquitoes towards MS standard aerosol. There was a significant difference ($P< 0.05$) of mortality and knockdown between 5% and 10% of essential oil aerosol and 5% and 10% essential oil between MS standard. For 5% essential oil, mean percentage (%) of knockdown and mortality of *Ae. aegypti* displayed slightly higher compared to *Ae. albopictus.* Spraying with 5% *M. cajuputi* essential oil aerosol indicated a knockdown of *Ae. aegypti* 5.60±1.18 and mortality of 22.90±4.22 while *Ae. albopictus* showed 4.60±0.89 knockdown and 20.00±2.85 mortality. The 10% essential oil concentration gave 23.60±1.68 knockdown and 48.05±0.37 mortality for *Ae. aegypti*. *Ae. albopictus* gave 23.00±3.16 knockdown and 44.20 ± 2.10 mortality respectively.

Conclusions: Extracts of essential oils does possessed an adulticidal effects and could be considered and utilized for future dengue vectors control.

Keywords: *Melaleuca cajuputi*, essentials oil, aerosol can, *Aedes aegypti*, *Aedes albopictus*

Introduction

To date, *Aedes aegypti* and *Ae. albopictus* mosquitoes are principal vectors of dengue fever (DF) and dengue haemorrhagic fever (DHF) in many tropical countries in urban, and semi-urban areas. In addition, chikungunya is yet another viral disease transmitted by *Ae. aegypti* and exhibits similar symptoms (Singh and Pavri 1967). At present there is no specific antiviral therapy or effective vaccines available for dengue and chikungunya virus (CDC 2006). Therefore, the only option is to prevent the diseases by controlling the vector mosquito population. This major approach of controlling the population vector relies mainly on insecticide application (Tikar et al. 2008).

A consequence of the widespread use of insecticides is the development of resistance in mosquitoes against insecticides. Because of these problems and concerns, the interest towards botanical insecticides derived from plants has been revived worldwide, and tre-

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mendous efforts are being made to isolate, screen and to develop phytochemicals possessing pesticidal activity (Mulla and Su 1999). Since ancient times, plant products were used in various aspects. However their use against pests decreased when chemical products become developed (Kallyaperumal 2008). However emphases are being made towards bio-products to replace synthetic insecticide for the control of dengue particularly in cases where susceptibility is decreasing (Chatiyasit and Choochote 2006).

Mosquito behavior may contaminate the aquatic environment (Kallyaperumal 2008). Natural pesticides especially those derived from plants are more promising in this aspects (Amer and Mehlhorn 2006). Earlier studies on larvicidal activities of leguminous seeds and grains against *Ae. aegypti* and *Culex pipiens pallens*, had shown larvicidal properties (Young-Su et al. 2002). In Malaysia, study on thirty species of plants extracts were tested for their ability as mosquitocidal activities against dengue vector has revealed the potential effects (Zaridah et al. 2006).

The objective of this study was to evaluate the effectiveness of *Melaleuca cajuputi* essential oil extract in aerosol spray against *Ae. aegypti* and *Ae. albopictus*, compared with Malaysia SIRIM standard (MS standard) aerosol under field conditions. The evaluation used MS standard aerosol as standard reference or guideline for the bio-efficacy of household insecticide testing.

Materials and Methods

Plant collections

Melaleuca cajuputi leaves were obtained from Port Dickson, Negeri Sembilan (2^{o} 31’22.07N 101^{o} 48’00.01E) in Peninsular Malaysia located about 120 km south of Kuala Lumpur (3^{o}09’00.76”N 101^{o}42’27.73”E). Verification of species identification was made by botanists from the Forest Research Institute of Malaysia (FRIM). The voucher specimens were deposited at the herbarium for reference.

Extraction

The whole process of extraction was conducted at FRIM. The extraction of essential oil from leaves specimens was conducted using steaming hydro-distillation. This continuously 8 hours process has enabled to isolate and produce essential oil from subjected samples, made up with a Clevenger-type apparatuses which were comprised with 500ml to 1000ml round bottomed distillation flask, oil separator tube and condenser. Fresh leaves were left to dry at room temperature and dried leaves were collected and grounded to a small particles to maximize the essential oil production. Samples were then transferred into the distillation flask and weigh were recorded. Samples then covered with distilled water and heated at 60–70^{o} C. The samples allowed boiling slowly until distillation process was completed.

