Susceptibility of *Aedes aegypti*, *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say to 19 Pesticides with Different Modes of Action

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ABSTRACT To access the relative potency of pesticides to control adult mosquitoes, 19 pesticides with various modes of action were evaluated against *Aedes aegypti*, *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say. On the basis of 24-h LD_{50} values after topical application, the only pesticide that had higher activity than permethrin was fipronil, with LD_{50} values lower than permethrin for 107-, 4,849-, and 2-fold against *Ae. aegypti*, *Cx. quinquefasciatus* Say, and *An. quadrimaculatus* Say, respectively. Abamectin, imidacloprid, spinosad, diazinon, and carbaryl showed slightly lower activity than permethrin (<20-fold). However, bifenazate showed very low activity against the three mosquito species tested, with LD_{50} values higher than permthrin for >1000-fold. On the basis of 24-h LD_{50} values, *Cx. quinquefasciatus* was the least susceptible species to nine pesticides tested (DNOC, azocyclotin, chlorfenapyr, carbaryl, spinosad, imidaclorid, diazinon, abamectin, and permethrin), whereas *Ae. aegypti* was the least susceptible species to six pesticides tested (dicofol, amitraz, propargite, hydramethylnon, cyhexatin, and diafenthiuron), and *An. quadrimaculatus* was the least susceptible species to four pesticides tested (bifenazate, pyridaben, indoxacarb, and fipronil). Our results revealed that different species of mosquitoes had different susceptibility to pesticides, showing the need to select the most efficacious compounds for the least susceptible mosquito species to achieve successful mosquito control.

KEY WORDS pesticide, mosquito control, *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles quadrimaculatus*

The mosquito *Aedes aegypti* L. (Diptera: Culicidae) transmits viral pathogens of humans, including yellow fever (Gillett and Ross 1955, Philip 1962, Soper 1967, Aitken et al. 1977) and dengue (Mattingly 1967, Rudnick 1967, Coleman and McLean 1973, Degallier et al. 1988), both of which can cause severe human morbidity and mortality. The mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) is the vector of the filarial parasite *Wuchereria bancrofti* (Cobbold) (Spirurida: Onchocercidae), which causes bancroftian filariasis in human (Sabatinelli et al. 1994, Samuel et al. 2004). *Cx. quinquefasciatus* Say is also a vector of West Nile virus (Godsey et al. 2005), Japanese encephalitis virus (Nitatpattana et al. 2005), and Saint Louis encephalitis virus (Jones et al. 2002). In North America, the common malaria mosquito *Anopheles quadrimaculatus* Say (Diptera: Culicidae) is a vector for human malaria (Box et al. 1953, Micks and Mc 1953).

The primary approach used for mosquito control has mainly relied on pesticides. However, very few types of pesticides are currently registered for mosquito control. Furthermore, many mosquito species have developed resistance to various classes of pesticides (Su and Mulla 2004, Tia et al. 2006, Xu et al. 2006), creating an urgent need to seek and identify new effective pesticides to control these important disease vectors. To search for pesticides that are effective as mosquito adulticides, we selectively chose 19 pesticides (Table 1) from the Insecticide Resistance Action Committee (IRAC) Mode of Action (MoA) classification list (http://www.irac-online.org/documents/IRAC%20MoA%20Classification%20v5_3.pdf), with each pesticide representing a different category of pesticide and evaluated their activities against three species of mosquitoes— *Aedes aegypti*, *Cx. quinquefasciatus*, and *An. quadrimaculatus*. Our results revealed that these three mosquitoes had different susceptibilities to various pesticides, showing the need to select the most efficacious compounds for the least susceptible mosquito species to achieve successful mosquito control.

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Table 1. Modes of action of the 19 selected pesticides used in the study

