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EFFECT OF CHLORPYRIFOS ON THE OVIPOSITION AND SURVIVAL OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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Abstract Phenomenal of high egg reproduction when mortality risk rose is very common in mosquito. Yet, the presence of this reproduction advantage in insecticide resistance mosquito remains unclear. In this study, we evaluated the effect of chlorpyrifos (CP) exposure on the oviposition and survival of *Aedes aegypti* using two strains. We found that CP exhibited no oviposition deterrent against female mosquitoes of both Lab and KH strains, given that the females did not show any decrease in their reproduction activity on CP-treated sites. High mortality was recorded in Lab strain after contacting the CP-treated oviposition sites on day 4. Before death, the mosquitoes oviposited large number of eggs indicating the adaptive strategy of the mosquito aimed at maintaining the progeny in the population. However, this reproductive effort was not seen in KH strain although ~60% of the mosquitoes were killed at 1.0 ppm CP. Mosquitoes of this strain are exposed to a variety of insecticide. Coping with the toxicity of insecticides can be costly, and thus energy and resource are redirected to survival instead of reproduction.

Key words Fitness cost, fecundity, selection, trade-off, dengue

INTRODUCTION

Aedes aegypti is the vector for dengue outbreak in the urban area of Southern of Taiwan. Pyrethroids and organophasphate are widely used in Taiwan to suppress the population of this mosquito. As a result, the mosquitoes are found resistance to these compounds. These mosquitoes often exhibited a series of trade-offs, most apparently on reproduction (Martins et al., 2012; Brito et al. 2013). High egg reproduction when mortality risk rose is common in mosquitoes. However, no study till date has investigated this reproductive effort in resistance strain of *A. aegypti*. In this study, we examined the oviposition trend of susceptible and resistance strain of *A. aegypti* on organophosphate insecticide.

MATERIALS AND METHODS

Two strains of *A. aegypti* were used in the study: 1) Kaohsiung laboratory strain (Lab). This standard susceptible strain to chemical insecticides was collected from Kaohsiung, Taiwan, and has been maintained until 243 generation in the insectarium of National Chung Hsing University at 25.0 ± 1.0°C, 65.0 ± 2.0% relative humidity (RH), and a photoperiod of 12:12 (L:D); and 2) Kaohsiung wild strain (KH). The population was collected from Kaohsiung Lingya district, and maintained in the insectarium as mentioned above. First filial generation (F_1) of KH strain was used in the study.

Briefly, a total of 20 blood-fed female mosquitoes aged between 6-7 days old were introduced into mosquito cage (60.0 cm length x 60.0 cm width x 60.0 cm height) with provision of 10% sucrose. Two days after the introduction of the mosquitoes, a plastic cup (6.0 cm diameter x 7.0 cm height) lined with towel paper (22.5 cm length x 7.0 cm width) was filled with 30 ml chlorpyrifos (CP), and placed in

the cage as oviposition site. The number of eggs laid and the mortality of the adults were recorded daily for three consecutive days. Oviposition site was replaced with new oviposition site daily.

Three concentrations of CP 95.3% (SINON Corporation, Taiwan R. O. C) were tested: 0.25 ppm, 0.5 ppm, and 1.0 ppm. For the control, the oviposition site was filled with distilled water. The test was replicated three times per each treatment. All the data were examined using the one-way analysis of variance (ANOVA), and means were separated by Tukey's honestly significant difference (HSD) test.

RESULTS

Adult female mosquitoes laid eggs on non-treated (control) and CP-treated oviposition sites. Regardless of concentrations, the total number of eggs laid per KH strain female on CP-treated sites (32.65 ± 7.06 to 37.32 ± 3.32 eggs per female) were similar to the control (33.92 ± 1.65 eggs per female) (Table 1). Each day, a female mosquito would oviposit ~ 2 to 20 eggs (Table 2). Conversely, large number of eggs were found laid by the Lab strain on day 4 on the CP-treated sites (Table 2), recorded ~ 40 eggs per female which was significantly higher than the control ($F = 9.747$; df = 6, 14; $P < 0.001$).This indirectly resulted in higher total number of eggs being produced by the Lab strain on the CP-treated sites throughout the study, e.g., total number of eggs laid on 0.25 ppm CP-treated site (83.69 ± 6.06) were significantly higher than the control ($F = 6.381$; df = 6, 14; $P = 0.002$) (Table 1). Although large numbers of eggs were also laid on 0.5 ppm and 1.0 ppm CP-treated sites, they were not significantly different from the control.

Table 1. Number of eggs laid by *A. aegypti* (mean ± se) on concentrations of CP-treated oviposition sites.

	Control	**CP (ppm)**		
		0.25	**0.5**	**1.0**
Lab	37.78 ± 1.52a(a)	83.69 ± 6.06b(a)	51.15 ± 10.31a(a)	48.68 ± 7.18a(a)
KH	33.92 ± 1.65ab(a)	37.32 ± 3.32b(b)	36.81 ± 0.70b(a)	32.65 ± 7.06a(a)

Mean values followed by the same letter within a row are not significantly different (Tukey's HSD; $P > 0.05$). Mean values followed by the same letter in parentheses within a column are not significantly different (Student's *t*-test; $P > 0.05$)

High mortality was recorded in Lab strain after contacting the CP-treated oviposition sites, registered the mortality of. ~ 70-80% on day 4 (Figure 1). This would also be the possible explanation on the sudden high egg reproduction on day 4 on the CP-treated sites. Both 0.25 ppm and 0.5 ppm CP resulted in ~ 20% mortality in KH strain, and ~ 50-60% of females were killed after contacting with 1.0 ppm CP-treated oviposition sites. The phenomenal of sudden egg reproduction was not seen in KH strain.

