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Effects of Piperonyl Butoxide on the Toxicity of Novel Selected Insecticides against *Aedes aegypti* L. (Diptera: Culicidae) Adults

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ABSTRACT

Background: A reliable and effective strategy is paramount to controlling *Aedes aegypti*, which are considered a major vector of dengue viruses. **Objective:** The synergistic effects of piperonyl butoxide (PBO) on the toxic activity of 16 insecticides [7 neonicotinoids (NNC), 3 pyrethroids, 1 organophosphorus, 1 pyrrole, and 4 insect growth regulators (IGR)] on *Aedes aegypti* adults were evaluated. **Results:** Fenvalerate was found to be the most potent insecticide tested (LC_{50}= 0.14, 0.071, and 0.0063 ng/cm^2, after 24, 48, and 72 hours of exposure, respectively). PBO was synergistic with all types of insecticides except insect growth regulators even after 48 and 72 hours of exposure. The largest synergistic ratio (SR) was measured for neonicotinoid insecticides (SR_{50} ranged from 363- to 941-fold after 72 hours of exposure) followed by pyrethroid and organophosphorus insecticides**. Conclusion:** The results strongly indicate that PBO and NNC are the most powerful combination in controlling *Aedes aegypti* adults. Further field experiments and biochemical and molecular biological investigation should be conducted to illustrate the synergistic mechanisms of PBO.

INTRODUCTION

Aedes aegypti (L.) is considered the major vector of dengue viruses worldwide and one of the most serious disease vectors to vector control programs (Araújo *et al*., 2015; Christofferson and Mores, 2015; DeRaedt *et al*., 2015; Dusfour *et al*., 2015; Engdahl *et al*., 2015). Insecticides play an essential role in controlling insect pests especially mosquitoes (Ahmed and Matsumura, 2012; Ahmed and Saba, 2014; Ahmed and Vogel, 2015; Ahmed *et al*., 2015). However, the intensive use of insecticides has led to severe environmental issues (Ahmed *et al*, 2014a&b), such as insecticide resistance (Rodríguez *et al*., 2014; Chapadense *et al*., 2015; Paiva *et al*., 2015; Wuliandari *et al*., 2015). Further, one of the essential biochemical mechanisms involved in the development of insecticide resistance is detoxification; mosquitoes build up resistance by making changes to their detoxification enzymes, such as oxidases, esterases, and transferases to allow them to more readily convert an insecticide to a nontoxic compound (Hemingway, 2000; Enayati *et al*., 2005; Ranson and Hemingway, 2005; Pereira *et al*., 2014).

Because mosquitoes can spread disease(s), it is very important to develop various strategies to preclude any further development of resistance to insecticides. One solution that integrated pest management (IPM) programs have implemented is the use of synergists in combination with insecticides that have various mode of action (Ishak *et al*., 2015). Piperonyl butoxide (PBO) is a potent synergist that inhibits cytochrome P450 monooxygenases' ability to detoxify insecticides and lowers insecticide resistance in *Aedes aegypti* (Paul *et al*.,

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2006). PBO also inhibits the binding sites of esterases and mixed function oxidases (MFOs), which can lead to improved insecticide effectiveness. In this study, we evaluated potential insecticides that considered new tools for reducing the incidence of dengue infection in the global mosquito control. we investigate the synergistic action of PBO with 16 insecticides [7 neonicotinoids (NNC), 3 pyrethroids (PY), 1 organophosphorus (OP), 1 pyrrole (PR), and 4 insect growth regulators (IGRs)] on *Aedes aegypti* adults after 24, 48, and 72 hours of exposure under laboratory conditions.

MATERIALS AND METHODS

Mosquitoes:

The FIELD strain (Fresno, CA, USA) of *Aedes aegypti* was acquired from the laboratory of Dr. Thomas W. Scott, University of California, Davis, CA, USA. This strain was utilized for all experiments. The FIELD strain was considered a resistant strain because it was exposed to multiple insecticides in the pyrethroid group (permethrin and deltamethrin) in the field. The resistance level was approximately 5- and 6-fold determined using the exposure to residue bioassay comparing the FIELD strain with a susceptible strain (ROCK strain) under laboratory conditions. However, the strain was maintained for one generation in the laboratory prior to the study. Ahmed and Vogel (2015) have previously described the rearing regime of the strain. All experimental procedures were reviewed by the Institutional Review Board (IRB) of the University of California, Davis.