Pesticide name	Modes of action	IRAC MoA group
Bifenazate	Neuronal inhibitors, unknown mode of action	25
Dicofol	Unknown	Unknown
Amitraz	Octopaminergic agonists	19
Propargite	Inhibitors of oxidative phosphorylation, disruptors of ATP formation	12C
Hydramethylnon	Mitochondrial complex III electron transport inhibitors	20
Cyhexatin	Inhibitors of oxidative phosphorylation, disruptors of ATP formation	12B
Diafenthiuron	Inhibitors of oxidative phosphorylation, disruptors of ATP formation	12A
DNOC	Uncouplers of oxidative phosphorylation via disruption of H^+ gradient	13
Azocyclotin	Inhibitors of oxidative phosphorylation, disruptors of ATP formation	12B
Pyridaben	Mitochondrial complex I electron transport inhibitors	21
Chlorfenapyr	Uncouplers of oxidative phosphorylation via disruption of H^+ gradient	13
Indoxacarb	Voltage Dependent Sodium channel blockers	22
Carbaryl	Acetylcholinesterase inhibitors (Carbamates)	1A
Spinosad	Nicotinic acetylcholine receptor agonists	5
Imidacloprid	Nicotinic acetylcholine receptor agonist/antagonists	4
Diazinon	Acetylcholinesterase inhibitors (Organophosphates)	1B
Abamectin	Chloride channel activators	6
Permethrin	Sodium channel modulators	3
Fipronil	GABA-gated chloride channel antagonists	2

Materials and Methods

Mosquitoes and Pesticides. All three species of mosquitoes were reared in the insectary of the Mosquito and Fly Research Unit at Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), USDA–ARS. *Ae. aegypti* and *An. quadrimaculatus* have been established in the insectary since 1952 from Orlando, FL, strains. *Cx. quinquefasciatus* has been established in the insectary since 1995 from a Gainesville, FL, strain. Female adults were used for all experiments because only this sex takes blood meals and is of concern to the general public. Mosquitoes were reared using standard procedures (Reinert et al. 1997, McCall and Eaton 2001, Pridgeon et al. 2007). Briefly, collected eggs were hatched in a flask, and the hatched larvae were held overnight in the flask and transferred to a plastic tray containing distilled water. Larval diet was added to each tray. Mosquitoes were reared in an environmental chamber set with a temperature profile representing a simulated summer day regimen (ranging from 22 to 30°C) and 80% RH. Incandescent lighting was set to a crepuscular profile with a photoperiod of 14 h:10 h (L:D), including 2 h of simulated dawn and 2 h of simulated dusk. Adults were held in a screened cage and provided 10% sucrose ad libitum. Bovine blood in 1% heparin that had been placed in a pig intestine and warmed to 37°C was provided to adults twice a week. Eggs were hatched, and larvae were reared in containers as described above. Nineteen pesticides with different modes of action were selected (Table 1). All pesticides were purchased in technical grade from Chem Service (West Chester, PA).

Adult Bioassays and Data Analysis. To determine precisely the activity of each pesticide against female mosquitoes, each chemical was serially diluted in acetone and topically applied to individual mosquitoes. Before pesticide application, 5- to 7-d-old females were briefly anesthetized for 30 s with carbon dioxide and placed on a 4°C chill table (BioQuip Products, Rancho Dominguez, CA). A droplet of 0.5 μl of pesticide solution was applied to the dorsal thorax using a 700 series syringe and a PB 600 repeating dispenser (Hamilton, Reno, NV). Six concentrations providing a range of 0–100% of mortality were used on 25–30 females per concentration. Tests were replicated three times. Control treatments with 0.5 μl of acetone alone gave control mortality rates of <10%. After treatment, mosquitoes were kept in plastic cups and supplied with 10% sucrose solution for 24 h before mortality was recorded. Temperature and humidity were maintained at 26°C and 80% RH, respectively. Every bioassay was conducted at 27°C and 80% RH and replicated three times. Bioassay data were analyzed using PoloPlus probit and logit analysis software (LeOra Software, Petaluma, CA). Control mortality was corrected using Abbott's formula. χ^2 goodness-of-fit tests were performed, and LD_{50}/LD_{95} values were calculated using PoloPlus program.

