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LABORATORY BIOASSAYS OF MALAYSIAN STANDARD MOSQUITO MAT FORMULATION AGAINST *AEDES AEGYPTI* (L.) AND *CULEX QUINQUEFASCIATUS* (SAY) USING TWO TEST METHODS

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Abstrak: Satu formulasi piawai mat (bahan aktif: d-allethrin 35.0 mg w/w dan piperonyl butoxide 35.0 mg w/w per mat) oleh Institut Piawai dan Penyelidikan Industri Malaysia (SIRIM) diuji terhadap nyamuk *Aedes aegypti* dan *Culex quinquefasciatus* pada selang masa 1, 2, 4, 6, 8 dan 10 jam selepas alat pemanas dipasang, berdasarkan protokol ujian piawai Malaysia yang dijalankan dalam peti kaca (70 x 70 x 70 cm) dan peti Peet Grady (180 x 180 x 180 cm). Nilai min KT_{50} yang diperolehi menunjukkan *Ae. aegypti* adalah lebih rentan kepada bahan aktif yang digunakan apabila dibanding dengan *Cx. quinquefasciatus*. Purata nilai KT_{50} keseluruhan bagi tempoh 10 jam di dalam peti kaca bagi *Aedes aegypti* dan *Culex quinquefasciatus* ialah 1.19 ± 0.07 dan 3.41 ± 0.35 minit, masing-masing. Apabila kandungan bahan aktif di dalam mat menurun dengan masa, penambahan beransur-ansur dalam nilai KT_{50} dengan selang tempoh pemanasan terhadap kedua-dua spesies nyamuk didapati. Selepas pendedehan awalan selama 20 minit dalam kedua-dua peti, kedua-dua spesies nyamuk yang dirawat menunjukkan nilai kematian selepas 24-jam rawatan yang amat rendah (<20%); ini menunjukkan penggunaan bahan aktif pemengsanan dalam formulasi mat. Keputusan makmal dan implikasinya dalam penggunaan formulasi mat di persekitaran tropika dibincang.

Abstract: A standard mat formulation (active ingredient: d-allethrin 35.0 mg w/w and piperonyl butoxide 35.0 mg w/w per mat) by the Standard and Industrial Research Institute of Malaysia (SIRIM) was tested against laboratory-cultured *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes at 1, 2, 4, 6, 8, and 10 hour intervals after the electric heater was switched on using Malaysian Standard test protocols conducted in both Glass Chamber (70 x 70 x 70 cm) and Peet Grady Chamber (180 x 180 x 180cm). The mean KT_{50} values obtained indicated that *Ae. aegypti* was more susceptible to the active ingredient used when compared to *Cx. quinquefasciatus*. The overall mean KT_{50} values over the 10 hour test interval in the glass chamber for *Ae. aegypti* and *Cx. quinquefasciatus* were 1.19 ± 0.08 and 3.41 ± 0.35 minutes, respectively. As the content of active ingredient in the mat decreased with time, there was a gradual increase in KT_{50} values with increasing exposure intervals to both species of mosquitoes. After the initial 20 minutes exposure to mat in both types of chambers, the treated mosquitoes of both species showed exceedingly low mortality values (<20%) at 24 hour posttreatment reflecting the use of knockdown active ingredients in the mat formulation. The laboratory results and their implication on the use of mosquito mat formulation in a tropical environment is discussed.

INTRODUCTION

Mosquito mat formulations with their accompanying electric heaters have been used in Malaysia for more than a decade. A questionnaire survey on household pests and household insecticide products conducted earlier (Yap and Foo 1984) indicated that Malaysian consumers used more aerosols (52.8%) and mosquito coils (39.0%) than mosquito mats (4.1%).

To date, there has been only one published report on the biological efficacy of mosquito mats (Chadwick & Lord 1977). This study was carried out to evaluate the biological efficacy of a standard mat formulation (Malaysian Standard, MS) against the two most common household mosquitoes in Malaysia, ie *Aedes aegypti* (L.) and *Culex quinquefasciatus* Say.