Chemicals:

Nitenpyram (99.9%), chlorfenapyr (99.6%), lufenuron (99.7%), diafenthiuron (99.9%), diflubenzuron (98.1%), novaluron (99.6%), and piperonyl butoxide (99%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Fenitrothion (98.6%), permethrin (technical, 47.6% cis- 50.4% trans-), deltamethrin (99%), fenvalerate (96%, mixture of isomers), dinotefuran (99.5%), imidacloprid (99.5%), clothianidin (98%), acetamiprid (99.5%), thiacloprid (99.5%), and thiamethoxam (99.5%) were obtained from Chem Service (West Chester, PA, USA).

Adult Bioassays:

Adult bioassays were conducted in glass jars (600 ml) with an internal surface area of 65 cm^2 that had been treated with 1 ml of insecticide solution on the inner walls. For control bioassays, the inner jar walls were treated with only 1 ml of acetone (insecticide carrier). Following coating of the jar, the acetone or insecticide solution was allowed to evaporate for 30 minutes. Then 20 adult mosquitoes (5-7 days old post-emergence) per glass jar were placed inside, and the opening was covered with double layered white mesh cheesecloth. Adults were considered dead if they were ataxic. Because of the slow-acting nature of some of these insecticides, mortality was determined after 24-72 hours of exposure.

Synergistic Action Bioassay:

The synergistic action bioassay was conducted as described above for adult bioassays. Controls were run concurrently with each series of tests. Synergism was determined by testing the lethal action (LC_{50}) of varying concentrations of test insecticide alone or co-administered with 10 µg/ml of PBO dissolved in 1 ml of acetone. Insecticide solutions were stirred briefly to ensure uniform mixture before being coated on glass jar walls. Initial tests indicated that 10 µg/ml of PBO was the maximum sublethal concentration at which mortality was not observed. Further, at least five concentrations of an insecticide were used for all bioassays, and every bioassay was held at 25^{o}C. Percentage mortality was recorded after 24, 48, and 72 hours of exposure.

Statistical Analysis:

Data from all tests were corrected by Abbott's formula (Abbott, 1925). Bioassay data were pooled and analyzed (LC_{50} and 95% CL values) by using IBM SPSS statistics 22 program (SPSS Inc., Chicago, IL). Synergism was determined to be significant ($P \leq 0.05$) by using *t-test* statistical analysis when the 95% CIs for the LC_{50} values for adults exposed to insecticide alone did not overlap with those for adults exposed to insecticide + PBO. The synergistic ratio (SR) was calculated by dividing the LC_{50} value of the test insecticide by that of the LC_{50} obtained with insecticide + PBO.

RESULTS AND DISCUSSION

Toxicities of selected insecticides and their synergistic action with PBO on *Aedes aegypti* adults after 24, 48, and 72 hours of exposure are shown in Tables 1-3, respectively. The PY fenvalerate was the most toxic insecticide (LC_{50}= 0.14, 0.071, and 0.0063 ng/cm^2, after 24, 48, and 72 hours, respectively), followed by PYs deltamethrin and permethrin. The toxicity of the organophosphorus (OP) insecticide fenitrothion was moderate (LC_{50}= 6.31, 4.63, and 2.79 ng/cm^2, after 24, 48, and 72 hours, respectively). The most potent neonicotinoid

(NNC) insecticide was thiamethoxam, especially after 48 and 72 hours of exposure (LC_{50}= 762, 68, and 32 ng/cm^2, after 24, 48, and 72 hours, respectively), whereas imidacloprid was the least toxic NNC insecticide, even after 48 and 72 hours of exposure. Chlorfenapyr showed moderate toxicity. Diafenthiuron was the most toxic insecticide among the insect growth regulator (IGR) insecticides tested and lufenuron was the lowest.

