DIFFERENTIAL TOXICITY OF PYRETHROID AND ORGANOPHOSPHATE INSECTICIDES TO THE HONEY BEE, *APIS MELLIFERA* AND THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

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ABSTRACT

Six insecticide active ingredients (AIs) and five commercial insecticide formulations were applied by topical application and onto filter paper strips to determine differential toxicity to *Aedes aegypti* (L.) and *Apis mellifera* (L.), and to evaluate their potential use in future insecticide resistance monitoring surveys. For topical application, 0.1 or 1 µl of the technical insecticide solution was applied to the *Ae. aegypti* and *A. mellifera* thorax, respectively. For insecticide-impregnated strips the insecticide amount varied, according with the commercial formulation. By topical application deltamethrin was the most toxic AI (LD_{50} = 0.057 µg/g) to *Ae. aegypti* and prallethrin was least toxic (LD_{50} = 19.42 µg/g). For *A. mellifera*, the most toxic AIs were deltamethrin (LD_{50} = 0.013 µg/g) and bifenthrin (LD_{50} = 0.156 µg/g); and the least toxic was chlorpyrifos (LD_{50} = 3.246 µg/g). When the insecticide-impregnated papers method was used, Mosquitomist Two (chlorpyrifos 24.6%) was the most toxic insecticide for *Ae. aegypti* (LC_{50} = 0.024 µg/cm^2), and Aqualuer (permethrin 20.6%, PBO 20.6%) was least toxic (LC_{50} = 0.408 µg/cm^2). For *A. mellifera* the most toxic commercial insecticide formulations were Talstar (bifenthrin 7.9%; LC_{50} = 0.288 µg/cm^2) and Mosquitomist Two (LC_{50} = 0.299 µg/cm^2), with no significant differences, and the least toxic commercial formulation was Deltagard (deltamethrin 2.0%; LC_{50} = 15.084 µg/cm^2). By topical application, more than 28 times of chlorpyrifos was needed to obtain the same mortality in *A. mellifera* as in *Ae. aegypti*. When using the insecticide-impregnated paper method, more than 206 times of Deltagard was needed to obtain the same mortality in *A. mellifera* as in *Ae. aegypti*. Even though Mosquitomist Two was the most toxic insecticide for both insect species, the honey bees were >12 times more tolerant to this insecticide, compared with the mosquitoes.

Key words: *Aedes aegypti*, *Apis mellifera*, insecticides, toxicity, topical application, insecticide-impregnated papers, mosquito control

INTRODUCTION

Aedes aegypti (L.), the yellow fever mosquito, is an important vector of numerous human arboviral diseases including dengue, Zika, chikungunya and yellow fevers (CDC 2020a, 2020 b, 2020c, 2020d). Dengue, Chikungunya, and Yellow fever viruses may cause long-lasting severe symptoms and death. The illness caused by Zika virus is usually mild but may cause serious brain defects including microcephaly in unborn babies. Local transmissions of dengue and Zika have been reported from several states in the United States, including Florida. The Chikungunya and Yellow fever viruses are not currently present in the United States, but the risk of (re)introduction is possible due to infected travelers and the presence of *Ae. aegypti* (FDOH 2020a, 2020b).

About two-thirds of the crops traded on the world market depend on pollinator services (Klein et al. 2007). Honey bees, *Apis mellifera* (L.), are the most valuable pollinators for agricultural crops and the elevated loss rates of managed honey bee colonies