MATERIALS AND METHODS

Samples of Malaysian Standard (MS) mosquito mat formulation with accompanying electric heaters used in this study were supplied by Fumakilla Malaysia Berhad, Penang, Malaysia. The MS mat formulation (active ingredient: d-allethrin 35.0 mg w/w and piperonyl butoxide 35.0 mg w/w per mat) was tested in the laboratory against *Ae. aegypti* and *Cx. quinquefasciatus* at 1, 2, 4, 6, 8 and 10 hour intervals after the electric heater was switched on using the Glass Chamber method (Malaysian Standard 1986a, b) and the modified Peet Grady Chamber method (Busvine 1971).

The test protocol for the glass chamber method used in this study follows that of the Malaysian Standard (1986a). Based on this test protocol, the mat samples were heated in a draft chamber with the accompanying electric heater (Malaysian Standard 1986b) which was subsequently introduced into the glass chamber (70 X 70 X 70 cm) for a three minute exposure, at each designated interval. A batch of twenty laboratory-cultured sucrose-fed female mosquitoes aged 2 to 5 days old were then released into the chamber at each exposure interval. Mosquito knockdown values (KT values) were read at indicated intervals up to 20 minutes. Criteria for knockdown were that the mosquitoes no longer maintain normal posture and were unable to fly or were on their backs. After the 20 minute exposure in the test chamber, the knockdown mosquitoes were then collected using a pair of fine forceps and placed in a clean polyethylene cup with a lid covered with cotton pad containing 10% sucrose. The mortality of the mosquitoes in the cup was observed after 24 hours. The mortality value was derived by dividing the total number of dead and moribund mosquitoes by the total number of mosquitoes initially released. A minimum of 80 insects were used for the test in four replicates. All test procedures were conducted in an air conditioned laboratory with a temperature of 27 $\pm$ 2°C and 70 $\pm$ 10% relative humidity.

The Peet Grady chamber (180 x 180 x 180 cm) used in the tests was essentially similar to that described earlier by Busvine (1971). The bioassay tests were based essentially on the test protocols used in the Glass Chamber method, with the following modifications: (1) The test mat was heated continuously throughout the experimental period of 10 hours with a small fan blowing underneath the test mat; (2) The ventilation ports of the chamber were opened and the exhaust fans were switched off; (3) Twenty-five mosquitoes were maintained in each of the four cylindrical cages constructed of iron frame covered with fine mesh netting (diameter = 30 cm; height = 30 cm); (4) Each of the cage was then hanged at the four corners of the Peet Grady chamber approximately 100 cm from the ground level of the chamber; (5) Knockdown mosquitoes were observed at indicated intervals up to ten hours and the *mortality values of each batch of exposed* mosquitoes were read at 24 hours post-treatment. A minimum of 400 sucrose-fed female mosquitoes were used for the test in four replicates.

The amount of active ingredient (ai.) present in the mat was 35 mg/mat d-allethrin (Pynamin-Forte[R], dl-3-allyl-2-methyl-4-oxo-2-cyclopentyl d-cis trans chrysanthemates) with 35 mg/mat piperonyl butoxide [2-(2-butoxyethoxy) ethyl 6-propylpiperonyl ether] acting as an evaporation retardant.

The colonies of *Ae. aegypti* and *Cx. quinquefasciatus* used in this study were established earlier using adult mosquitoes collected from Georgetown and Teluk Kumbar, Penang Island, respectively. These colonies have been reared at the Vector Control Research Unit, Universiti Sains Malaysia since the 1980s. For all bioassays conducted, only sucrose-fed adult female mosquitoes aged between 2 to 5 days were used.

The time response data collected were analyzed by probit analysis (Finney 1963) using a computer program (Daum 1970).

In addition to the above experiment, the changes in the a.i. content in the mat at different time intervals were also determined. For the a.i. analysis, a mat was cut into small pieces (0.5 x 0.5 cm each) and dissolved in 95% analytical grade acetone after different heating periods. This was followed by the analysis of the amount of a.i. remaining using a standard liquid chromatography method (Malaysian Standard 1986b).

