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ORIGINAL ARTICLE

Permethrin treated clothing to protect outdoor workers: evaluation of different methods for mosquito exposure against populations with differing resistance status

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ABSTRACT
Minimizing arthropod exposure (e.g. mosquito and tick bites) is vital to protect health of outdoor workers. Personal protective measures can help protect against exposure. Here, the quantity of permethrin was evaluated for different fabric types after washing. Cone and petri dish exposure assays were used to investigate the knockdown/mortality of permethrin-susceptible and permethrin-resistant populations of mosquitoes. Permethrin-treated clothing was effective against the tested mosquito population that was susceptible to permethrin but not a permethrin-resistant population. Permethrin quantity was significantly highest in the 100% cotton fabric and for the 0 wash group. Permethrin quantity in fabrics decreased with washing. No significant differences ($p > 0.05$) were observed in knockdown/mortality rates for either exposure method. The protective effect of permethrin-treated clothing against mosquitoes is impacted by many factors, e.g. wash frequency, fabric type, and the susceptibility/resistance status of local mosquito populations.

KEYWORDS
Bioassay; insecticide resistance; knockdown; occupational health; pyrethroid; worker protection

Introduction

Actions to prevent arthropod exposure are vital to protect the public from diseases such as malaria (mosquito), West Nile encephalitis (mosquito), dengue (mosquito), Lyme disease (tick), and others. For instance, dengue fever impacts about 40% of the world's population [1] and malaria is one of the world's most common mosquito-borne diseases. In 2015, there were > 200 million malaria cases and > 400,000 malaria deaths [2]. This number has decreased compared to previous years, likely due to better prevention and control measures (e.g. early diagnosis/treatment, long-lasting insecticidal nets, and indoor residual insecticide spraying; however, mosquito-borne illness remains a global threat to public health [2].

Commonly reported tick-borne diseases are Lyme disease, spotted fever group rickettsiosis, ehrlichiosis, and anaplasmosis [3]. The reason for the high number of tick-borne disease cases in recent years may be due to a lack of public knowledge of tick bite prevention methods [4]. A serosurvey done on United States National Park Service employees revealed that 22% of employees were seropositive from past exposure to spotted fever group rickettsiae, 3% were seropositive from past exposure to *Ehrlichia chaffeensis*, and 8% of employees were seropositive from past exposure to *Anaplasma phagocytophylium* [5]. Most of the participants spent 26% of their time working outdoors. This same study also looked at mosquito-borne pathogens and found that 1.5% were seropositive from past exposure to West Nile virus, 12.6% were seropositive from past exposure to La Crosse virus, and 2.2% were seropositive from a flavivirus (type not specified) [5]. A participant who was infected with La Crosse virus was reported to have spent 38% of his time working outdoors [5].

Personal protection methods, such as wearing insecticide-treated clothing, can be used to minimize arthropod bites and can be the first line of defense against vector-borne diseases [6]. In a study on German forestry workers [7], personal protection measures, particularly the use of long-sleeved shirts/trousers and daily application of repellants to exposed skin and/or clothing, were effective in reducing the risk of Lyme borreliosis. Remembering to tuck trousers into socks, checking one's body for ticks after potential exposure, and removing ticks right away once discovered are also other personal protection measures identified [7].

A study [22] tested four different types of permethrin impregnation methods used in school uniforms in Thailand. The four samples used were: (1) factory dipped clothing (polymer coated), (2) home dipped clothing, (3) microencapsulated clothing, and (4) factory dipped

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school uniforms (polymer coated). Clothes were washed up to 30 times and exposed to previously sugar fed *Ae. aegypti* female mosquitoes using the WHO cone test. The factory treated clothing not only produced higher knockdown rates compared to the other two fabrics but also retained more permethrin after 30 washes [22]. Military personnel are particularly vulnerable to biting arthropods (e.g. mosquitoes, sand flies, ticks) due to the nature of their work in the field. Insecticide-treated tents, bed nets, and clothing are used by military personnel to protect against arthropod exposure [8]. Most previous studies on permethrin-treated clothing have involved military items [8–15].

Different mosquito species exhibit different blood feeding preferences and not all mosquitoes transmit pathogens that cause diseases. *Aedes aegypti* L. prefers to blood feed on humans while *Ae. albopictus* Skuse is an opportunistic feeder that feeds on a variety of animals, including humans. Others have shown differences in insecticide resistance between mosquito populations [16,17]. Hence, identifying and testing different mosquito populations against permethrin-treated clothing is important when determining efficacy.

Permethrin is used as a repellant and an insecticide and is effective against ticks, mosquitoes and other arthropods [18]. Permethrin is commonly used for pest control in forestry, agriculture, residential, and public health settings, including for head lice and mosquito control [18]. Synthetic pyrethroids, such as permethrin, can be applied to various fabrics (e.g. bed net, clothing) for protection against arthropods. Permethrin is the most commonly used synthetic pyrethroid used in fabric; however, other pyrethroids including bifenthrin, deltamethrin, and cyfluthrin have been tested [19]. Cotton, jute, polyester, and nylon fibers may react differently to insecticides, e.g. deltamethrin showed better results with cotton material compared to other pyrethroids, while cyfluthrin was better for treating jute fibers [8]. The loss of insecticides in treated fabrics can be due to various factors such as: (1) method of impregnation, (2) number of washes, (3) exposure to light, and others [12,20].

