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Field evaluation of the bioefficacy of diflubenzuron (Dimilin®) against container-breeding *Aedes* sp. mosquitoes

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Abstract. The inhibitory activity of diflubenzuron, a chitin synthesis inhibitor, on the ecdysis of *Aedes* sp. larvae was evaluated in earthen jars and automobile tires. Two formulations of diflubenzuron were used in this study: Dimilin® WP (wettable powder), 25% and Dimilin® GR (granular), 2%. The equivalent rate of 25 g/ha, 50 g/ha and 100 g/ha active ingredients for both WP and GR formulations were used in this study. Generally, at the higher dosage of 100 g/ha, both formulations were more effective against *Aedes* mosquitoes. On the whole, the WP formulation appeared to perform better than the GR formulation in terms of residual activity.

INTRODUCTION

Dengue was first reported in Malaya by Skae (1902), who described an epidemic in Penang. Since the first nationwide dengue outbreak in 1973, the incidence rate of dengue has been on the increase. Rapid industrial and economic developments over the last 3 decades have brought about massive infrastructure development, creating man-made environment for breeding of *Aedes* mosquitoes. Currently, dengue is the most important mosquito-borne viral disease in Malaysia.

Dengue viruses are transmitted to human through the bite of an infected *Aedes* mosquito. For many years, members of subgenus *Stegomyia*, especially *Aedes aegypti* and *Aedes albopictus*, have been recognized as the primary vector of dengue (Boromisa *et al.*, 1987; Gubler *et al.*, 1987). The mosquitoes are also capable of transovarial transmission of dengue virus as reported by Rosen *et al.* (1978).

As effective dengue vaccine and specific dengue treatment are still not available, chemical insecticides still play an important role in the control of dengue vectors. Unfortunately, *Ae. aegypti* and *Ae. albopictus* resistance against major classes of chemical insecticides has been an increasing problem in the past decades worldwide.

Dimilin® (diflubenzuron) is an insect growth regulator of the benzoyl urea family that inhibits the systhesis of chitin and hence interferes with molting. Mosquito larvae treated with diflubenzuron die during ecdysis because the ecdysing larvae fail to completely shed the old cuticles; those that survive die in the pupal stage or during eclosion of adults (WHO, 2006).

Diflubenzuron WP has been used to control mosquito larvae since the mid-1970's at the WHOPES recommended dosages of 25-100 g/ha. This study was undertaken to assess the efficacy of diflubenzuron against container-breeding *Aedes aegypti* and *Ae. albopictus* (WHO, 2006).

MATERIALS AND METHODS

Test site

The study was conducted at the surrounding area of the Medical Entomology Unit, Institute for Medical Research (IMR), Jalan Pahang, Kuala Lumpur (N03°10.167', E101°41.919'). This site was an empty land covered by vegetation, which provided ideal resting places for mosquitoes.

Insecticide (Diflubenzuron – Dimilin®)
Two formulations of diflubenzuron were used in this study: Dimilin® WP (wettable powder), 25% and Dimilin® GR (granular), 2%. The equivalent amount of 25 g/ha, 50 g/ha and 100 g/ha diflubenzuron active ingredients for both WP and GR formulations were used in this study.

Test containers
Earthen jars and automobile tires were used as mosquito breeding containers in this study. Five replicates of each were used in each research arm of the study. Initially, each earthen jar held 20 L tap water. Addition of water was not done unless the water level fell below the original level. In tires, 4L of water was added. Before initiating the study, all containers were washed with tap water and tested for the presence of any larvicidal contaminant by introducting 30 lab-bred *Ae. aegypti* (L3) larvae. The larvae were observed daily until complete emergence. This was done prior to the actual testing of Dimilin against the natural population.

Trial procedures
The containers were set up as mentioned. The natural mosquito density was monitored for 2 weeks; after which diflubenzuron was applied in all the test containers. The amount of the larvicide to be applied was carefully measured and applied to the surface of the water. The larval/pupal populations in all containers were monitored 1, 2, 4 days post-treatment and thence after weekly, with minimal disturbances (tires, especially, should not be moved as the insecticide in the cracks of the tyre may no longer be available if the water in the container is displaced) until they returned to the original pre-control levels or in 3 month's time. All the pupae were collected daily into labeled and screened vials, and kept in the laboratory. The emergence rate of adults was recorded. Use of a torch to collect pupae, with minimum disturbance of the tires may be necessary.

Since all containers were examined daily and all pupae removed immediately, the risk of accidental release of adults was minimal or non-existing.