RESULTS

Comparative time-response values of the standard mat formulation against both mosquito species tested indicated a similar trend of relative susceptibility (Tables 1 and 2). The results showed that *Ae. aegypti* appeared to be more susceptible with lower KT_{50} values (knockdown time that achieved 50% KT of test mosquitoes) when compared to *Cx. quinquefasciatus* for both the Glass Chamber and Peet Grady Chamber methods at the same interval of exposure (Tables 1 and 2). However, the biological efficacy based on the KT_{50} values of the mats against the two mosquito species in the Glass Chamber tests was observed to be better than that of the Peet Grady Chamber test, (Tables 1 and 2). Overall, the results from the 10-hour exposure also indicated that *Ae. aegypti* (mean KT_{50} = 1.19 ± 0.08 minutes) was about 2.9 times more susceptible than *Cx. quinquefasciatus* (mean KT_{50} = 3.41 ± 0.35 minutes) when exposed to d-allethrin using the Glass Chamber method (Table 1). A similar trend was also observed in the Peet Grady Chamber tests with mean KT_{50} values for *Ae. aegypti* and *Cx. quinquefasciatus* of 14.49 ± 1.69 minutes and 34.02 ± 4.01 minutes, respectively (Table 2). Mortality values at 24 hours post-treatment also showed that *Ae. aegypti* was more susceptible than *Cx. quinquefasciatus*. However, no discernible correlation was observed between the mortality values and exposure intervals (Tables 1 and 2).

In both test methods, KT_{50} values against *Ae. aegypti* and *Cx. quinquefasciatus* measured with a 3-minute exposure in the chambers at time intervals up to 10 hours on the same mosquito mats increased gradually throughout the test period (Tables 1 and 2). The increase appeared to be more pronounced in *Cx. quinquefasciatus* when compared with *Ae. aegypti*. Analysis of active ingredient (d-allethrin) by gas liquid chromatography showed a gradual decrease of a.i. after the heating of the mat with its accompanying heater (Table 3). The decrease became more drastic with greater elapsed time. At the 10 and 12- hour exposure of a mat on the heating unit, the a.i. levels were 27.5 and 17.4% of the original, respectively (Table 3).

DISCUSSION

Based on the KT_{50} values and the percentage mortality, it was observed that the *Ae. aegypti* mosquito is more susceptible than *Cx. quinquefasciatus* to the Malaysian Standard mat formulation (Tables 1 and 2). The test results corresponded well with the mosquito coil biological efficacy studies against both species of mosquitoes by Yap and Chung (1987). Similarly, Ogami *et al.* (1970) had also reported on the slow knockdown of *Cx. pipiens pallens*, (a related *Culex pipiens* complex species), when tested using coils with d-allethrin as the active ingredient. The low mortality values achieved for both species of mosquitoes by the mats reflect the active ingredient used (d-allethrin) which is basically a knockdown agent.

The efficacy of mosquito coil does not vary with time due to the continuous and uniform release of a.i. through the burning period (MacIver 1963, Anonymous 1987). However, in the case of the mosquito mat, due to the continuous vaporization of a.i. from the same piece of mat, the decrease in the

Table 1: Bioassay of Malaysian Standard mat (d-allethrin 35 mg w/w and PBO 35 mg w/w per mat) against *Aedes aegypti* and *Culex quinquefasciatus* using the Glass Chamber method[1].