The mixture of oil and water was allowed to settle for 24 hours to ensure separation of water and oil layers. The water was slowly drawn off and the remained oil layer were slowly collected into a glass beaker and dried over anhydrous sodium sulfate. The remained oil was pipetted out into an amber-colored bottle and final volume was recorded and kept at 4–5 ^{o}C. The extraction was repeated accordingly to make stock. A total of 500ml essential oil was needed to meet the capacity needs for the aerosol study. The successively extracted essential oils were collected and sent to the Homesafe Products (M) Sdn Bhd Malaysia to produce aerosol spray cans. Concentrations of *M. cajuputi* aerosols used in the study were 5% and 10% respectively. The 5% *M. cajuputi* extract dissolved in 37% kerosene and 58% Liquid Petroleum Gas (LPG) while the 10% *M. cajuputi* extract dissolved 32% kerosene and 58% LPG respectively. MS standard aerosol was used as positive control which

contain 0.07% prallethrin and 0.05% d-phenothrin+40% kerosene+60% LPG. Whereas aerosol which contained only 40% kerosene and 60% LPG was used as untreated or negative control.

Specimens Preparation

Species used in the study were *Ae. aegypti* and *Ae. albopictus.* Both species were colonized and maintained continuously at 25–30 ºC in the insectarium at the Department of Biomedical Science, Faculty of Allied Health Sciences, University Kebangsaan Malaysia. Larvae were fed with 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-pig for breeding purposes. Adult 3–5 days age females fed only with sucrose 10% were used in the study. A total of hundred and twenty five adult mosquitoes were used for every single replicate. Twenty-five mosquitoes were then transferred into each 5 of cylindrical net.

Bioassay

Method for the evaluation of biological efficacy of household space spray insecticidal aerosol against adult mosquitoes was assayed following a version of MS standard method MS 1221:1991 UDC 632.982.2. As the method was design for the laboratory evaluation, modification was made to adapt the field conditions. Four blocks of low cost housing estate which have 4 storey medium flats were chosen in this study namely Block A, Block B, Block C and Block D. Each block has 24 units of houses with 6 units of houses at each floor. Distances from each block were about 20 metres. Block A, Block B, Block C and Block D were represent of 5%, 10%, MS standard and negative control aerosols respectively. Four houses from every block were selected as replicate. Each houses composed with a living hall, 2 bedrooms (sizes 4.0m x 4.05m x 2.20m each), kitchen and toilet. One appropriate room was specified throughout the evaluation period of 5 months. The spraying was conducted alternately between *Ae. aegypti* and *Ae. albopictus*.

The room was decontaminated with diluted methanol to disinfect and remove traces of insecticide or unknown household chemical products residual if any. The floor and wall were cleaned each pre and post spraying. Mosquitoes in cylindrical nets were hung at the room's ceiling for 1 hour prior spraying for acclimatization and were fed with sugar cube. Aerosol cans were weighed pre and post spraying to determine the discharge rate. Aerosols were sprayed vertically for 5 seconds for each samples tested. The room was closed immediately after spraying.

Observations were made for 1, 5, 10, 15 and 20 minutes for knockdown and 24 hours for mortality. Determination of knockdown was made when mosquitoes were failed to fly and not being able to move its body. Mosquitoes are not at its normal position and at their backs with legs facing upwards for the first of 1 hour. Definition for mortality was when mosquitoes were motionless and dead within 24 hours. All those that were alive were transferred into 250 ml paper cups (2.5 cm bottom diameter and 4.0 cm diameter opening). The cups were covered with a piece of net layer and mosquitoes were fed with wet cotton wool (wets with 10% sucrose). The wet cotton wools were then placed onto the net for mosquitoes accessible.

A total number of knockdown and mortality was counted and recorded after 1 hour and 24 hours respectively to determine the percentage. The experiment replicated 4 times with different houses and repeated with both species of mosquitoes. Results obtained were then compared to the MS Standard and negative control. The results were statistically analyzed (SPSS 11.5 determination computer program) to obtain the

knockdown values and regression slope using a probit analysis. Knockdown Time (KT_{50} and KT_{95}) is the value of time taken for aerosols to give knockdown effects of the 50% and 95% sample populations. Mean and least significant difference test were used for knockdown, mortality and dose concentrations applied. In cases where the mortality in tests control populations ranged from 5-20%, the observed percentage mortality (M%) was corrected by Abbott's formula (Abbott 1925):

$$M\% = \frac{\text{test mortality\% - control mortality\%}}{\text{100 - control mortality\%}} \times 100$$