Results and Discussion

To determine the susceptibility of *Ae. aegypti* to the 19 selected pesticides, topical application bioassays were performed. The bioassay results are summarized in Table 2. Our results revealed that, among the 19 pesticides tested, fipronil, a gamma amino butyric acid (GABA)-gated chloride channel antagonist, was the most toxic pesticide against *Ae. aegypti*, with an LD_{50} value as low as 4.6×10^{-7} μg/mg of mosquito (Table 2). The order of the next most toxic pesticides against *Ae. aegypti* was as follows: permethrin, a sodium channel modulator ($LD_{50} = 4.9 \times 10^{-5}$ μg/mg) > abamectin, a chloride channel activator ($LD_{50} = 4.6 \times 10^{-4}$ μg/mg) > diazinon, an acetylcholinesterase inhibitor representing organophosphates ($LD_{50} = 6.7 \times 10^{-4}$ μg/mg) > imidacloprid, a nicotinic acetylcholine receptor antagonist ($LD_{50} = 7.7 \times 10^{-4}$ μg/mg) > spinosad, a nicotinic acetylcholine receptor agonist ($LD_{50} = 8.8 \times 10^{-4}$ μg/mg) > carbaryl, an acetylcholinesterase inhibitor representing carbamates ($LD_{50} = 9.5 \times 10^{-4}$ μg/mg) > indoxacarb, a voltage

Table 2. Toxicities of 19 pesticides against female adults of *Ae. aegypti* × topical application

Pesticide name	LD_{50} (95% CI)[a]	LD_{95} (95% CI)[a]	Slope (SE)	χ^2
Bifenazate	1.5×10^{0} (1.2×10^{0}–1.9×10^{0})	5.7×10^{0} (4.0×10^{0}–1.0×10^{1})	2.83 (0.38)	1.67
Dicofol	4.8×10^{-1} (3.4×10^{-1}–7.0×10^{-1})	2.7×10^{0} (1.5×10^{0}–7.5×10^{0})	2.21 (0.27)	4.02
Amitraz	4.1×10^{-1} (2.8×10^{-1}–6.4×10^{-1})	5.3×10^{0} (2.3×10^{0}–3.6×10^{1})	1.48 (0.31)	0.28
Propargite	2.4×10^{-1} (1.8×10^{-1}–3.1×10^{-1})	1.3×10^{0} (8.6×10^{-1}–2.5×10^{0})	2.25 (0.33)	1.49
Hydramethylnon	2.0×10^{-1} (1.4×10^{-1}–2.6×10^{-1})	1.1×10^{0} (7.0×10^{-1}–2.3×10^{0})	2.29 (0.40)	1.26
Cyhexatin	5.6×10^{-2} (4.6×10^{-2}–7.0×10^{-2})	1.9×10^{-1} (1.4×10^{-1}–3.0×10^{-1})	3.14 (0.43)	1.30
Diafenthiuron	4.8×10^{-2} (4.2×10^{-2}–6.3×10^{-2})	2.1×10^{-1} (1.4×10^{-1}–4.0×10^{-1})	2.65 (0.38)	1.88
DNOC	2.8×10^{-2} (2.3×10^{-2}–3.4×10^{-2})	9.1×10^{-2} (7.0×10^{-2}–1.4×10^{-1})	3.23 (0.42)	0.59
Azocyclotin	8.8×10^{-3} (7.0×10^{-3}–1.1×10^{-2})	3.9×10^{-2} (2.8×10^{-2}–6.5×10^{-2})	2.54 (0.32)	2.14
Pyridaben	3.0×10^{-3} (2.5×10^{-3}–3.6×10^{-3})	9.2×10^{-3} (6.9×10^{-3}–1.4×10^{-2})	3.37 (0.47)	0.25
Chlorfenapyr	1.9×10^{-3} (1.6×10^{-3}–2.2×10^{-3})	4.5×10^{-3} (3.7×10^{-3}–6.3×10^{-3})	4.32 (0.68)	0.53
Indoxacarb	1.5×10^{-3} (1.3×10^{-3}–1.8×10^{-3})	6.0×10^{-3} (4.3×10^{-3}–1.1×10^{-2})	2.78 (0.41)	0.22
Carbaryl	9.5×10^{-4} (6.7×10^{-4}–1.2×10^{-3})	4.2×10^{-3} (2.9×10^{-3}–9.1×10^{-3})	2.48 (0.45)	0.59
Spinosad	8.9×10^{-4} (7.7×10^{-4}–1.1×10^{-3})	2.1×10^{-3} (1.6×10^{-3}–3.1×10^{-3})	4.46 (0.63)	0.89
Imidacloprid	7.7×10^{-4} (4.6×10^{-4}–1.2×10^{-3})	3.9×10^{-3} (2.2×10^{-3}–1.8×10^{-2})	2.32 (0.33)	3.38
Diazinon	6.7×10^{-4} (5.3×10^{-4}–8.4×10^{-4})	3.4×10^{-3} (2.2×10^{-3}–7.4×10^{-3})	2.29 (0.33)	2.07
Abamectin	4.6×10^{-4} (3.2×10^{-4}–6.0×10^{-4})	3.0×10^{-3} (1.8×10^{-3}–7.0×10^{-3})	2.03 (0.32)	2.33
Permethrin	4.9×10^{-5} (2.9×10^{-5}–8.8×10^{-5})	1.2×10^{-4} (7.4×10^{-5}–1.1×10^{-3})	4.14 (0.61)	2.34
Fipronil	4.6×10^{-7} (3.9×10^{-7}–5.6×10^{-7})	1.8×10^{-6} (1.3×10^{-6}–3.0×10^{-6})	2.78 (0.38)	1.91

