MOSQUITOCIDAL ACTIVITIES OF MALAYSIAN PLANTS

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ZARIDAH, M. Z., NOR AZAH, M. A. & ROHANI, A. 2006. Mosquitocidal activities of Malaysian plants. Extracts from about 30 species of plants in Malaysia were tested for their ability to kill the larvae or to repel or knock down the adults of *Aedes aegypti,* the vector mosquito for dengue and dengue hemorrhagic fever. Observation of mortality was made after 24 hours of exposure to the plant extract/essential oil to obtain the median lethal concentration (LC_{50}) of the plant extract/essential oil tested. In repellency and knock-down effects of adult mosquitoes, median effective concentration (EC_{50}) was obtained after each test. The three best extracts for killing larvae were the essential oils of *Zanthoxylum acanthopodium* stem, *Aquilaria malaccensis* wood and *Pelargonium citrosum* plant. For repelling adult mosquitoes, the most effective was the leaf essential oil of *Cymbopogon nardus*, followed by that of *A. malaccensis*. Knock-down ability was best with mosquito coils made from the seed kernel of *Azadirachta indica*, followed by the leaf of *C. nardus* and the wood of *Fernandoa adenophylla*.

Keywords: Larvicidal, adulticidal, knock-down effect, repellency effect

ZARIDAH, M. Z., NOR AZAH, M. A. & ROHANI, A. 2006. Aktiviti antinyamuk tumbuh-tumbuhan Malaysia. Ekstrak daripada 30 spesies tumbuhan Malaysia diuji keupayaannya membunuh larva atau menghalau atau memengsankan nyamuk dewasa *Aedes aegypti* iaitu vektor demam denggi dan demam denggi berdarah. Pemerhatian untuk kematian dilakukan 24 jam selepas pendedahan nyamuk kepada ekstrak tumbuhan atau minyak pati bagi memperoleh kepekatan maut median (LC_{50}) ekstrak tumbuhan atau minyak pati yang diuji. Dalam kesan menghalau dan kesan memengsankan nyamuk, kepekatan berkesan median (EC_{50}) diperoleh. Tiga ekstrak terbaik bagi membunuh larva ialah minyak pati daripada batang *Zanthoxylum acanthopodium*, kayu *Aquilaria malaccensis* dan tumbuhan *Pelargonium citrosum*. Minyak pati daripada daun *Cymbopogon nardus* paling berkesan untuk menghalau nyamuk, diikuti oleh *A. malaccensis*. Keupayaan memengsankan nyamuk paling baik dengan lingkaran nyamuk yang diperbuat daripada isirong biji *Azadirachta indica*, diikuti oleh daun *C. nardus* dan kayu *Fernandoa adenophylla*.

Introduction

The Forest Research Institute Malaysia (FRIM) is actively exploring the use of plant extracts and essential oils as one of the methods in controlling vector mosquito especially *Aedes aegypti*, the vector of dengue and dengue hemorrhagic fever in Malaysia. This is to promote natural cure. The use of synthetic insecticides, in the long run, produces negative effects. The effects include a number of environmental problems and other consequences such as non-biodegradable chemical insecticide residue. They also include excessive mortality and a reduced reproductive potential in birds, fish and other organisms (Koeman 1978). Another important issue is the occurrence of mosquito resistant species especially *Aedes aegypti* and *A. albopictus* after extensive utilization of chemical insecticides (Rohani *et al.* 2001). Prolonged exposure to these synthetic insecticides may lead to irritation, severe allergic dermatitis, systemic allergic reactions and large amounts may cause nausea, vomiting, tinnitus, headache and other central nervous system disturbances (Reynolds 1994). Due to these circumstances, the effectiveness of plant derivatives, either crude extracts or essential oils, in controlling mosquito was studied.

Plant selection in the study was based on the literature. A number of publications have reported the utilization of plant extracts and essential oils in controlling mosquito (Joshi *et al.* 1978, Thangam & Kathiresan 1988, Mohsen *et al.* 1990, Schmutterer 1990, Mwaiko & Savaeli 1994).

The life cycle of the mosquito has to be understood before any control method is applied. Its life cycle has a complete metamorphosis, from eggs to larvae, pupae and adults. The cycle is completed in 7 to 10 days. The control method should aim at the weakest link of the life cycle of the mosquito,

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which is the mosquito larva. However, control of adult mosquito has to be considered too, either by adulticiding or by prevention method such as repellency or mosquito coil burning. On the whole, plant extracts have been utilized to control destructive insects and vectors of diseases (Matsumura 1975).