Results

Table 1 demonstrates the total average of discharge and discharge rates of each aerosol sprayed towards *Ae. aegypti* and *Ae. albopictus*. For *Ae. aegypti* the ranges were from 2.56 gm/sec to 2.64 gm/sec. Whereas *Ae. albopictus* showed ranges between 2.55 gm/sec to 2.61 gm/sec. Table 2 shows mean percentage of knockdown and mortality of both species which have been treated with 5%, 10% of essential oils extract, MS standard and negative control aerosols. The 5% essential oils on *Ae. aegypti* gave 5.60±1.18 of knockdown and 22.90±4.22 of mortality. *Aedes albopictus* values were lower, 4.60± 0.89 of knockdown and 20.00±2.85 of mortality. Essential oils with 10% concentration gave the mean percentage of knockdown and mortality slightly higher than 5% of essential oils. Nevertheless the values pattern were consistent with 5% of essential oil where *Ae. aegypti* showed a small different of mean percentage compared to *Ae. albopictus*. The values were 23.60±1.68 of knockdown and 48.05±0.37 of mortality on *Ae. aegypti*. *Ae. albopictus* gave values of 23.00±3.16 knockdown and of 44.20±2.10 mortality. MS standard aerosols showed highest effect of knockdown and mortality against both species. The mean percentage of knockdown and mortality for *Ae. aegypti* was 94.60±1.00 and 96.60± 0.50 respectively. *Ae. albopictus* gave a mean percentage of 93.00±2.68 knockdown and of 97.00±1.10 mortality.

Negative control showed no values of knockdown for both *Aedes* spp. Mortality for mean percentage showed values of 0.50± 0.29 for *Ae. aegypti* and 0.25±0.25 for *Ae albopictus* which are less than 20%. Both species shows no correlation of knockdown and mortality towards discharge rates ($r< 0.40$). The r values for knockdown and mortality of *Ae. aegypti* were 0.215 and 0.066 whereas r values of *Ae. albopictus* were -0.168 and -0.055 respectively. There were non significant difference ($P> 0.05$) between *Ae. aegypti* and *Ae. albopictus* for knockdown and mortality mean percentage. There were significant differences ($P< 0.05$) between doses applied of 5%, 10% essential oils and MS standard towards knockdown and mortality of *Ae. aegypti* and *Ae. albopictus.*

Table 3 shows the values of Knockdown Time, KT_{50} and KT_{95} for both *Aedes* spp. after treatment with *M. cajuputi* essential oil in aerosol cans using 5% and 10% concentrations and the comparison with MS standard and negative control. The best and effective aerosols gave lower values of KT_{50} and KT_{95} respectively. From the results obtained, 10% concentration gave best effect compared to 5% concentration. *Aedes aegypti* was more susceptible compared to *Ae. albopictus* towards essential oils extract. At 5% KT_{50} of *Ae. aegypti* was 311.96 min and *Ae. albopictus* 550.81min. While at 10% concentrations, KT_{50} were lower with 122.98 min for *Ae. aegypti* and 174.36 min for *Ae. albopictus* respectively. MS standard aerosols gave the best and lowest KT_{50} values of 4.775min for *Ae. aegypti* and 3.581min for *Ae. albopictus*. There were no KT values for negative control can be analyzed as no knockdown effect was observed during evaluation.

Table 1. Total average of discharge and discharge rates of Melaleuca cajuputi, MS standard and untreated aerosol sprays

Discharges	*Aedes aegypti*				*Aedes albopictus*			
	5%	10%	MS	Untreated	5%	10%	MS	Untreated
Total Average of Discharge (gm)	13.21	2.81	3.14	13.20	12.74	13.03	12.80	13.00
Discharge Rates (gm/sec)	2.64	2.56	2.63	2.64	2.55	2.61	2.56	2.6

Table 2. Mean percentage (%) knockdown and mortality *Aedes aegypti* and *Aedes albopictus* after spraying with *Melaleuca cajuputi* extract in aerosol sprays

Doses	*Aedes aegypti*			*Aedes albopictus*		
	Discharge Rates (gm/sec)	Knockdown (%) Mean ± SE	Mortality (%) Mean ± SE	Discharge Rates (gm/sec)	Knockdown (%) Mean ± SE	Mortality (%) Mean ± SE
5%	2.64[c]	5.60 ± 1.18[a]	22.90 ± 4.22[b]	2.55[d]	4.60 ± 0.89	20.00 ± 2.85
10%	2.56[c]	23.60 ± 1.68[a]	48.05 ± 0.37[b]	2.61[d]	23.00 ± 3.16	44.20 ± 2.10
MS Standard	2.63[c]	94.60 ± 1.00[a]	96.60 ± 0.50[b]	2.56[d]	93.00 ± 2.68	97.00 ± 1.10
Neg Control	2.64 [c]	-	0.50 ± 0.29 [b]	2.60 [c]	-	0.25 ± 0.25 [b]

[a b] P>0.05 non significant difference compared to *Ae. albopictus*
[c d] r<0.40 no correlation with knockdown and mortality (Pearson's correlation)

Table 3. Values of KT_{50} and KT_{95} of essential oil extract of *Melaleuca cajuputi* to *Aedes aegypti* and *Aedes albopictus* adult females in the field which has been sprayed for 5 seconds