[a] LD_{50} and LD_{95} values are in units of micrograms of pesticide per milligram of mosquito (average weight of 7-d-old female *Ae. aegypti* was 2.85 mg).

dependent sodium channel blocker ($LD_{50} = 1.5 \times 10^{-3}$ $\mu g/mg$) > chlorfenapyr, an uncoupler of oxidative phosphorylation through disruption of H^+ gradient ($LD_{50} = 1.9 \times 10^{-3}$ $\mu g/mg$) > pyridaben, a mitochondrial complex I electron transport inhibitor ($LD_{50} = 3 \times 10^{-3}$ $\mu g/mg$; Table 2). Our results also revealed that the least toxic pesticide against *Ae. aegypti* was bifenazate, a neuron inhibitor currently registered as miticide with unknown mode of action, with an LD_{50} value as high as 1.49 $\mu g/mg$. The activity order of the next least toxic pesticides tested were dicofol, a registered miticide with unknown mode of action ($LD_{50} = 0.48$ $\mu g/mg$) < amitraz, an insecticide and acaricide to control red spider mites and control bollworms acting as an octopaminergic agonist ($LD_{50} = 0.41$ $\mu g/mg$) < propargite, a registered miticide acting as an inhibitor of oxidative phosphorylation and ATP synthase ($LD_{50} = 0.24$ $\mu g/mg$) < hydramethylnon, a mitochondrial complex II electron transport inhibitor ($LD_{50} = 0.2$ $\mu g/mg$) < cyhexatin, a miticide acting as an inhibitor of oxidative phosphorylation and ATP synthase ($LD_{50} = 5.6 \times 10^{-2}$ $\mu g/mg$) < diafenthiuron, an inhibitor of oxidative phosphorylation and ATP synthase ($LD_{50} = 4.8 \times 10^{-2}$ $\mu g/mg$) < DNOC (*di*nitro-*o*-cresol), a pesticide registered for killing locusts and spider mites acting as an uncoupler of oxidative phosphorylation through disruption of H^+ gradient ($LD_{50} = 2.5 \times 10^{-2}$ $\mu g/mg$) < azocyclotin, a miticide acting as an inhibitor of oxidative phosphorylation and ATP synthase ($LD_{50} = 8.8 \times 10^{-3}$ $\mu g/mg$) (Table 2).

To study whether different mosquito species have various susceptibilities to the 19 selected pesticides, topical application bioassays were performed against female adults of *Cx. quinquefasciatus* and *An. quadrimaculatus.* The bioassay results are presented in Tables 3 and 4. Among the 19 pesticides tested, fipronil, the most toxic pesticide against *Ae. aegypti,* was also the most toxic pesticide against *Cx. quinquefasciatus* and *Anopheles quadrimaculatus*, with an LD_{50} value of 3.3×10^{-7} and 6.8×10^{-5} $\mu g/mg$, respectively (Tables 3 and 4). However, to our surprise, *An. quadrimaculatus* was the least susceptible species to fipronil, with 206-fold higher LD_{50} value than *Cx. quinquefasciatus* and 148-fold higher LD_{50} value than *Ae. aegypti.* This could be simply because of species variability. An alternative explanation is that the *An. quadrimaculatus* strain might have previous exposure to pesticides with similar modes of action as fipronil (i.e., GABA-gated chloride channel antagonist). The second most toxic pesticide tested against all three mosquito species was permethrin. However, the three species showed different susceptibilities against permethrin, with *Cx. quinquefasciatus* as the least susceptible species, whereas *Ae. aegypti* was the most susceptible species (Table 5). Three relatively new pesticides (spinosad, imidacloprid, and abamectin) showed slightly lower activities against all three mosquito species than permethrin, with activities <20-fold lower than permethrin (Table 5). However, when LD_{50} values were compared, *Cx. quinquefasciatus* was the least susceptible species to the three pesticides (Table 5). Furthermore, *Cx. quinquefasciatus* also showed the least susceptibility to six other pesticides tested (carbaryl, diazinon, permethrin, chlorfennapyr, azocyclotin, and DNOC; Table 5). The relatively low susceptibility of *Cx. quinquefasciatus* to nine pesticides tested (DNOC, azocyclotin, chlorfenapyr, carbaryl, spinosad, imidaclorid, diazinon, abamectin, and permethrin) may be simply caused by natural species-specific tolerance to the nine pesticides. Different susceptibility of various mosquito species to pesticides has been previously reported (Pampiglione et al. 1985, Campos and Andrade 2003, Somboon et al. 2003). For example, it has been reported that, when female mosquitoes engorged blood from mice injected subcutaneously 12 h previously with avamectin MK-933 at 82 mg (AI)/kg, mortality rates after 36 h were 100% for *An. stephensi,*