In this paper, we report the results of mosquitocidal activities of selected plant extracts and essential oils with the aim of reducing or eliminating chemical usage.

Materials and methods

Plant material

Plant samples (Table 1) were collected from various locations in Malaysia. Voucher specimens were deposited at the herbarium of FRIM, Kepong. The samples were left to air dry over two days. Plant materials were then subjected either to successive extraction or hydro distillation. Plant extracts and essential oils obtained from the extraction method were subjected to larvicidal, repellency and knock-down assessments.

Larvicidal bioassay

The vector mosquito, *Aedes aegypti*, was used as test organism. Mosquito eggs were obtained from the Institute for Medical Research (IMR). The eggs were soaked in unchlorinated and filtered tap water for them to develop into first instar larvae. The larvae were given boiled liver as food half a day after hatching. Development of larvae to third and fourth instars were within three to four days. Late third instar larvae were used.

The bioassay was according to WHO (1981) guidelines with slight modifications. A known amount of essential oil was dissolved in 95% ethanol to provide a stock solution. From this stock solution, concentrations of 200, 100, 50, 25 and 12.5 mg l^{-1} (ppm) were prepared by dilution and each concentration was replicated three times. Appropriate amount of stock solution was mixed with distilled water to make a final test solution of 200 ml. Test solution was placed in a 250 ml beaker and 20 late third instar larvae of *A. aegypti* were introduced into the beaker. Each experiment set contained three replicate controls, which consisted of 2 ml ethanol and 198 ml of distilled water. All beakers were kept at room temperature and mortality was recorded after 24 hours' exposure during which no food was offered to the test organisms. The concentration lethal to 50% (LC_{50}) and 90% (LC_{90}) of test organisms, 95% confidence interval and their slopes of probit regression line were determined by probit analysis program (Raymond 1985) to compare their effectiveness.

Repellency evaluation

Mosquito repellency activity was assessed using the test cage described in the American Society for Testing and Materials Standard E951-83 for laboratory testing of non-commercial mosquito repellent formulations on the skin (Anonymous 1983). The test procedure was similar to that described by Buescher *et al.* (1982) and Gupta *et. al.* (1989). The flexor regions of the forearms of volunteers were outlined with five circular 29 mm diameter test areas. A volume of 0.025 ml of serial dilutions of the essential oils in ethanol (95%) (0.0006–0.0379 mg cm^{-2}) and 0.025 ml of the diluent was applied randomly on the marked areas of the first, second, fourth and fifth circles. Ethanol (95%) was applied on the middle circle, which was the third circle as the control test. The test cage was positioned securely on the arms of each volunteer with Velcro tapes to ensure that only the test areas were exposed to mosquito bites. Fifteen female mosquitoes between three and seven days old were introduced into each cage and the number of biting was recorded at the end of 120 s. Percentage repellency was determined by the formula described by Weaving and Sylvester (1967).

$$\text{Percentage repellency} = 100 - \left[\frac{\text{Total number of bites on treated arm}}{\text{Total number of bites on control arm}} \times 100 \right]$$

The test procedure was replicated three times for each oil sample and statistically reliable estimates of their effective concentrations (EC_{50} and EC_{90}) were obtained by probit analysis (Raymond 1985).

Knock-down evaluation

Preparation of test coil was done according to the Malaysian Standard Specification MS23 established by the Standards and Industrial Research Institute of Malaysia Berhad (SIRIM 1986) with minor modifications using selected plant material as organic filler (35%), wood powder of *Cinnamomum iners* as binding materials (30%) and coconut shell powder as burning material (35%). Different coils containing selected plant powder were cut down to pieces weighing 1.5 g each and introduced into the glass chamber. A blank coil containing only *C. iners* (65%) and coconut shell (35%) was used as Control 1.