Doses	*Aedes aegypti*			*Aedes albopictus*		
	KT_{50} and Confidence Limit (min)	KT_{95} and Confidence Limit (min)	Regression Coefficient±SE	KT_{50} and Confidence Limit (min)	KT_{95} and Confidence Limit (min)	Regression Coefficient± SE
5%	311.96* (200.28 – 694.02)	1785.58* (776.94 – 8065.24)	0.942 ± 0.14	550.81* (261.62 – 3340.08)	5041.04* (1251.04 – 148712.84)	1.711 ± 0.36
10%	122.98* (128.65 – 143.12)	740.29* (551.84 – 1065.48)	2.110 ± 0.12	174.36* (143.18 – 225.50)	1805.94* (1123.24 – 3375.52)	1.620 ± 0.11
MS Standard	4.775* (4.307 – 5.253)	42.398* (34.461–54.671)	1.734 ± 0.05	3.581* (2.929 – 4.232)	45.710* (33.553 – 69.711)	1.486 ± 0.04
Neg Control	0	0	0	0	0	0

*A heterogeneity factor is used in the calculation of confidence limit

Discussion

In context of pollution environment such as residue problem, health risks of the consumer and the development of insect resistance to synthetic insecticides, recent interest among researchers has developed to investigate and explore potential of plants extract that are environmentally safe and target specific but still effective to control vector populations.

From the study, it was observed that aerosol of MS standard were the most effective followed by essential oil of 10% and 5% concentration respectively. Pearson's correlation analysis showed that the discharge rates had no correlation on the effectiveness (knockdown and mortality) of the aerosols. Of all MS standard gave highest mean percentage of knockdown and mortality of *Ae. aegypti* and *Ae. albopictus* compared to the essential oils extract. The mean mortality percentage for negative control was not more than 20% thus can be said that new formulations of essential oil have contributed the knockdown and mortality effect. Previous studies by Azlinda (2009) on the evaluation of *M. cajuputi* extract essential oil in aerosol sprays against dengue vectors in laboratory had similar findings. That study demonstrated high value of mean percentage of knockdown and mortality at concentration of 10% with highest value recorded for knockdown were 37.5±1.33% and mortality were 64.0±5.72%. In comparison with the present study in the field trial, the highest number has shown slightly lower of knockdown (23.60±1.68) and mortality (48.05± 0.37). In spite of that, it indicates the potential of *M. cajuputi* essential oil extract as botanical insecticides in the laboratory as well as in the field. The bio-efficacy of the products however depends on many factors such as mode of application and local conditions like temperature and humidity (Tawatsin et al. 2001).

Study on the evaluation of effectiveness for household insecticide products performances knockdown time (KT) values is one of the parameters which contributes not less important. The knockdown effect were calculated to estimate the number of knocked down mosquitoes in tested population, as recommended by WHO (1975) for testing space spraying insecticides such as aerosol. Values of KT_{50} and KT_{95} were lower when aerosols take shorter time to give knockdown effect. MS standard aerosols give the lowest values of KT_{50} and KT_{95} compared to the essential oil extracts. However, essential oil with 10% concentration performs better than 5% concentration. Similar ported on a study with *Callosobruchus maculates* of which the knockdown effect varied with concentrations (Keita et al. 2000). Variation in knockdown time and mortality were also observed between *Ae. aegypti* and *Ae. albopictus*. This probably due to the different reaction to chemical compound in active ingredients of essential oil which act as a reversible inhibitor of a acetyl cholinesterase, capable of disrupting the function of neurotransmitter in insects like mosquito, thus caused knockdown effect (Ryan and Byrne 1988). Recent investigations indicate that some chemical constituents of many plants extracted essential oils interfere with the octopaminergic nervous system in insects. Hence, meet the criteria for 'reduced risk' pesticides, as this target site is not shared with mammals (Koul et al. 2008).

From the knockdown and mortality values, *Ae. aegypti* showed to be more susceptible than *Ae. albopictus* which means greater number of knockdown and mortality were noted. Since *Ae. aegypti* plays a very important role in dengue and dengue haemorrhagic fever transmission in Southeast Asia and tropical regions (Harinasuta 1984)

this study could provide a useful information in future research.

In conclusion, this study confirmed that extract of *M. cajuputi* (Family: Myrtaceae) essential oil possesses some adulticidal effects on dengue vectors with most effective concentration was 10% for controlling vectors. Similarly, study on the efficacy of essential oil extract as green pesticides, has observed that essential oil may require greater application or frequent reapplication compared to synthetic pesticides (Koul et al. 2008). Thus, in addition of development for commercial application of plant essential oil based pesticides, considerations to include are availability of sufficient quantities of plant resources, standardization and refinement of pesticide products and regulatory approval (Isman 2005).The results obtained suggest that, further studies are considerable essential that will lead to improve formulations with enhanced activity which may eventually acceptable as mosquito control.

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