threaten the pollination services they provide (Bruckner et al. 2018, Klein et at. 2007, López-Uribe and Simone-Finstrom 2019). For that reason, there is global concern about the decline of honey bee populations which is attributed to a range of factors such as "Colony Collapse Disorder" (Williams et al. 2010), pathogens, and pesticides (Ostiguy et al. 2019). Since worker honey bees can forage up to 12 km around their hive and reach urban areas (Beekman and Ratnieks 2000), they can be exposed to insecticides used in public health to manage mosquitoes. *Ae. aegypti* is closely associated with urban and suburban domestic habits (Jansen and Beebe 2010), and insecticides are regularly applied to control them (Farook et al. 2018). Some studies concluded that barrier or ground insecticide applications to control host-seeking mosquitoes may also affect nontarget insects such as honey bees (Qualls et al. 2010, Drake et al. 2016). Adding to the challenges faced by mosquito control districts, *Ae. aegypti* is becoming increasingly resistant to pyrethroids (Smith et al. 2016, Estep et al. 2018 Casey et al. 2020), which are the active ingredients (AIs) of choice in many adulticides available for mosquito control. As such, novel ways are needed to control mosquitoes with minimal impacts on non-target organisms.

The first objective of the studies presented here was to determine the differential toxicity of one organophosphate and five pyrethroid AIs and one organophosphate and four pyrethroid commercial insecticide formulations for *Ae. aegypti* and *A. mellifera*. The second objective was to evaluate two bioassay methods for potential use in insecticide resistance monitoring surveys. This information is needed to help evaluate the impact of insecticide applications on both *Ae. aegypti* and *A. mellifera*.

MATERIALS AND METHODS

Insects rearing and maintenance. Pyrethroid-susceptible *Ae. aegypti* (ORL1952 strain) pupae in 473 ml (16 oz) deli cups were obtained from colonies maintained at the United States Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology (USDA CMAVE) in Gainesville, FL, USA. Pupae and emerging adults were maintained in adult colony cages in an environmental chamber at 26+2°C (79+3°F), 50-80% RH and a photoperiod of 12:12 (Light:Dark). *Apis mellifera* were obtained from an apiary managed according to common practices for North Central Florida by the Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL, USA. Female adult *Ae. aegypti* were collected 3-4 days after they had emerged from the pupal stage and used for the insecticide assays. Female adult worker *A. mellifera* were at least 3 days old and collected from three separate hives by shaking off adults crawling on hive frames. Throughout the experiments, adult *Ae. aegypti* and *A. mellifera* were provided with 10% and 50% sucrose solution *ad libitum,* respectively.

Active ingredient experiments. The following six commonly used mosquito adulticidal technical AIs (Sigma-Aldrich, USA) were used in the experiments: Phenothrin (94.6 %), prallethrin (96 %), deltamethrin (99.7%), chlorpyrifos (99.3%), permethrin (96.7%) and bifenthrin (99.1%). For range-finding experiments, 10-fold serial dilutions in acetone from $1.0x10^{4}$ - $1.0x10^{-1}$ ng/µL were applied topically onto the thorax of adult female *Ae. aegypti* and *A. mellifera*. Intermediate dilutions were included for the determination of the LD_{50}.

For *Ae. aegypti*, topical toxicity bioassays were performed based on the method of Pridgeon et al. (2008). For each of 5 replicate assays per treatment, 10 adult female *Ae. aegypti* were knocked down using CO_2 for 15 s, and then treated with 0.1 µl of insecticide preparation using a 5 µl syringe (Hamilton Co. Reno NV) with a repeat dispenser (Hamilton PB 600-1). Each group of treated mosquitoes were transferred to a 20-ml scintillation vial, which was covered with mesh to prevent escape. Control insects were treated with acetone only. Mortality was assessed 24 h after exposure to insecticides. The replicates were performed on different days with

4-5 doses over the critical portion of the dose curve for Probit analysis.

For *A. mellifera*, topical toxicity bioassays were performed based on the method of the Organization for Economic Co-operation and Development (OECD 1998). For each of 6-7 replicate assays per treatment, 10 adult female workers from three separate hives were knocked down with CO_2 for 20 s and then treated with 1.0 µl of insecticide preparation using a 50 µl syringe (Hamilton Co. Reno NV) with a repeat dispenser (Hamilton PB 600-1). Each group of treated insects were transferred to a 120-ml Mason jar which was then closed with a lid that was modified with glued-in mesh. Negative controls were treated with acetone only. Mortality was assessed 24 h after exposure to insecticides. The replicates were performed on different days with 4-5 doses over the critical portion of the dose curve for Probit analysis.