The bioassay was conducted in a glass chamber measuring 140 × 120 × 60 cm following the method of Chadwick (1975). Mosquitoes in the polyethylene cup and coils were introduced into the chamber through a 30 × 30 cm sliding window at the mid-bottom on one side of the chamber. The mosquito coil was kept on a stand in the middle of the chamber and allowed to burn for 2 min before 30 sucrose-fed mosquitoes was released into the chamber. Knock-down mosquitoes (i.e. those that no longer maintained normal posture and were unable to fly or were on their backs) were recorded at 1-min intervals up to 3 hours or until total knock-down was achieved. Knocked-down mosquitoes was placed in a clean container containing cotton wool soaked with 5% sucrose solution and the mortality of the mosquitoes was observed after 24 hours. The above procedures were carried out in triplicates for each coil formulation. Control 1 was performed by exposing the mosquitoes to the smoke of a blank coil. A test carried out without coil served as Control 2. Knock-down times [KD_{50} and KD_{90}, as the time (min)needed to knock-down 50 and 90% of the mosquitoes respectively] were determined by the probit analysis.

Probit analysis

All data obtained were subjected to log-probit analysis (Raymond 1985) to obtain the lethal concentration value (LC) or effectiveness concentration value (EC) from 1 to 99 (LC_1 or EC_1 to LC_{99} or EC_{99}). These computer generated program provided LC value or EC value with appropriate regression line and slope, their range of confidence interval at 95% confidence limit, variance of the LC_{50} and the heterogeneity of the test. The LC_{50} and LC_{90} or EC_{50} and EC_{90} were chosen as comparison value of the activity.

Results and discussion

The stem essential oil of *Zanthoxyllum acanthopodium* exhibited the strongest larvicidal activity, recording the lowest LC_{50} (19.87 mg l^{-1}) value after 24-hour observation on late third instar larvae (Table 1). These were followed by the gaharu oil of *Aquilaria malaccensis* with LC_{50} value of 20.19 mg l^{-1} and *Pelargonium citrosum* essential oil (LC_{50} = 24.97 mg l^{-1}). The rest of the essential oils and extracts exhibited LC_{50} values ranging from 25.58 to 105.90 mg l^{-1}. The result corresponded with those of Marr and Tang (1992), who reported the insecticidal properties of some *Zanthoxyllum* essential oils. The fragrant wood of *A. malaccensis,* well known for its gaharu or agar wood oil, is usually used as incense (Chang *et al.* 2002). The results showed that the oil can also be utilized for controlling mosquito larvae. The mosquito repellent plant, *P. citrosum,* was reported to be ineffective against mosquito (Jensen *et al.* 2000). However, this study showed that the essential oil extracted from the plant had potential activity against mosquito larvae.

Table 1 Larvicidal properties of different plant samples against late third instar larvae of *Aedes aegypti*

Plant	Locality/Source	Tested	LC_{50} (mg l^{-1}) (95% CI)	LC_{90} (mg l^{-1}) (95% CI)	Slope ± SE
Homalomena propinqua	Bukit Lagong	Rhizome oil	42.34 (38.43–46.64)	67.06 (59.26–80.19)	6.42 ± 0.78
Aquilaria malaccensis	Gombak	Wood oil	20.19 (18.25–22.31)	32.93 (28.95–39.75)	6.04 ± 0.74
Cymbopogon nardus	Gua Musang	Leaf oil	32.97 (31.20–34.70)	42.46 (39.90–46.25)	11.66 ± 1.33
Cymbopogon nardus	Bukit Hari	Leaf oil	43.08 (41.08–45.15)	54.05 (50.57–60.64)	13.01 ± 2.07
Cymbopogon nardus	Kuala Krai	leaf oil	40.43 (36.3–50.4)	64.88 (51.6–115.6)	6.24 ± 1.42
Cinnamomum pubescens	Cameron Highlands	Leaf oil	25.58 (23.20–28.08)	40.74 (35.98–49.34)	6.34 ± 0.86
C. pubescens	Cameron Highlands	Bark oil	51.83 (50.00–53.42)	58.63 (56.50–62.46)	23.95 ± 4.14
C. pubescens	Cameron Highlands	Twig oil	54.61 (44.28–58.28)	65.25 (62.23 – 70.94)	16.58 ± 4.90
C. kuntsleri	Cameron Highlands	Leaf oil	105.90 (95.70–117.27)	153.05 (135.24 – 187.73)	8.01 ± 1.28
C.scortechinii	Cameron Highlands	Bark oil	70.10 (54.31–90.19)	110.75 (68.84 – 183.17)	6.45 ± 2.01
C. sintoc	Taiping	Bark oil	39.96 (26.70–51.13)	49.88 (30.16 – 83.38)	9.85 ± 3.05
C. sintoc	Taiping	Leaf oil	35.58 (27.66–45.60)	47.23 (33.10 – 69.52)	10.43 ± 2.51
C. iners	Kepong, FRIM	Leaf oil	62.84 (48.81–81.20)	77.80 (52.11 – 122.50)	13.82 ± 4.33
C. zeylanicum	Kepong, FRIM	Leaf oil	86.79 (77.78–97.42)	142.80 (122.29 – 185.22)	5.93 ± 0.91
Derris sp.	Perak	Root (hexane extract)	60.67 (49.86–69.07)	94.22 (81.11 – 129.15)	6.71 ± 1.56
Derris sp.	Perak	Root (methanol extract)	62.77 (42.96–96.96)	198.51 (119.29 – 844.58)	2.56 ± 0.69
Pelargonium citrosum	Sg. Buloh	Whole plant oil	24.97 (22.60–27.57)	40.50 (35.66 – 48.63)	6.10 ± 0.73
Xylopia caudata	Pasoh	Leaf oil	29.83 (21.87–37.45)	60.33 (48.04 – 82.47)	4.19 ± 0.69
Xylopia ferruginea	Pasoh	Leaf oil	74.51 (68.39–86.52)	106.45 (90.23 – 159.78)	8.27 ± 1.88
Zanthoxyllum acanthopodium	Cameron Highlands	Stem oil	19.87 (18.58–21.19)	29.87 (27.39 – 33.63)	7.24 ± 0.75