Briefly, PY insecticides were generally the most potent insecticide group on *Aedes aegypti* adults followed by OP, NNC, and IGR insecticides. These results agree with previous studies (Paul *et al*., 2006; Ahmed and Matsumura, 2012; Ahmed and Vogel, 2015). Furthermore, in a recent study, the deltamethrin + PBO + neonicotinoid mixtures [(tricomponent mixtures consist of thiamethoxam, nitenpyram and thiacloprid (70.7, 64.9 and 55.9% mortality, respectively)] were more efficient than the deltamethrin + PBO mixture (Darriet and Chandre, 2013). Interestingly, a notable finding from this study was that the lethal toxicity of IGR insecticides on *Aedes aegypti* adults suggested that IGRs may have another mechanism of toxicity than just inhibiting chitin synthesis (Paul *et al*., 2006; Ahmed and Vogel, 2016).

In the presence of the cytochrome P450 inhibitor PBO, fenvalerate was still the most potent insecticide tested (LC_{50}= 0.023, 0.0076, and 0.00054 ng/cm^2, after 24, 48, and 72 hours of exposure, respectively) and lufenuron was the lowest (LC_{50}= 11292, 7087, and 1929 ng/cm^2, after 24, 48, and 72 hours, respectively). Thiamethoxam + PBO had the greatest synergistic ratio (SR), especially after 48 and 72 hours of exposure (819- and 941-fold, respectively), whereas the SR for fenvalerate was moderate (6.09-, 9.34-, and 11.67-fold after 24, 48, and 72 hours, respectively). The lowest SR observed was for lufenuron (0.79-, 0.89-, and 1.13-fold after 24, 48, and 72 hours, respectively).

As a whole, PBO was strongly synergistic with NNC, PY, and OP insecticides. In contrast, it greatly limited the toxicity of IGR insecticides on adult *Aedes aegypti*. In this regards, Paul *et al*. (2006) demonstrated that PBO was strongly synergistic with imidacloprid (SR >2000-fold on *Aedes aegypti* adults after 48 hours exposure), while PBO was slightly ineffective with chlorfenapyr which decreased its toxicity and the SR were 0.93, 1.13, 1.21-fold after 24, 48, and 72 hours, respectively.

Figure 1 shows the time-dependent changes in the SR_{50} affected by PBO. With increasing time of exposure, there was a trend for the SR to rise with most insecticides. The exception was clothianidin after 72 hours of exposure, at which point the SR decreased. PBO was not synergistic with IGR insecticides, suggesting that the toxicity of these insecticides is not restricted by P450 detoxification.

Table 1: Toxicity of selected insecticides and synergism with PBO on *Aedes aegypti* adults after 24 hours of exposure.

Group	Insecticide	Insecticide			Insecticide + PBO[e]		
		LC_{50} (95% CL)[b]	Slope (SE)	n[a]	LC_{50} (95% CL)[c]	Slope (SE)	SR[d]
NNC	Imidacloprid	4384 (3652-6754)	3.2 (0.2)	360	33 (27-51)	2.8 (0.3)	133*
	Dinotefuran	932 (791-1163)	3.5 (0.4)	360	1.74 (0.86-3.04)	3.1 (0.2)	536*
	Thiamethoxam	765 (504-974)	4.1 (0.3)	360	1.23 (0.62-2.45)	3.7 (0.6)	622*
	Thiacloprid	1982 (1321-2672)	2.8 (0.6)	360	4.24 (2.73-6.97)	2.6 (0.1)	467*
	Acetamiprid	2740 (1970-3682)	3.6 (0.4)	360	9.01 (6.91-11.05)	3.2 (0.4)	304*
	Clothianidin	2034 (1893-2507)	3.1 (0.5)	360	4.83 (2.78-6.67)	2.7 (0.2)	421*
	Nitenpyram	403 (391-634)	3.8 (0.3)	360	0.63 (0.27-1.65)	3.6 (0.4)	640*
PY	Permethrin	0.36 (0.14-1.08)	2.9 (0.2)	360	0.086 (0.035-0.24)	2.8 (0.3)	4.19*
	Deltamethrin	0.23 (0.042-0.87)	3.0 (0.4)	360	0.040 (0.021-0.17)	2.9 (0.1)	5.75*
	Fenvalerate	0.14 (0.0072-0.59)	3.6 (0.2)	360	0.023 (0.015-0.083)	3.1 (0.2)	6.09*
OP	Fenitrothion	6.31 (2.73-8.74)	3.9 (0.1)	360	2.01 (1.53-4.09)	3.5 (0.1)	3.14*
PR	Chlorfenapyr	232 (176-312)	4.2 (0.3)	360	250 (196-397)	3.8 (0.2)	0.93
IGR	Diafenthiuron	631 (511-892)	4.1 (0.1)	360	734 (572-911)	3.7 (0.6)	0.86
	Diflubenzuron	3172 (2906-4861)	3.5 (0.7)	360	3486 (2964-4976)	3.4 (0.1)	0.91
	Novaluron	6376 (4856-7980)	4.3 (0.2)	360	6856 (4740-8416)	4.1 (0.2)	0.93
	Lufenuron	8921 (7753-9709)	4.4 (0.3)	360	11292 (10763-12903)	4.3 (0.4)	0.79