Table 3. Toxicities of 19 pesticides against female adults of *Cx. quinquefasciatus* by topical application

Pesticide name	LD_{50} (95% CI)[a]	LD_{95} (95% CI)[a]	Slope (SE)	χ^2
Bifenazate	1.6×10^{0} (1.1×10^{0}–2.4×10^{0})	1.7×10^{1} (8.3×10^{0}–6.7×10^{1})	1.61 (0.29)	1.93
Dicofol	3.1×10^{-1} (2.2×10^{-1}–4.1×10^{-1})	2.4×10^{0} (1.4×10^{0}–6.2×10^{0})	1.84 (0.30)	0.65
Amitraz	2.4×10^{-1} (1.7×10^{-1}–3.6×10^{-1})	1.4×10^{0} (6.9×10^{-1}–1.5×10^{1})	2.10 (0.58)	0.18
Propargite	1.0×10^{-1} (7.4×10^{-2}–1.5×10^{-1})	6.4×10^{-1} (3.2×10^{-1}–6.2×10^{0})	2.07 (0.58)	0.42
Hydromethylnon	7.9×10^{-2} (5.9×10^{-2}–1.4×10^{-1})	4.1×10^{-1} (2.0×10^{-1}–3.94×10^{0})	2.29 (0.61)	0.10
Cyhexatin	3.2×10^{-2} (2.6×10^{-2}–3.8×10^{-2})	1.4×10^{-1} (1.0×10^{-1}–2.3×10^{-1})	2.56 (0.35)	3.95
Diafenthiuron	3.5×10^{-2} (2.8×10^{-2}–5.0×10^{-2})	2.7×10^{-1} (1.4×10^{-1}–8.5×10^{-1})	1.87 (0.29)	1.60
DNOC	3.5×10^{-2} (2.5×10^{-2}–4.0×10^{-2})	1.1×10^{-1} (7.9×10^{-2}–1.9×10^{-1})	3.12 (0.48)	1.09
Azocyclotin	4.6×10^{-2} (3.3×10^{-2}–1.0×10^{-1})	2.6×10^{-1} (1.1×10^{-1}–5.5×10^{0})	2.17 (0.63)	0.09
Pyridaben	2.6×10^{-3} (2.0×10^{-3}–3.3×10^{-3})	1.1×10^{-2} (7.4×10^{-3}–2.5×10^{-2})	2.57 (0.43)	0.85
Chlorfenapyr	6.9×10^{-3} (5.5×10^{-3}–8.9×10^{-3})	2.6×10^{-2} (1.6×10^{-2}–7.8×10^{-2})	2.81 (0.62)	0.02
Indoxacarb	1.7×10^{-3} (1.3×10^{-3}–2.1×10^{-3})	6.4×10^{-3} (4.6×10^{-3}–1.2×10^{-2})	2.79 (0.46)	1.33
Carbaryl	5.0×10^{-3} (3.4×10^{-3}–1.0×10^{-2})	4.9×10^{-2} (1.8×10^{-2}–7.6×10^{-1})	1.65 (0.40)	0.64
Spinosad	3.2×10^{-3} (2.4×10^{-3}–5.0×10^{-3})	2.7×10^{-2} (1.2×10^{-2}–1.7×10^{-1})	1.79 (0.39)	0.77
Imidacloprid	1.2×10^{-3} (8.9×10^{-4}–2.0×10^{-3})	6.4×10^{-3} (3.0×10^{-3}–5.8×10^{-2})	2.29 (0.62)	0.02
Diazinon	7.4×10^{-3} (5.0×10^{-3}–2.3×10^{-2})	4.2×10^{-2} (1.6×10^{-2}–1.8×10^{0})	2.16 (0.67)	0.11
Abamectin	3.0×10^{-3} (2.3×10^{-3}–4.5×10^{-3})	2.1×10^{-2} (1.1×10^{-2}–9.8×10^{-2})	1.93 (0.40)	0.40
Permethrin	1.6×10^{-3} (1.2×10^{-3}–3.2×10^{-3})	6.9×10^{-3} (3.3×10^{-3}–5.7×10^{-2})	2.66 (0.70)	0.35
Fipronil	3.3×10^{-7} (2.3×10^{-7}–7.4×10^{-7})	3.5×10^{-6} (1.2×10^{-6}–6.7×10^{-5})	1.60 (0.40)	0.15