Commercial insecticide experiments. The following five commercial insecticides were tested using an insecticide-impregnated paper method: Mosquitomist Two™ (chlorpyrifos 24.6%; Clarke Roselle, IL), Aqualuer® 20-20 (permethrin 20.6%, PBO 20.6%; AllPro Vector Group, St Joseph, MO), Deltagard® (deltamethrin 2.0%; Bayer Cropscience, Cary, NC), Duet® (Prallethrin 1.0%, Phenothrin 5.0%, PBO 5.0%; Clarke, Roselle, IL) and Talstar P (Bifenthrin 7.9%; FMC, Philadelphia PA). For range-finding experiments, 10-fold serial dilutions from $1.0x10^{0}$ - $1.0x10^{-5}$ % were prepared using different diluents depending on the miscibility of the pesticide formulation. Mosquitomist Two and Aqualuer were diluted in acetone; Deltagard and Talstar in distilled water; and Duet in mineral oil. Intermediate dilutions were included for the determination of the LC_{50}.

Each insecticide preparation was applied to filter paper strips (Whatman filter paper #2). For *A. mellifera*, the strips were 14 cm^2 (2x7 cm), and for *Ae. aegypti* the strips were 5 cm^2 (1x5 cm). To ensure the same amount of AI/cm^2, the volume of insecticide solution applied was adjusted based on the size of the paper strip and the solvent used. *A. mellifera* strips were treated with 90 µl of Mosquitomist Two or Aqualuer preparations; 140 µl of Deltagard or Talstar preparations, or 70 µl of Duet preparation. *Ae. aegypti* strips were treated with 32 µl of Mosquitomist Two or Aqualuer, 50 µl of Deltagard or Talstar, or 25 µl of Duet preparations (Sanchez-Arroyo et al., 2019). The negative control strips were treated with the diluents of the corresponding insecticides.

Aedes aegypti were knocked down using CO_2 for 15 s and transferred to 20-ml scintillation vials, which were then covered with mesh secured by rubber bands. Ten females were used in each concentration replicate and housed in the same vial. After 30 minutes and complete insect recovery from CO_2, an insecticide-treated filter paper strip was introduced to the middle of the scintillation vial, with both sides available for mosquitoes to rest. The strips remained in the vial for the duration of the experiment. Five replicates were carried out on separated days.

Apis mellifera were knocked down using CO_2 for 20 s and transferred to 120-ml glass jars which were then secured with a mesh. Ten worker bees were used in each concentration replicate and housed in the same jar. After 30 minutes and complete recovery from CO_2, a filter paper strip treated with insecticide was introduced to the center of the jar with both sides exposed. The strip remained in the jar for the duration of the experiment. Any bees that were not walking at the time the insecticide-treated paper strip was added, were not included in the experiment. Five to seven replicates were carried out on separate days.

At least 350 *Ae. aegypti* or *A. mellifera* were assayed against each insecticide. For both insects, mortality was assessed 24 h after exposure to insecticides.

Statistical Analysis. To determine the LD_{50} and LC_{50} for each AI and insecticide formulation, respectively, a probit analysis was performed with SAS version 9.4 (SAS Institute Inc., Cary, NC), and significance was determined by non-overlap of 95% confidence limits. If negative control mortality was >5% , mortality data of the corresponding treatments were corrected with Abbott's formula (Abbott 1925).

RESULTS

Toxicity of the active ingredients by topical application. For *Ae. aegypti*, deltamethrin was the most toxic of the 6 AIs, followed by bifenthrin, chlorpyriphos, phenothrin, permethrin, and prallethrin (Table 1, Table 3). For *A. mellifera,* the most toxic AI was deltamethrin, followed by bifenthrin, permethrin, phenothrin, prallethrin, and chlorpyriphos which was the least toxic AI (Table 1, Table 3). The honey bee tolerance index was largest for chlorpyriphos (28.72), followed by phenothrin (11.44), permethrin (3.95), bifenthrin (2.64), deltamethrin (0.228), and prallethrin (0.14) (Table 1). This means that much more chlorpyriphos was needed to kill *A. mellifera* than susceptible *Ae. aegypti* but, conversely, much less prallethrin or deltamethrin. Phenothrin, permethrin, and bifenthrin were moderately to slightly less toxic to *A. mellifera* than to *Ae. aegypti.*