[a] *n*, no. of adults tested including control.
[b] Concentrations are expressed in ng/cm^2 and the response determined after 24 hours of exposure.
[c] Concentration of synergist was 10 µg/ml.
[d] SR, synergistic ratio. Calculated by dividing the lethal concentration (LC) for an insecticide by the LC of the insecticide + PBO.
[e] Adults exposed to insecticides and synergist simultaneously.
[*] SR significantly different from 1.0 ($P \leq 0.05$).

Table 2: Toxicity of selected insecticides and synergism with PBO on *Aedes aegypti* adults after 48 hours of exposure.

Group	Insecticide	Insecticide			Insecticide + PBO[e]		
		LC_{50} (95% CL)[b]	Slope (SE)	n[a]	LC_{50} (95% CL)[c]	Slope (SE)	SR[d]
NNC	Imidacloprid	563 (384-692)	3.9 (0.2)	360	2.14 (1.84-3.65)	3.2 (0.3)	263*
	Dinotefuran	108 (86-203)	3.2 (0.1)	360	0.15 (0.085-0.73)	2.8 (0.1)	720*
	Thiamethoxam	68 (47-94)	3.6 (0.4)	360	0.083 (0.035-0.16)	3.2 (0.6)	819*
	Thiacloprid	193 (116-274)	4.1 (0.3)	360	0.36 (0.14-0.64)	3.9 (0.1)	536*
	Acetamiprid	257 (197-305)	3.8 (0.1)	360	0.62 (0.21-0.93)	3.5 (0.3)	415*
	Clothianidin	218 (158-312)	3.2 (0.7)	360	0.38 (0.17-0.72)	2.9 (0.3)	574*
	Nitenpyram	207 (169-308)	4.6 (0.1)	360	0.29 (0.17-0.48)	4.2 (0.2)	714*
PY	Permethrin	0.21 (0.13-0.96)	2.9 (0.3)	360	0.033 (0.019-0.076)	2.6 (0.2)	6.36*
	Deltamethrin	0.16 (0.075-0.64)	2.8 (0.2)	360	0.023 (0.0083-0.046)	2.5 (0.4)	6.96*
	Fenvalerate	0.071 (0.045-0.13)	3.3 (0.4)	360	0.0076 (0.0053-0.018)	3.0 (0.2)	9.34*
OP	Fenitrothion	4.63 (2.98-6.12)	3.6 (0.5)	360	0.86 (0.75-1.32)	3.2 (0.5)	5.38*
PR	Chlorfenapyr	121 (96-183)	4.2 (0.3)	360	107 (86-138)	3.9 (0.4)	1.13
IGR	Diafenthiuron	374 (286-411)	4.3 (0.1)	360	378 (293-516)	4.1 (0.3)	0.99
	Diflubenzuron	1532 (1273-2052)	4.1 (0.2)	360	1266 (1172-1409)	3.7 (0.4)	1.21
	Novaluron	4761 (3583-6821)	4.7 (0.4)	360	3401 (2795-4323)	4.3 (0.2)	1.40
	Lufenuron	6307 (4903-8051)	4.5 (0.2)	360	7087 (6743-9065)	4.2 (0.3)	0.89

[a] *n*, no. of adults tested including control.
[b] Concentrations are expressed in ng/cm^2 and the response determined after 48 hours of exposure.
[c] Concentration of synergist was 10 µg/ml.
[d] SR, synergistic ratio. Calculated by dividing the lethal concentration (LC) for an insecticide by the LC of the insecticide + PBO.
[e] Adults exposed to insecticides and synergist simultaneously.
[*] SR significantly different from 1.0 ($P \leq 0.05$).