[a] LD_{50} and LD_{95} values are in units of micrograms of pesticide per milligram of mosquito (average weight of 7-d-old female *Cx. quinquefasciatus* was 2.02 mg).

>60% for *Ae. aegypti*, and >50% for *Cx. quiquefasciatus* (Pampiglione et al. 1985). Similarly, our results also showed that *Cx. quiquefasciatus* was the least susceptible species to abamectin with the highest LD_{50} value, followed by *Ae. aegypti* and *An. quadrimaculatus* (Table 5), although we used a different bioassay method.

Although the three mosquito species showed different susceptibility to certain pesticides, they also showed similar susceptibility to some other pesticides. For example, DNOC, a registered pesticide used agriculturally as a larvicide, ovicide, and pesticide against locusts and other insects, had very similar activity against *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. quadrimaculatus*, with LD_{50} values of 2.5×10^{-2}, 3.5×10^{-2}, and 3.5×10^{-2} μg/mg, respectively. Another example was bifenazate, the active ingredient in acramite to control mites on a variety of fruit crops. The LD_{50} values of bifenzate against *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. quadrimaculatus* were 1.49, 1.6, and 2.46 μg/mg, respectively. These results suggest that the three species of mosquito tested had no previous exposure to either bifenzate or DNOC.

On the basis of 24-h LD_{50} values, the most toxic pesticide tested was fipronil and the least toxic pesticide tested was bifenzate. Our results revealed that the three mosquito species had very similar susceptibility to relatively new pesticides such as DNOC and bifenzate. However, the three mosquito species also showed various susceptibilities to some pesticides such as fipronil and permethrin. Therefore, it is evi-

Table 4. Toxicities of nineteen pesticides against female adults of *An. quadrimaculatus* by topical application