Toxicity of insecticide formulations by paper bioassay. When the insecticide-impregnated papers method was used, Mosquitomist Two was most toxic to *Ae. aegypti*, followed by Talstar, Duet, Deltagard, and Aqualuer (Table 2, Table 3). For *A. mellifera*, the most toxic commercial insecticide formulation was Talstar, followed by Mosquitomist Two , Duet, Aqualuer, and Deltagard (Table 2, Table 3). The honey bee tolerance indexes show that *A. mellifera* was more tolerant than *Ae. aegypti* to all five insecticide formulations, with Deltagard being the least toxic, and Talstar the most toxic (Table 2). This means, for example, that > 200 times of Deltagard was needed to kill *A. mellifera* than susceptible *Ae. aegypti.*

Insects were not only observed for mortality 24 h post treatment, but behavior was also assessed during the time of exposure. Both insect species behaved differently when exposed to the pyrethroids as opposed to the organophosphate formulation. The insects walked for only shorts periods of time on the pyrethroid-impregnated papers; apparently trying to avoid them. This behavior was not observed when the insects were exposed to chlorpyrifos.

Table 1. Lethal doses (LD_{50}) of six insecticides topically applied to *Aedes aegypti* and *Apis mellifera* adults.

	Aedes aegypti			*Apis mellifera*			
Insecticide	LD_{50} (µg/g insect)	(95% CI)	Slope (SE)	LD_{50} (µg/g insect)	95% CI	Slope (SE)	Honey Bee Tolerance Index[1]
Deltamethrin*	0.057a	0.050-0.067	1.88 (0.149)	0.013a	0.008-0.018	2.27 (0.39)	0.228
Bifenthrin*	0.059a	0.051-0.069	2.10 (0.183)	0.156b	0.137-0.177	2.71 (0.24)	2.64
Chlorpyrifos*	0.113b	0.105-0.158	1.55 (0.169)	3.246d	2.902-3.622	3.52 (0.35)	28.72
Phenothrin*	0.168bc	0.150-0.187	3.25 (0.307)	1.922c	1.703-2.160	2.08 (0.23)	11.44
Permethrin*	0.194c	0.170-0.221	2.63 (0.28)	0.767b	0.0674-0.887	2.66 (0.25)	3.95
Prallethrin*	19.42d	17.53-21.51	3.55 (0.32)	2.603d	2.290-2.950	3.56 (0.35)	0.134

[1]*A. mellifera* LD_{50} / *Ae. aegypti* LD_{50}

*Means followed by the same letter within a treatment group for each species are not significantly different.

Table 2. Lethal concentrations (LC_{50}) of commercial insecticides to *Aedes aegypti* and *Apis mellifera* adults, using insecticide-impregnated paper method.

	Aedes aegypti				*Apis mellifera*				
Insecticide	LC_{50} (µg/cm²)	95% CI	Slope (SE)	N	LC_{50} (µg/cm²)	95% CI	Slope (SE)	N	Honey Bee Tolerance Index[1]
Deltagard* (AI deltamethrin)	0.073a	0.059-0.091	1.65 (0.160)	360	15.084a	9.37-23.66	2.29 (0.426)	490	206.6
Talstar* (AI bifenthrin)	0.030b	0.026-0.035	2.44 (0.214)	360	0.288b	0.259-0.323	3.26 (0.314)	350	9.6
Aqualuer* (a.i permethrin)	0.408c	0.363-0.461	3.142 (0.282)	360	4.647c	2.65-9.249	1.168 (0.175)	490	11.39
Mosquitomist Two* (AI chlorpyrifos)	0.024b	0.021-0.027	3.06 (0.320)	360	0.299b	0.267-0.333	3.04 (0.250)	420	12.45
Duet* (AIs sumithrin and prallethrin)	0.069a	0.054-0.070	2.59 (0.247)	460	2.302d	2.043-2.627	2.75 (0.245)	490	33.36

[1]*A. mellifera* LC_{50} / *Ae. aegypti* LC_{50}

*Means followed by the same letter within a treatment group for each species are not significantly different.