Data analysis
The indicators of effectiveness of diflubenzuron for container-breeding *Aedes* were:

a) changes in population levels between treated and untreated container i.e. mean number pupae in each type of containers at various diflubenzuron dosages,

b) residual activities of each dosage, and

c) percentage emergence inhibition (= 100 – rate of emergence in treated container/ rate of emergence in untreated container x 100).

Statistical software (SPSS v10) was used to analysis the data.

RESULTS

Earthen Jars
The natural *Ae. albopictus* larval/pupal populations was established quickly in about 11 days after placement of the earthen jars outdoor. The pre-treatment mosquito population was monitored for 2 weeks, after which diflubenzuron was added in accordance to pre-determined dosages. The pupal populations in treated and untreated earthen jars were monitored daily.

Table 1 showed the weekly mean number of pupae (from 5 jars of each dosages) collected from earthen jars treated with diflubenzuron and untreated control, while Table 2 indicated the post-treatment mean pupal populations in the 2 months' trial period. Within the first 4 week of post-treatment, in earthen jars treated with Dimilin GR 25 g/ha, no significant difference in the reduction of pupal populations was observed compared with the untreated control ($p = 0.989$). However, within the same period, significant pupae reduction was observed for Dimilin GR 50 g/ha ($p = 0.050$), Dimilin GR 100 g/ha ($p = 0.036$),

Table 1. Mean number of pupae per week obtained from earthen jars placed outdoor

Jar	Pre-treatment	Week							
		1	2	3	4	5	6	7	8
C - Untreated	11.12	10.00	16.20	20.67	21.33	14.50	12.17	11.75	10.33
Treated									
GR 25g/ha	6.38	3.50	12.40	28.00	24.67	20.17	17.83	14.25	13.83
GR 50g/ha	6.25	1.50	5.60	11.33	13.33	13.50	14.00	16.25	16.17
GR 100g/ha	5.38	6.25	6.80	6.67	14.00	21.33	19.83	19.25	18.50
WP 25g/ha	6.50	2.25	0.00	0.00	0.00	16.17	18.00	13.25	12.17
WP 50g/ha	5.13	1.75	0.00	0.00	0.00	1.30	13.50	17.50	13.85
WP 100g/ha	4.75	0.50	0.00	0.00	0.00	0.33	2.17	17.75	15.50

Table 2 Mean pupae numbers ± SE for every 4 weeks post treatment in earthen jars placed outdoor

Jar	Post-treatment			
	1 – 4 weeks		5 – 8 weeks	
	Pupae number (mean ± SE)	t-test	Pupae number (mean ± SE)	t-test
C - Untreated	17.05 ± 2.61	–	12.19 ± 0.87	–
Treated				
GR 25g/ha	17.14 ± 5.65	$p = 0.989^a$	16.52 ± 1.51	$p = 0.048^c$
GR 50g/ha	7.94 ± 2.70	$p = 0.050^b$	14.98 ± 0.72	$p = 0.048^c$
GR 100g/ha	8.43 ± 1.86	$p = 0.036^b$	19.73 ± 0.60	$p = 0.000^c$
WP 25g/ha	0.56 ± 0.56	$p = 0.000^b$	14.90 ± 1.34	$p = 0.141^a$
WP 50g/ha	0.44 ± 0.44	$p = 0.000^b$	11.54 ± 3.53	$p = 0.864^a$
WP 100g/ha	0.13 ± 0.13	$p = 0.000^b$	8.94 ± 4.48	$p = 0.503^a$

$P > 0.05$ = No significant different
$P < 0.05$ = Significant different
[a] = No significant different between number of pupae obtained from untreated and treated containers
[b] = Pupae obtained from treated containers significantly less than untreated containers
[c] = Pupae obtained from treated containers significantly more than untreated containers

Dimilin WP 25 g/ha ($p = 0.000$), Dimilin WP 50 g/ha ($p = 0.000$) and Dimilin WP 100 g/ha ($p = 0.000$) at $p < 0.05$ in comparison to the untreated containers.