Pesticide name	LD_{50} (95% CI)[a]	LD_{95} (95% CI)[a]	Slope (SE)	χ^2
Bifenazate	2.5×10^{0} (2.0×10^{0}–3.0×10^{0})	9.8×10^{0} (7.0×10^{0}–1.7×10^{1})	2.74 (0.40)	1.81
Dicofol	1.8×10^{-1} (1.0×10^{-1}–2.8×10^{-1})	6.0×10^{0} (2.4×10^{0}–4.0×10^{1})	1.08 (0.20)	0.78
Amitraz	3.7×10^{-1} (2.3×10^{-1}–5.4×10^{-1})	7.0×10^{0} (2.9×10^{0}–5.3×10^{1})	1.28 (0.27)	1.14
Propargite	1.7×10^{-1} (1.0×10^{-1}–3.7×10^{-1})	9.9×10^{0} (2.3×10^{0}–3.3×10^{2})	0.93 (0.20)	0.25
Hydramethylnon	6.3×10^{-2} (5.2×10^{-2}–9.4×10^{-2})	3.2×10^{-1} (1.8×10^{-1}–1.1×10^{0})	2.35 (0.44)	1.13
Cyhexatin	8.9×10^{-3} (6.3×10^{-4}–1.7×10^{-2})	9.4×10^{-2} (3.5×10^{-2}–1.3×10^{0})	1.62 (0.39)	0.22
Diafenthiuron	1.5×10^{-2} (1.1×10^{-2}–2.3×10^{-2})	1.1×10^{-1} (5.3×10^{-2}–6.1×10^{-1})	1.87 (0.40)	0.34
DNOC	3.5×10^{-2} (2.7×10^{-2}–5.2×10^{-2})	1.9×10^{-1} (1.0×10^{-1}–7.5×10^{-1})	2.22 (0.44)	0.46
Azocyclotin	1.4×10^{-2} (1.1×10^{-2}–1.9×10^{-2})	7.3×10^{-2} (4.2×10^{-2}–2.2×10^{-1})	2.31 (0.42)	0.73
Pyridaben	7.8×10^{-3} (5.2×10^{-3}–1.6×10^{-2})	1.4×10^{-1} (4.7×10^{-2}–2.0×10^{0})	1.30 (0.28)	0.53
Chlorfenapyr	1.5×10^{-3} (9.9×10^{-4}–3.3×10^{-3})	1.4×10^{-2} (5.1×10^{-3}–2.3×10^{-1})	1.67 (0.41)	0.54
Indoxacarb	9.9×10^{-3} (7.8×10^{-3}–1.3×10^{-2})	4.6×10^{-2} (2.9×10^{-2}–1.1×10^{-1})	2.48 (0.42)	0.27
Carbaryl	1.0×10^{-3} (8.9×10^{-4}–1.3×10^{-3})	3.0×10^{-3} (2.2×10^{-3}–5.7×10^{-3})	3.62 (0.66)	1.34
Spinosad	1.5×10^{-3} (1.2×10^{-3}–1.9×10^{-3})	9.9×10^{-3} (6.3×10^{-3}–2.1×10^{-2})	1.97 (0.25)	0.71
Imidacloprid	3.8×10^{-4} (3.1×10^{-4}–5.2×10^{-4})	1.5×10^{-3} (8.9×10^{-4}–5.2×10^{-3})	2.74 (0.60)	0.08
Diazinon	5.7×10^{-4} (4.3×10^{-4}–8.9×10^{-4})	2.8×10^{-3} (1.5×10^{-4}–1.8×10^{-2})	2.39 (0.61)	0.34
Abamectin	3.0×10^{-4} (1.5×10^{-4}–9.4×10^{-4})	5.2×10^{-2} (8.9×10^{-3}–1.7×10^{-1})	0.73 (0.12)	0.13
Permethrin	1.1×10^{-4} (7.3×10^{-5}–2.1×10^{-4})	2.0×10^{-3} (6.8×10^{-4}–2.6×10^{-2})	1.29 (0.27)	0.25
Fipronil	6.8×10^{-5} (5.1×10^{-5}–1.1×10^{-4})	4.1×10^{-4} (1.9×10^{-4}–4.5×10^{-3})	2.13 (0.59)	0.21

[a] LD_{50} and LD_{95} values are in units of micrograms of pesticide per milligram of mosquito (average weight of 7-d-old female *An. quadrimaculatus* was 1.92 mg).

Table 5. Toxicity comparison of the 19 selected pesticides against *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. quadrimaculatus*