Table 3. Comparative Toxicity of Active Ingredients Tested by Topical Application and Surface Treatment.

	Ae. aegypti		*A. mellifera*	
	Topical Application	Filter paper strips	Topical Application	Filter Paper Strips
Most toxic	Deltamethrin (a)	Mosquitomist Two (a) (AI chlorpyriphos)	Deltamethrin (a)	Talstar (a) (AI bifenthrin)
	Bifenthrin (a)	Talstar (a) (AI bifenthrin)	Bifenthrin (b)	Mosquitomist Two (a) (AI chlorpyriphos)
↓	Chlorpyriphos (b)	Duet (AIs sumithrin, prallethrin) (b)	Permethrin (b)	Duet (AIs sumithrin, prallethrin) (b)
	Phenothrin (c)	Deltagard (AI deltamethrin) (b)	Phenothrin (c)	Aqualuer (AI permethrin) (c)
Least toxic	Permethrin (c)	Aqualuer (AI permethrin) (c)	Prallethrin (d)	Deltagard (AI deltamethrin) (d)
	Prallethrin (d)		Chlorpyriphos (d)	

*Means followed by the same letter within a treatment group for each species are not significantly different.

DISCUSSION

The pyrethroid AIs deltamethrin and bifenthrin were most toxic to both *Ae. aegypti* and *A. mellifera*, when applied topically. Bifenthrin was also most toxic when the insects were exposed to treated filter paper strips, but deltamethrin was much less toxic (Table 3). Instead, the organophosphate, chlorpyrifos, was very toxic to both insect species when exposed to treated filter paper strips. Chlorpyrifos had an intermediate insecticide toxicity for *Ae. aegypti* and was least toxic for *A. mellifera* when applied topically (Table 3).

In the topical application method, immobilized insects are treated with insecticide, and the doses are independent of insect activity (Moses and Gfeller 2001). In the insecticide-impregnated method, insects are actively exposing themselves to insecticide when walking on the treated strips, and the amount of insecticide picked up is a function of time spent on the treated surface. For *Ae. aegypti* and both methods of insecticide application, < 1 µg of active ingredient or formulation/g insect resulted in 50% mortality, with the notable exception of prallethrin. For *A. mellifera*, insecticide-impregnated paper strips tended to be less toxic than topically applied insecticides, with the notable exception of Mosquitomist Two (Tables 1 and 2). One possible reason could be that the insects walked for longer periods of time on the chlorpyrifos-treated papers than on the pyrethroid-treated papers, and hence picked up more chlorpyriphos AI by tarsal contact. This may, in part, explain why chlorpyrifos was more toxic than three of the four pyrethroid insecticides. Danka et al. (1986) also suggested that insecticide cuticular penetration in honey bees is slower for applications made to the thorax than tarsi due to differences in sclerotization in those areas. On the other hand, when summarizing the toxicity data of insecticides, Hardstone and Scott (2010) reported that while honey bees can be sensitive to individual insecticides, they are not highly sensitive to insecticides overall, or even to specific classes of insecticides.

There are few reports in the literature on the toxicity of modern insecticides to honey bees. Greig-Smith et al. (1994) reported LD_{50} of 0.59 µg/g bee for chlorpyrifos, and Hardstone and Scott (2010) an LD_{50} range from 0.590 to 1.14 µg/g bee for the same insecticide. In this research we reported an LD_{50} of 3.24 µg/g for honey bees. For permethrin, Inglesfield (1989) reported an LD_{50} of 1 µg/g bee, meanwhile Danka (1986) reported an LD_{50} of 0.15 µg/g bee. In our study we obtained an LD_{50} of 0.767 µg/g bee, an intermediate value.