Mosquito Species	Time intervals (hours)	KNOCKDOWN VALUES			% Mortality at 24 hour post-treatment
		KT50 & Confidence limit (min)	KT90 & Confidence limit (min)	Regression Slope ± Std Errors	
Aedes aegypti	1	0.96 (0.75-1.22)	1.5 (1.24-2.69)	5.96 ± 0.67	8
	2	1.28 (1.24-1.32)	1.98 (1.89-2.08)	6.78 ± 0.28	5
	4	1.07 (1.03-1.12)	2.13 (2.02-2.26)	4.30 ± 0.15	14
	6	1.27 (1.15-1.40)	2.32 (2.06-2.70)	4.91 ± 0.27	20
	8	1.10 (1.06-1.14)	1.77 (1.68-1.86)	6.23 ± 0.26	15
	10	1.48 (1.43-1.54)	2.87 (2.74-3.02)	4.47 ± 0.14	11
	1 - 10	1.19 ± 0.08[2]			
Culex quinquefasciatus	1	2.71 (2.63-2.80)	5.19 (5.01-5.39)	4.55 ± 0.13	0
	2	2.62 (2.54-2.71)	4.86 (4.68-5.05)	4.80 ± 0.15	1
	4	3.28 (3.17-3.38)	7.17 (6.90-7.48)	3.77 ± 0.10	0
	6	3.30 (3.20-3.39)	6.43 (6.21-6.68)	4.42 ± 0.12	5
	8	3.54 (3.44-3.64)	6.79 (6.54-7.08)	4.53 ± 0.14	6
	10	5.02 (4.89-5.14)	9.57 (9.22-9.95)	4.57 ± 0.12	4
	1 - 10	3.41 ± 0.35[2]			

[1] A minimum of 80 insects were used for the test and 4 replicates were conducted.
[2] Mean value (± S.E.) of KT_{50} over 10 hours

Table 2: Bioassay of Malaysian Standard mat (d-allethrin 35 mg w/w and PBO 35 mg w/w per mat) against *Aedes aegypti* and *Culex quinquefasciatus* using the Peet Grady Chamber method[1].

Mosquito Species	Time intervals (hours)	KNOCKDOWN VALUES: KT50 & Confidence limit (min)	KNOCKDOWN VALUES: KT90 & Confidence limit (min)	KNOCKDOWN VALUES: Regression Slope ± Std Errors	% Mortality at 24 hour post-treatment
Aedes aegypti	1	10.67 (10.40-10.95)	21.16 (20.37-22.04)	4.31 ± 0.08	10
	2	11.53 (11.23-11.83)	23.74 (22.86-24.71)	4.05 ± 0.07	9
	4	12.56 (12.27-12.85)	26.26 (25.44-27.15)	4.00 ± 0.06	6
	6	12.66 (12.36-12.96)	26.62 (25.72-27.60)	3.97 ± 0.06	9
	8	14.21 (13.79-14.64)	28.12 (26.99-29.38)	4.32 ± 0.09	16
	10	19.77 (19.42-20.12)	36.87 (26.06-37.72)	4.74 ± 0.07	11
	1 - 10	14.49 ± 1.69[2]			
Culex quinquefasciatus	1	25.54 (25.28-25.80)	49.84 (49.14-50.57)	4.41 ± 0.04	3
	2	25.69 (25.43-25.94)	49.45 (48.76-50.16)	4.51 ± 0.04	3
	4	30.25 (29.96-30.53)	57.30 (56.46-58.18)	4.62 ± 0.05	5
	6	32.00 (31.36-32.64)	64.06 (61.91-66.46)	4.25 ± 0.09	9
	8	43.28 (42.77-43.81)	96.33 (93.63-99.26)	3.69 ± 0.05	6
	10	49.10 (48.51-49.73)	101.20 (98.27-104.40)	4.08 ± 0.06	3
	1 - 10	34.02 ± 4.01[2]			

[1] A minimum of 400 insects were used for the test and 4 replicates were conducted.
[2] Mean value (± S.E.) of KT_{50} over 10 hours.

Table 3: Changes in the content of active ingredient of MS Mat at various heating time on its accompanying electric heating unit at 160°C[1].