The emergence inhibition is shown in Figure 1. In earthen jar treated with Dimilin GR 25 g/ha, emergence inhibition of the *Aedes* pupae was effective for 2 weeks, after which no inhibition was observed, though the degree of effectiveness had declined to 23.46%. At Dimilin GR 50 and 100 g/ha, the inhibition lasted 4 – 5 weeks. In the Dimilin WP series, all the 3 dosages caused complete inhibition up to 4 weeks, after which the 2

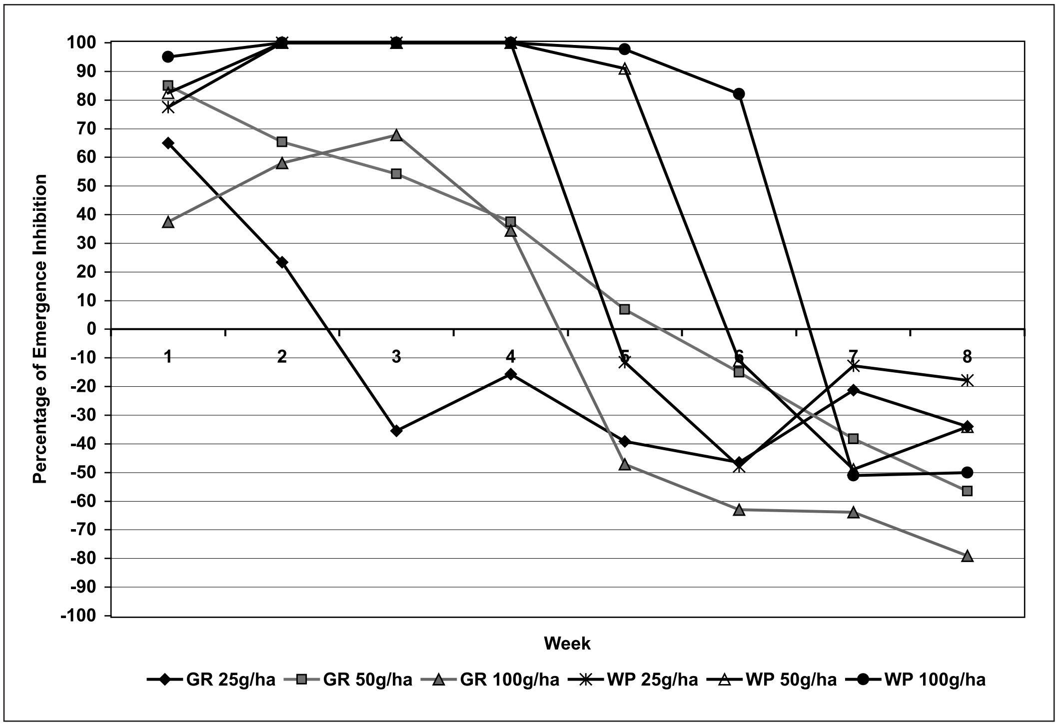

Figure 1. Percent emergence inhibition of *Aedes* larvae obtained from outdoor jars placed outdoor.

higher concentrations of 50 and 100 g/ha lasted another 1 – 2 weeks, still causing 91.03% and 82.17% of inhibition, respectively.

Tires

About 30 days after placement of the automobile tires outdoor, significantly high pupal and larval populations of *Ae albopictus* were observed. The mosquito populations were further monitored for 26 days as pre-treatment population density. The tires were then treated with various pre-determined dosages of Dimilin® in accordance to test protocol. Table 3 indicated changes in the pupae populations in various tires in the 8-week trial period. In the first 4 weeks post-treatment (Table 4), significant pupae reduction was observed for Dimilin GR 50 g/ha ($p = 0.002$), Dimilin GR 100 g/ha ($p = 0.003$), Dimilin WP 50 g/ha ($p = 0.004$) and Dimilin WP 100 g/ha ($p = 0.000$) in comparison to the untreated tires. In the next 4 weeks, no significant pupae reduction was observed in all treated tires, except tires treated with Dimilin WP 100 g/ha ($p = 0.000$).

The emergence inhibition is shown in Figure 2. In tires treated with Dimilin GR 25 g/ha, Dimilin GR 50 g/ha and Dimilin GR 100 g/ha, there was generally inhibition of emergence for 6 weeks. In tires treated with WP 25 g/ha, the effect of emergence inhibition appeared to be inconsistent, in which the effect was seen on week 1 & 4 only, but not in other weeks. In tires treated with Dimilin WP 50 g/ha, the inhibition lasted up to 4 weeks. However, tires treated with Dimilin WP 100 g/ha exhibited the longest emergence inhibition effects throughout the trial period of 8 weeks.