LC_{50} = lethal concentration to 50% test organisms, LC_{90} = lethal concentration to 90% test organisms, CI = confidence interval at 95% confidence level, SE = standard error

For repellency evaluation, the strongest value was shown by *Cymbopogon nardus* leaf essential oil from Gua Musang (EC_{50}=0.0009 mg cm^{-2}) (Table 2) when tested against adult *A. aegypti*, followed by Bukit Hari *C. nardus* leaf essential oil (EC_{50}=0.0015 mg cm^{-2}) and essential oil of *A. malaccensis* (EC_{50}=0.0016 mg cm^{-2}). A previous study on *C. nardus* had shown effective and almost complete protection against *Anopheles culicifacies* and other anopheline species (Ansari & Razdan 1995). This study reconfirms the findings of the previous study. The rest of the essential oils and extracts exhibited EC_{50} values ranging from 0.0023 to 0.0065 mg cm^{-2}. On account of the effectiveness of the *C. nardus* citronella oil, FRIM in collaboration with SIRIM Berhad has taken the initiative to produce an insect repellent cream from citronella oils (Nor Azah *et al.* 2003).

The seed kernel of *Azadirachta indica* gave the strongest median knock-down (KD_{50}) value at 40.25 min, followed by the leaf of *C. nardus* (45.02 min) and wood of *Fernandao adenophylla* (57.51 min) [Table 3]. For mean mortality value after 24 hours, the leaf of *C. nardus* gave the highest mortality, i.e. 15.7% ± 0.3 from 30 female mosquitoes tested. This was followed by *Eurycoma longifolia* (10.9% ± 0.2) and *F. adenophylla* (10.6% ± 0.2). From the results obtained, all the active plant samples can be utilized as organic filler in mosquito coil formulation. The incorporation of active plant samples can reduce utilization of synthetic pyrethroids such as D-allethrin and D-*trans*-allethrin in mosquito coil formulation.

Table 2 Effective concentration value (EC_{50} and EC_{90}) of repellency assessment against *Aedes aegypti* mosquitoes