Topical application is a method where the insecticide is deposited directly onto the insect thorax, and allows the development of defined toxicological data for calculation of resistance ratios, a measure that World Health Organization (WHO 2009, 2018) and CDC bioassays were not designed to produce (Waits et al. 2017). This data is useful in comparing topical application with Ultra Low Volume (ULV) application, either using truck-mounted equipment or any kind of aircraft (Mount et al. 1996), since the droplets directly impinge the insect body. In the present study, the only difference is the insect size, since the honey bees are about 20 times bigger than mosquitoes.

The insecticide-impregnated papers method was originally developed to evaluate discriminating doses. In this method, the insects expose themselves to the insecticide; the more they move, the more insecticide they pick up by their tarsi. Additionally, it has been reported that the insecticide applied to the mosquito tarsomeres of the hind leg spread out across all the tarsomeres, the tibia, and a portion of the femur of the hind leg (Aldridge et al. 2016). Insecticide contact with appendages such as the leg resulted in much lower mortality from both permethrin and malathion and suggest that topical bioassay techniques used to evaluate mortality to *Culex quinquefasciatus* (Say) may be modified to include other body areas without reducing comparability to mesothorax studies (Aldridge et al. 2016). Insecticide toxicity determined by exposure to insecticide-impregnated filter paper is useful for comparison with barrier treatments, since in this type of operational insecticide application (either using a backpack sprayer or a

truck mounted-mist sprayer) we expect the insect to pick up the lethal amount of insecticide by their tarsi (VanDusen et al. 2016, Richards et al. 2017).

Irritation produced by pyrethroid insecticides may have prevented the insects from staying in contact with the insecticide-impregnated papers for a longer time. Since chlorpyrifos did not cause irritation, this may explain why it is more toxic to both insects, because they move freely or rest on insecticide-impregnated papers until they get a lethal dose. This toxicity may not be correlated with the insect's body weight (Robertson et al. 2017). From a practical point of view, it could be more useful to use insecticide-impregnated papers rather than topical treatments in order of generate more useful information about field insecticide effects on these species. The exposure to insecticide-impregnated papers has been proposed to carry out toxicological studies for monitoring of *Triatoma infestans* populations, and other insects (Remón et al. 2017).

Atkins et al. (1973, 1975; cited by Danka et al. 1986) reported that most referenced insecticide results are topical or contact, and the LD_{50} concentrations obtained by topical application are relatively lower. Felton et al. (1986) suggested that the data on the acute contact and oral toxicity of pesticides to honey bees should be expressed as LD_{50} and should be considered as one of the elements for assessment of danger to foraging honey bees. However, the current study provides evidence that the insecticide-impregnated paper method has value in determining which residual insecticides have the least effect on field nontarget species such as honey bees. Since commercial formulations were used in the insecticide-impregnated method, the results could provide guidance on which insecticides to use in the field. This information is needed to eliminate, as much as possible, non-target effects on honey bees which have comparatively few genes encoding detoxification enzymes (Claudianos et al. 2006).

Pyrethroids are the most common insecticides used for adult mosquito control, which has led to widespread resistance globally. Resistance to permethrin and other pyrethroids in mosquitoes were recently documented in Florida (Coleman et al. 2017, Estep et al. 2018, Parker et al. 2020). Honey bees are moderately sensitive to deltamethrin and permethrin, and more sensitive to bifenthrin (Hardstone and Scott 2010), and the application of these insecticides when pollinators are not foraging to avoid mortality of honey bee and other non-target insects becomes even more important when targeting pyrethroid-resistant mosquitoes. Correct application timing combined with better insecticide application techniques can further increase safety of mosquitocidal applications. Aerial ultra-low volume applications using high-pressure nozzle system reduced environmental insecticide contamination with Naled and leads to decreased bee mortality (Zhong et al. 2004). Similar studies can lead to improved application techniques that can be used in the control of mosquitoes in the field with lower risk for honey bees and other non-target insects.

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