DISCUSSION

The results in earthen jars indicated that both formulations of Dimilin® exhibited various degrees of inhibition on the *Aedes* larvae, albeit complete inhibition of pupation/ emergence for several weeks. Generally, there was indication of dose-response relationship, i.e. higher dosages tend to exhibit higher inhibition with longer effective period. The general lack of complete effect of Dimilin was due to the low dosages used in this trial. The dosages applied in the

Table 3. Mean number of pupae per week obtained from tires placed outdoor

Tires	Pre-treatment	Week							
		1	2	3	4	5	6	7	8
C – Untreated	14.69	10.00	10.5	8.50	9.75	6.33	7.68	6.67	6.00
Treated									
GR 25g/ha	4.02	4.00	5.00	10.50	5.25	3.67	4.67	9.00	7.33
GR 50g/ha	6.31	2.50	6.50	4.50	2.25	6.33	7.33	9.00	7.67
GR 100g/ha	5.27	0.75	6.75	3.50	2.25	4.33	4.00	6.67	7.67
WP 25g/ha	18.63	2.25	11.25	9.50	5.50	9.33	8.67	7.33	9.00
WP 50g/ha	14.88	3.00	7.50	4.00	4.75	7.00	15.33	10.00	9.67
WP 100g/ha	11.92	0.00	1.75	1.00	0.00	2.57	1.67	1.67	0.67

Table 4. Mean pupae numbers ± SE for every 4 weeks post treatment in tires placed outdoor

Tires	Post-treatment			
	1 – 4 weeks		5 – 8 weeks	
	Pupae number (mean ± SE)	t-test	Pupae number (mean ± SE)	t-test
C - Untreated	9.69 ± 0.43	–	6.67 ± 0.36	–
Treated				
GR 25g/ha	6.19 ± 1.46	p = 0.061[a]	5.17 ± 1.78	p = 0.440[a]
GR 50g/ha	3.96 ± 0.98	p = 0.002[b]	7.58 ± 0.55	p = 0.216[a]
GR 100g/ha	3.31 ± 1.28	p = 0.003[b]	5.67 ± 0.89	p = 0.338[a]
WP 25g/ha	7.13 ± 2.02	p = 0.261[a]	8.58 ± 0.44	p = 0.015[c]
WP 50g/ha	4.81 ± 0.96	p = 0.004[b]	10.50 ± 1.74	p = 0.075[a]
WP 100g/ha	0.69 ± 0.43	p = 0.000[b]	1.65 ± 0.39	p = 0.000[b]

$P > 0.05$ = No significant different
$P < 0.05$ = Significant different
[a] = No significant different between number of pupae obtained from untreated and treated containers
[b] = Pupae obtained from treated containers significantly less than untreated containers
[c] = Pupae obtained from treated containers significantly more than untreated containers

earthen jars and tires were based on previous recommended dosages of 25, 50 & 100 g/ha which when converted into mg ai/L were 0.018 mg ai /L, 0.022 mg ai/L and 0.043 mg ai/L for the WP formulation, and 0.0013 mg ai/L, 0.0026 mg ai/ha and 0.0052 mg/L for the GR formulation.

Ho *et al.* (1990) reported trials in which diflubenzuron used at a dosage of 0.5 mg/L was able to reduce 97% of *Ae. albopictus* larvae. In fact, the EC50 of diflubenzuron against *Ae. aegypti* was 0.045 mg/L and the EC95 was 0.255 mg/L (Montada Dorta *et al.*, 1989), and for *Ae. albopictus* the EC90 was 0.027 mg/L (Baruah & Das, 1996).

Lam (1990) in Malaysia reported the application of Dimilin® WP-25 in septic tanks for the control of *Ae. albopictus* breedings.