Treatment	Locality/Source	Tested	EC_{50} (mg cm^{-2}) (95% CI)	EC_{90} (mg cm^{-2}) (95% CI)	Slope ± SE
Cymbopogon nardus	Gua Musang	Leaf oil	0.0009 (0.0003–0.0024)	0.0209 (0.0095–0.1289)	0.94 ± 0.19
Cymbopogon nardus	Bukit Hari	Leaf oil	0.0015 (0.0013–0.0017)	0.0035 (0.0029–0.0048)	3.51 ± 0.51
Cinnamomum mollisimum	Pasoh	Leaf oil	0.0065 (0.0055–0.0075)	0.0181 (0.0072–0.0477)	2.89 ± 0.66
Litsea elliptica	Pasoh	Leaf oil	0.0060 (0.0045–0.0075)	0.0440 (0.0273–0.1245)	1.56 ± 0.31
Pelargonium citrosum	Sg. Buloh	Leaf oil	0.0051 (0.0012–0.0088)	0.3356 (0.1027–16.6985)	0.71 ± 0.19
Pogostemon cablin	Perak	Leaf oil	0.0023 (0.0017–0.0029)	0.0195 (0.0126–0.0410)	1.42 ± 0.20
Aquilaria malaccensis	Pahang	Wood oil	0.0016 (0.0002–0.0118)	0.0190 (0.0018–0.2338)	1.20 ± 0.36
Ocimum tenuiflorum	Kuala Selangor	Leaf oil	0.0024 (0.0021–0.0028)	0.0105 (0.0083–0.0141)	2.01 ± 0.15
Dimethyl phthalate	Aldrich Chemical Co., Inc.	Commercial repellent	0.0007 (0.0005–0.0008)	0.0026 (0.0022–0.0034)	2.42 ± 0.26
Deet	Aldrich Chemical Co., Inc.	Commercial repellent	0.0005 (0.0003–0.0009)	0.0015 (0.0006–0.0038)	2.76 ± 0.54

EC_{50} = effective concentration to 50% test organisms, EC_{90} = effective concentration to 90% test organisms, CI = confidence interval at 95% confidence level, SE = standard error

Table 3 Knock-down assessment values (KD_{50} and KD_{90}) of plants against *Aedes aegypti* mosquitoes

Treatment	Locality/Source	Tested	KD_{50} (min) (95% CI)	KD_{90} (min) (95% CI)	Mortality (%)
Aloe vera	Kepong, FRIM	Leaf	60.57 (60.10–60.86)	129.47 (129.09–129.78)	7.8 ± 0.2
Alstonia angustifolia	Kepong, FRIM	Wood	130.24 (128.93–131.59)	168.95 (167.08–171.11)	5.0 ± 0.2
Antiaris toxicaria	Kepong, FRIM	Wood	145.38 (144.85–146.10)	>180.00	8.0 ± 0.3
Azadirachta indica	Penang	Seed kernel	40.25 (40.12–40.34)	67.57 (67.14–67.89)	8.5 ± 0.3
Cinnamomum zeylanicum	Kepong, FRIM	Wood	125.34 (124.86–125.82)	160.65 (159.36–162.00)	8.4 ± 0.1
Cinnamomum javanicum	Pasoh	Wood	129.22 (127.98–130.12)	167.21 (166.67–167.82)	8.9 ± 0.3
Curcuma domestica	Kepong, FRIM	Rhizome	113.33 (112.35–114.44)	159.00 (157.98–159.88)	10.4 ± 0.3
Cymbopogon nardus	Sabak Bernam	Leaf	45.02 (44.78–45.37)	62.02 (61.88–62.32)	15.7 ± 0.3
Eupatorium odoratum	Kepong, FRIM	Wood	125.48 (124.78–126.06)	175.98 (173.83–177.68)	9.7 ± 0.1
Eurycoma longifolia	Pasoh, NS	Wood	97.54 (96.97–98.90)	153.35 (153.30–153.40)	10.9 ± 0.2
Fernandao adenophylla	Perlis	Wood	57.51 (57.38–57.70)	98.32 (97.95–98.65)	10.6 ± 0.2
Morinda citrifolia	Kepong, FRIM	Wood	168.93 (168.00–169.68)	>180.00	7.3 ± 0.2
Oroxylum indicum	Perlis	Wood	155.98 (155.10–156.79)	>180.00	7.5 ± 0.1
Scorodocarpus borneensis	Kepong, FRIM	Leaf	135.87 (135.22–136.80)	170.55 (169.15–172.25)	8.2 ± 0.3
Control 1 (blank coil)		–	–	–	6.7 ± 0.2
Control 2 (no coil)		–	–	–	0.0 ± 0.0

EC_{50} = effective concentration to 50% test organisms, EC_{90} = effective concentration to 90% test organisms, CI = confidence interval at 95% confidence level, SE = standard error

Conclusions

The results from this study suggested that even from the same plant sample, the value for each activity was quite different. This suggests that the major component in each plant sample may contribute to its larvicidal, repellency and knock-down effects. In the search for alternative chemical insecticide, other factors have to be considered such as evaluation for the safety of non-target and beneficial organisms as well as sensitive indicator species, their resistance potential, performance in actual field conditions and their residual life span before any commercial biopesticidal agents can be developed.

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