Table 3: Toxicity of selected insecticides and synergism with PBO on *Aedes aegypti* adults after 72 hours of exposure.

Group	Insecticide	Insecticide			Insecticide + PBO[e]		
		LC_{50} (95% CL)[b]	Slope (SE)	n[a]	LC_{50} (95% CL)[c]	Slope (SE)	SR[d]
NNC	Imidacloprid	327 (287-490)	3.4 (0.2)	360	0.90 (0.47-1.84)	3.2 (0.2)	363*
	Dinotefuran	78 (59-113)	4.2 (0.3)	360	0.096 (0.057-0.31)	3.8 (0.3)	813*
	Thiamethoxam	32 (28-51)	2.8 (0.1)	360	0.034 (0.012-0.082)	3.1 (0.1)	941*
	Thiacloprid	91 (77-126)	3.8 (0.2)	360	0.15 (0.086-0.93)	3.5 (0.2)	607*
	Acetamiprid	136 (104-218)	3.2 (0.1)	360	0.27 (0.16-1.072)	2.9 (0.4)	504*
	Clothianidin	119 (89-225)	3.6 (0.4)	360	0.23(0.17-0.98)	3.2 (0.1)	517*
	Nitenpyram	106 (82-196)	4.4 (0.1)	360	0.13 (0.072-0.39)	4.1 (0.2)	815*
PY	Permethrin	0.12 (0.078-0.64)	2.7 (0.1)	360	0.017 (0.0094-0.047)	2.5 (0.2)	7.06*
	Deltamethrin	0.097 (0.026-0.29)	2.6 (0.5)	360	0.011 (0.0083-0.033)	2.4 (0.3)	8.82*
	Fenvalerate	0.0063(0.0028-0.014)	2.9 (0.1)	360	0.00054 (0.00018-0.0015)	2.7 (0.3)	11.67*
OP	Fenitrothion	2.79 (1.02-4.71)	3.4 (0.2)	360	0.41 (0.17-0.78)	3.0 (0.2)	6.80*
PR	Chlorfenapyr	63 (48-86)	4.2 (0.3)	360	52 (37-118)	3.9 (0.1)	1.21
IGR	Diafenthiuron	193 (103-286)	4.6 (0.2)	360	162 (96-257)	4.2 (0.3)	1.19
	Diflubenzuron	761 (582-974)	4.1 (0.3)	360	567 (374-708)	3.7 (0.1)	1.34
	Novaluron	1031 (862-1802)	3.9 (0.2)	360	589 (431-836)	3.6 (0.1)	1.75
	Lufenuron	2180 (1765-2940)	4.1 (0.1)	360	1929 (1682-2517)	3.8 (0.3)	1.13

[a] *n*, no. of adults tested including control.
[b] Concentrations are expressed in ng/cm^2 and the response determined after 72 hours of exposure.
[c] Concentration of synergist was 10 µg/ml.
[d] SR, synergistic ratio. Calculated by dividing the lethal concentration (LC) for an insecticide by the LC of the insecticide + PBO.
[e] Adults exposed to insecticides and synergist simultaneously.
[*] SR significantly different from 1.0 ($P \leq 0.05$).