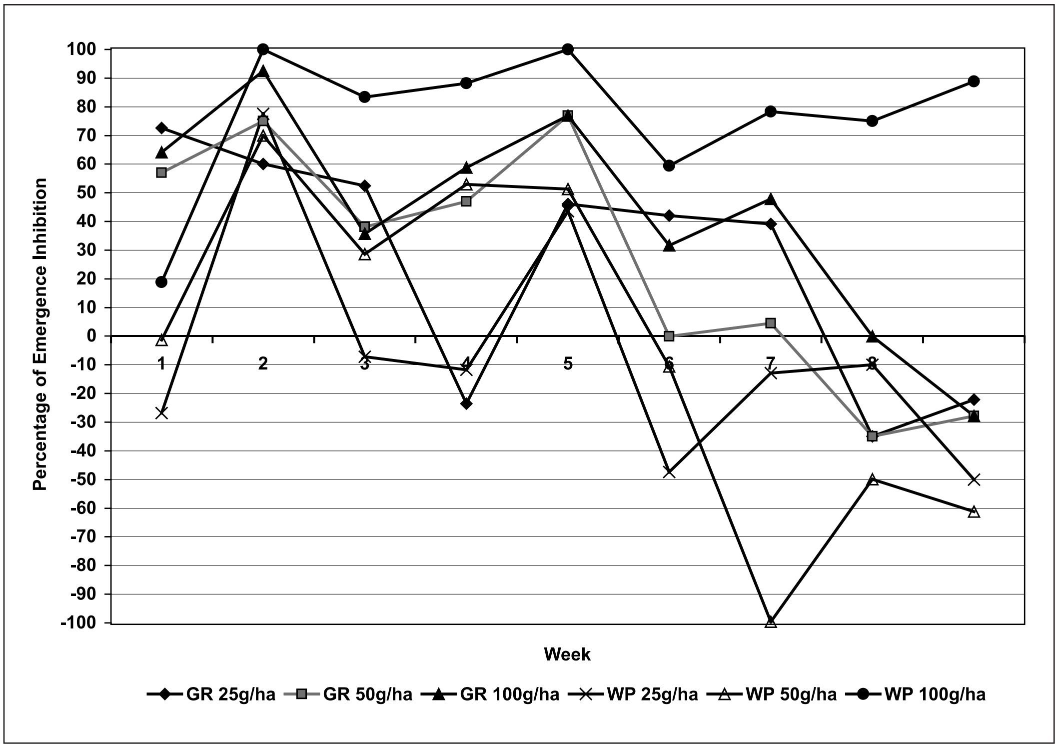

Figure 2. Percent emergence inhibition of *Aedes* larvae obtained from tires placed outdoor.

Effective control of up to 8 weeks post-treatment was obtained at dosage rates of 1 mg ai/L, 2 mg ai/L and 4 mg ai/L. Complete inhibition of pupation was achieved for 8 weeks at an application rate of 4 mg ai/L. It is thus clear that if both Dimilin WP and GR are to be used to control container breeding *Aedes* in which the water is expected to be devoid of organic matters, the application rate is estimated to be 0.5-1.0 mg ai/L.

Beside this, Cetin *et al.* (2006) also conducted a study on diflubenzuron (25% wettable powder and 4% granular formulation) by evaluated it against late 2nd and early 3rd instars of *Cx. pipens* in a single-family septic tanks. Both formulations were tested at 0.01, 0.02 and 0.03 mg a.i./L. The results indicated that both formulations applied at the rate of 0.02 and 0.03 mg a.i./L achieved 100% adult inhibition up to 28 days (4 weeks) post treatment. Septic tanks treated with 0.01 mg a.i./L WP and GR formulations resulted in complete adult inhibition through 2 weeks and 3 weeks, respectively.

Similarly in tires treated at 25, 50 and 100 g/ha equivalent of Dimilin WP and GR, which may account for the short residual effects as well as incomplete inhibition of pupation and emergence.

Despite the lower dosages applied in all containers, both Dimilin® formulations exhibited various degree of inhibition against container-breeding *Aedes*. Although pupation continued to occur, some degree of emergence inhibition was also evident.

In terms of user preference, both Dimilin formulations mixed well with the water. The WP formulation did not give rise to any turbidity in the treated water and the appearance of GR treated water was also normal. These features will not deter consumers from using an IGR such as Dimilin. However, because of the mode of action of IGR, larvae are still present & alive after application of the chemical and this may discourage use of IGR in the control of container-breeding *Aedes* because in certain countries, the presence of *Aedes* larvae is ground for the enforcement officers to take

Table 5. Residual effect of Dimilin GR and WP formulation at 25 g/ha and 100 g/ha based on 50% emergence inhibition of *Aedes*

Dosage of Dimilin Formulation (g/ha)	Duration for 50% emergence inhibition (week)	
	Earthen jar	Tires
GR 25	1	2
GR 50	3	4
GR 100	3	4
WP 25	4	Inconsistent
WP 50	5	4
WP 100	6	8

legal action against the house-owners in spite of the use of IGR in containers since the larvae will still be actively moving around until affected by the chemical later on. Hence, the user and the enforcer should be educated on the use of IGR in container-breeding *Aedes* control.

The residual effects of both Dimilin GR and WP formulations at 25 g/ha and 100 g/ha based on the 50% emergence inhibition of *Aedes* mosquitoes is summarised in Table 5. Generally, at the higher dosage of 100 g/ha, both formulation is more effective against *Aedes* mosquitoes. On the whole, the WP formulation appeared to perform better than the GR formulation in terms of residual activity.

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