Heating interval (hours)	Active ingredient (mg) (Mean ± SE)	% ai remaining
0	36.26 ± 0.09	100
0.5	34.33 ± 0.21	95
1	32.96 ± 0.59	91
2	30.08 ± 0.23	83
4	26.20 ± 0.47	72
6	18.85 ± 0.82	52
8	15.98 ± 0.69	44
10	9.96 ± 1.25	27
12	6.30 ± 0.58	17

[1] Mean results of 6 replicates using different sets of mats with accompanying heating unit.

remaining a.i. content (Table 3) will imply that less a.i. would be vaporized in the test chambers as time progressed. An earlier report (Anonymous 1987) indicated that the heating temperature, design of heating device as well as mat substrate were the main factors that contributed to the differences in the rate of decline of a.i. emission from the mat over the exposure period. In this study, the correlation of the bioassay results with the remaining a.i. content of the mat formulation over the test period of 10 hours indicated corresponding decrease both in biological efficacy and a.i. contents. Chadwick and Lord (1977) also reported similar findings and postulated that the temperature of the heating device will affect the rate of a.i. released.

The usage of mosquito mats in Malaysian household was reported to be just 4.1% of the total usage of household insecticide products (Yap & Foo 1984). A more recent survey conducted in 1991 did not show any major increase in the mosquito mat usage in the Malaysian household insecticide market (Yap HH, unpublished data). In comparison with mats, the use of mosquito coils for the purpose of preventing man-mosquito contacts in living premises indoors in Malaysia was much higher (Yap and Foo 1984).

In Japan, Koda (1980) had reported that the consumption of mats was increasing exponentially and was gradually replacing mosquito coils in the Japanese market, since its introduction in 1967. Within 12 years after its introduction, mats have taken over a sizeable market from the coils in Japan. In fact, in 1982, the usage of mosquito mats had exceeded the usage of mosquito coils, with 1.064 billion pieces of mats compared to only 0.68 billion pieces of coils used annually (Sumitomo Chemical Co, Osaka, Japan, personal communication). In Malaysia, the usage profile appears to indicate that there is some resistance by the consumers to the use of mosquito mats over existing coils. This may be due to the following reasons: (1) When the first brand of mosquito mat (Vape®) was initially introduced in Malaysia in 1978, the active ingredient used (d-allethrin 35 mg w/w per mat and PBO 35 mg w/w) was exactly the same as those used in Japan. The same mat may provide adequate efficacy in the rooms in Japanese living premises which are relatively small with little ventilation when compared with rooms in living premises in Malaysia and other ASEAN countries where bigger rooms with ample ventilation are the norm. The size of living premises reflects more on the weather condition and living cost of Japan as compared to the situation in ASEAN countries. With the different sizes of living premises in ASEAN countries, whether mats with the same a.i. can provide adequate field efficacy in such contrasting environments is still not determined. (2) The mat formulation with its accompanying electric heating device imply that the supply of electricity is universal in Japan. However, this is not true of the situation in many rural areas in Malaysia and other ASEAN countries such as Indonesia, Philippines and Thailand. (3) The family income in Malaysia and related ASEAN countries is lower than that in Japan. Hence, consumers may not switch to a new device (mat with its heating unit) that cost more than the existing product (coils) for the purpose of preventing man-mosquito contact in their living premises.

The active ingredients used in both mats and coils in Malaysia and other Southeast Asian countries are dominated by the short-lived knockdown pyrethroids including d-allethrin and d-transallethrin. In the case of the mats, the common a.i. d-allethrin and d-trans allethrin are 35 mg/mat and 23 mg/mat, respectively (Yap 1988). More recently, another knockdown agent, namely prallethrin [(s)-2-methyl-4-oxo-3(2-propynyl) cyclopent-2-enyl (IR)-cis, trans-chrysanthemate] has also been introduced as an a.i. for mat.

The bioassay methods described in this study have been tested and developed at the Vector Control Research Unit, Universiti Sains Malaysia with the assistance of the chemical industries since 1981. It has provided reproducible test results and good differentiation response for different test formulations against mosquitoes. The Glass Chamber method was also adopted by the Malaysian Standard Institute as the Malaysian Standard test method for mosquito mats (Malaysian Standard 1986a). In addition, the adoption of the Peet Grady Chamber method described in this study is being deliberated by the Household Pesticide Committee at the Malaysian Standard Institute and should become a Malaysian Standard in due course.

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