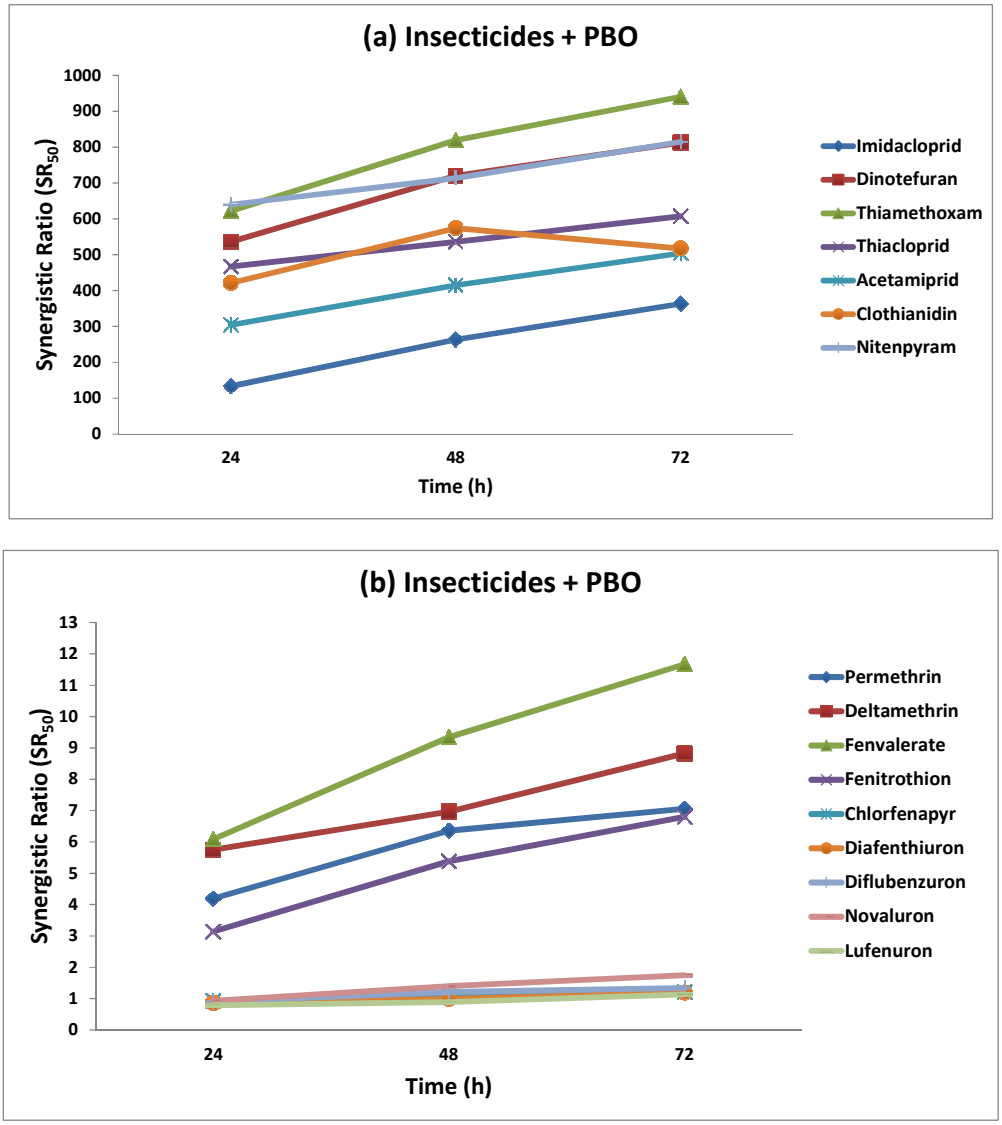

Fig. 1: Time-dependent changes in the synergistic ratio (SR_{50}) as calculated from LC_{50} values from Tables 1-3 for the combined treatments of **(a)** NNC insecticides and **(b)** PY, OP, PR, and IGR insecticides as determined after 24, 48, and 72 hours of exposure.

Conclusions:

Currently, many insect pests have developed resistance to insecticides. However, synergists can increase the toxicity of these insecticides with the benefit of keeping the use of the insecticides to a minimum level. Based on our results, PBO is a promising tool in the management of resistant pests. PBO and NNC are the most powerful combination in controlling *Aedes aegypti* adults. This study sheds light on the importance of synergists in the control of *Aedes aegypti*. Further field experiments including biochemical and molecular biological investigations are needed to illustrate the synergistic mechanisms of PBO.

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