Table 2. Number of eggs laid by *A. aegypti* (mean ± se) daily on concentrations of CP-treated oviposition sites.

Strain	Larvicide (ppm)		Day 3	Day 4	Day 5
Lab	Control		10.37 ± 1.84a(ab)	17.95 ± 1.48b(a)	9.47 ± 1.00a(a)
	CP	(0.25)	10.32 ± 2.22a(ab)	45.81 ±5.75b(c)	35.33 ± 3.18b(b)
		(0.50)	5.73 ± 0.85a(a)	34.60 ± 5.33b(bcd)	12.33 ± 9.67ab(a)
		(1.0)	4.22 ± 1.43a(a)	39.48 ± 6.88b(cd)	0.50 ± 0.50a(a)
KH	control		2.22 ± 0.48a(a)	18.27 ± 0.55b(a)	13.43 ± 2.23b(a)
	CP	(0.25)	10.67 ± 2.04a(ab)	15.53 ± 3.03a(a)	11.40 ± 1.88a(a)
		(0.50)	9.25 ± 2.99a(ab)	18.25 ± 0.89a(a)	8.66 ± 3.25a(a)
		(1.0)	13.53 ± 3.25a(b)	12.40 ± 2.50a(a)	3.69 ± 0.93a(a)

Mean values followed by the same letter in the same row are not significantly different (Tukey's HSD; $P > 0.05$).Mean values followed by the same letter in parentheses in the same column within a strain are not significantly different (Tukey's HSD; $P > 0.05$).

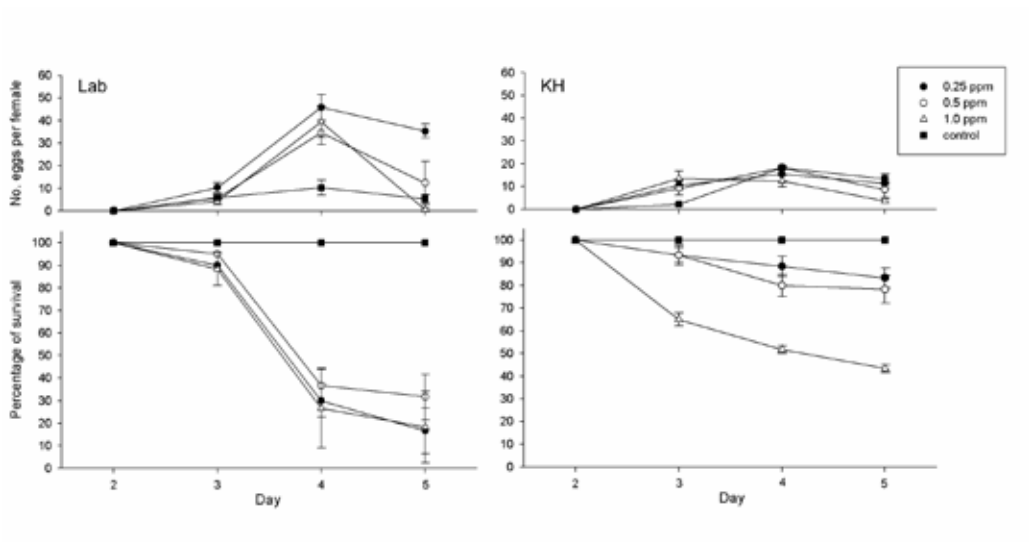

Figure 1. Number of eggs laid (solid line) by Lab strain and KH strain *A. aegypti* on CP-treated oviposition sites and the survival of *A. aegypti* (dotted line) after contacting on the oviposition sites.

DISCUSSION

We found that CP exhibited no oviposition deterrent against female mosquitoes of both Lab and KH strains, given that the females did not show any decrease in their reproduction activity on CP-treated sites. In fact, large number of eggs was found oviposited by the Lab strain on the CP-treated sites.

The adults were found to pick up lethal dose when ovipositing on CP-treated sites. However, KH strain reflected more tolerance towards CP than the Lab strain as the females reacted to 1.0 ppm CP, registered the mortality of ~50% at day 4, while the Lab strain registered the mortality of ~70-80% at all concentrations. Similarly, only 1.0 ppm CP showed ovicidal and larvicidal effects on the KH strain (data not shown). Interestingly, the Lab strain female was shown to increase its reproduction effort by laying substantial number of eggs just before died. This phenomenal was commonly seen in insects during life

threatening situations. For example, in the rove beetle, *Paederus fuscipes* Curtis, high reproduction was registered in the strain with short life span (Bong et al., 2012). Also, the parasitized male *Drosophila nigrospiracula* Patterson and Wheeler was found mated repeatedly before death (Polak and Starmer 1998). These strategies were believed to be adopted to sustain their progenies in the population when mortality risk rose. However, such trend was not exhibited in the KH strain. It was believed that the wild strain possibly has developed resistance to certain insecticides (unpublished data) as insecticide applications, e.g. thermal fogging were frequently used especially during summer to suppress the mosquito population in Lingya district. Such actions are costly and requires energy which redirected towards survival instead of reproduction (Kliot and Ghanim 2012). There were numerous studies on the effect of insecticides on insects reproduction, yet it remains highly controversial. On the one hand, some reported the increased of reproduction rate (Sutherland et al. 1967, Chelliah et al. 1980, Liu et al. 1986), while many reported otherwise (Hunter et al., 1958; Duncan 1963; Georghiou, 1965; Kumar and Chapman, 1984). These authors have overlooked the fact that resource might redirect for longevity instead of fecundity in insects which are under insecticide stress. So, the present study provided an important information that reacted to this controversial that was unanswered in decades.

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