Pesticide name	LD_{50} values[a]			Activity compared with permethrin (fold)[b]		
	Ae. aegypti	*Cx. quinquefasiatus*	*An. quadrimaculatus*	*Ae. aegypti*	*Cx. quinquefasiatus*	*An. quadrimaculatus*
Bifenazate	1.5×10^{0}	1.6×10^{0}	2.5×10^{0}	−30,408	−1,000	−22,364
Dicofol	4.8×10^{-1}	3.1×10^{-1}	1.8×10^{-1}	−9,796	−194	−1,636
Amitraz	4.1×10^{-1}	2.4×10^{-1}	3.7×10^{-1}	−8,367	−150	−3,364
Propargite	2.4×10^{-1}	1.0×10^{-1}	1.7×10^{-1}	−4,898	−63	−1,546
Hydramethylnon	2.0×10^{-1}	7.9×10^{-2}	6.3×10^{-2}	−4,082	−49	−573
Cyhexatin	5.6×10^{-2}	3.2×10^{-2}	8.9×10^{-3}	−1,143	−20	−81
Diafenthiuron	4.8×10^{-2}	3.5×10^{-2}	1.4×10^{-2}	−980	−22	−127
DNOC	2.5×10^{-2}	3.5×10^{-2}	3.5×10^{-2}	−510	−22	−318
Azocyclotin	8.8×10^{-3}	4.6×10^{-2}	1.4×10^{-2}	−180	−29	−127
Pyridaben	3.0×10^{-3}	2.6×10^{-3}	7.8×10^{-3}	−61	−2	−71
Chlorfenapyr	1.9×10^{-3}	6.9×10^{-3}	1.5×10^{-3}	−39	−4	−14
Indoxacarb	1.5×10^{-3}	1.7×10^{-3}	9.9×10^{-3}	−31	−1	−90
Carbaryl	9.5×10^{-4}	5.0×10^{-3}	1.0×10^{-3}	−19	−3	−9
Spinosad	8.8×10^{-4}	3.2×10^{-3}	1.5×10^{-3}	−18	−2	−14
Imidacloprid	7.7×10^{-4}	1.2×10^{-3}	3.8×10^{-4}	−16	−1	−4
Diazinon	6.7×10^{-4}	7.4×10^{-3}	5.7×10^{-4}	−14	−5	−5
Abamectin	4.6×10^{-4}	3.0×10^{-3}	3.0×10^{-4}	−9	−2	−3
Permethrin	4.9×10^{-5}	1.6×10^{-3}	1.1×10^{-4}	1	1	1
Fipronil	4.6×10^{-7}	3.3×10^{-7}	6.8×10^{-5}	+107	+4,849	+2

[a] LD_{50} values are in units of micrograms of pesticide per milligram of mosquito.

[b] Activity is calculated according to the formula: Activity (fold) = (LD_{50} value of permethrin ÷ LD_{50} value of pesticide) if the pesticide has higher toxicity than permethrin or Activity (fold) = (LD_{50} value of pesticide/LD_{50} value of permethrin) if the pesticide has lower activity than permethrin. "−" symbol means the activity is lower than permethrin; "+" symbol means the activity is higher than permethrin.

dent that the evaluation and selection of the most efficacious compound for the least susceptible mosquito species is an important step for effective mosquito control. Based on activity, fipronil seems to be the best compound of the 19 chemicals tested for successful mosquito control. However, fipronil is a broad-specturm pesticide that is also very toxic to aquatic nontargets (Overmyer et al. 2007). Therefore, it is not likely that fipronil will be approved as arial sprays. Permethrin, one of the pyrethroids currently registered for mosquito control, is the second highest active compound against all three mosquito species. Therefore, unless field strains have developed resistance, pyrethroids are still highly recommended for mosquito control. Of the 19 pesticides tested, 5 (carbaryl, spinosad, imidacloprid, diazinon, and abamectin) showed slightly lower activity than permethrin (<20-fold). Carbary and diazion are both currently registered as effective arial sprays for mosquito control. Therefore, they are recommended as alternative mosquito control compounds unless resistance has been reported. Abamectin, a relatively new pesticide not currently registered for mosquito control, is a natural fermentation product of soil bacterium *Streptomyces avermitilis.* Because abamectin showed only slightly lower activity than permethrin (<10-fold), we propose that abamectin is a compound worthy of pursuing as a mosquito adulticide. Imidacloprid, another relatively new pesticide, showed slightly lower activity than permethrin (<20-fold) against the three mosquito species tested. However, use of imidacloprid is highly controversial because it is believed to be responsible for high losses in bees. Therefore, its registration as a mosquito adulticide is not likely. Spinosad (spinosyn A and spinosyn D), a new chemical class of pesticides that are registered by the EPA to control a variety of insects, also showed slightly lower activity than permethrin (<20-fold). Because the active ingredient of spinosad is derived from a naturally occurring soil dwelling bacterium *Saccharopolyspora spinosa* and spinosad has very low impact to mammals, the environment, birds and predatory beneficials, we propose that spinosad is also worthy of pursuing as a mosquito adulticide.

In summary, we evaluated the potency of 19 pesticides with different modes of action against adult *Ae. aegypti*, *Cx. quinquefasciatus* Say, and *An. quadrimaculatus* Say. Our results revealed that different species of mosquitoes had different susceptibility to different pesticides, showing the need to select the most efficacious compound for the least susceptible mosquito species to achieve successful mosquito control.

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