Others have shown that higher starting permethrin concentration in insecticide-treated clothes results in higher residual permethrin content after repeated washing cycles, compared to clothing that started with lower permethrin content [12,13,21,22]. Another study examining fabric types obtained from different vendors (Insect Shield 100% cotton t-shirts, ExOfficio 15% cotton/85% polyester t-shirts, Sol's Monarch 100% cotton-shirts, and Labonal socks), and found that the Labonal socks had the highest initial permethrin concentration of 4300 mg/m^2, followed by Sol's Monarch t-shirts with 1310 mg/m^2, Insect Shield t-shirts with 1300 mg/m^2, and ExOfficio t-shirts with 870 mg/m^2 [21]. The same study showed that, after 100 launderings, Labonal socks' residual content went down by 58.1% (1800 mg/m^2). The lower the initial permethrin concentration on the fabric, the lower the residual permethrin content after washing [21]. Insect Shield t-shirts had an initial permethrin concentration of 1300 mg/m^2 and, after 100 launderings, the residual content was 20 mg/m^2 (98.5% reduction) [21]. A study [13] tested Australian military disruptive pattern camouflage uniform (DPCU) (washed up to 50 times) against two different permethrin impregnation methods: factory treatment (polymer coated) and dipping emulsion. In the same study, after 1, 3, 5, 10, 30, and 50 wash cycles, swatches were cut and unfed female *Ae. aegypti* and *Anopheles farauti* L. mosquitoes were exposed to swatches of the uniforms that were placed into a cylinder provided by the WHO susceptibility test kit. Higher concentrations of residual permethrin were found in the factory treated clothing after washing compared to the fabric that was dipped [13].

One important issue in using permethrin-treated fabric is the persistence of the insecticide in the fabric once it has been washed and/or worn several times. In a Thailand study [22], two washing methods were used: the WHO washing technique (hand washing) and machine washing. The WHO washing technique entailed submerging fabric swatches in one liter bottles of water/soap, placing in a 30 °C shaking water bath for 10 min, and drying at 30 °C for 45 min [22]. In the aforementioned study, machine washing consisted of washing fabrics for 30 min at a water temperature of 30º C, followed by air drying. In the same study, the WHO washing technique decreased permethrin content by 41.3% after five washes and then by 97.2% after 30 washes. Machine washing decreased permethrin content by 28% after five washes and then by 81% after 30 washes. Ironing and/or exposure to ultraviolet (UV) light also impacted the concentration of permethrin [22].

Insecticide susceptibility of mosquitoes exposed to fabrics can be tested using protocols such as shown in an archived Environmental Protection Agency (EPA) document which commented on a petri dish method submitted by an outside entity or the World Health Organization (WHO) cone method. The petri dish method involves placing each fabric swatch into a petri dish covered with a lid that has a small opening on top (for introducing mosquitoes) [23]. The mosquitoes remain in the petri dish (in close contact with fabric) for 2–3 min and the fabric is then pulled out from the dish at the end of the exposure time [23]. Mosquito knockdown is recorded at 15 and 60 min post-exposure. For the WHO method, a polyvinyl chloride (PVC) cone is placed over each fabric swatch [24]. Mosquitoes are introduced into the cone through a hole at the top. At the end of a 2–3 min exposure time, mosquitoes are transferred to other containers, placed into an incubator, and provided a sugar solution [24]. The mosquito knockdown rate is measured after 60 min and mortality is measured 24 h post-exposure [24]. Published research studies that compare the petri dish and cone methods are lacking, hence our study represents the first known attempt to compare these methods.

The findings of the current study will contribute to current research on mosquito knockdown/mortality by permethrin-treated clothing. Personal protection is one of the first steps taken that can reduce the risk of exposure to arthropods. Understanding the extent to which fabrics retain permethrin is important as this could lead to development of fabric blends that maximize permethrin retention. Insecticide resistance in mosquitoes is an important concern for efficacy studies of permethrin-treated clothing and is considered here.

Materials and methods

Mosquito colonies

Mosquito eggs (*Ae. albopictus,* generation $F_{7,}$ Savannah, Georgia; *Ae. aegypti,* generation $F_{3,}$ Anna Marie Island, Florida) were obtained for this study and reared to adults using standard procedures. Briefly, eggs were hatched in 24 × 34 cm plastic pans (BioQuip, Rancho Dominguez, CA) with 1.0 L of tap water and 200 mg of larval food (1:2 mixture of brewer's yeast and liver powder). Larvae were fed every other day until pupation. The pupae were transferred from the pans to water-holding cups and placed into square cages (30.5 cm^3; BioQuip). Adults were fed with 20% sucrose solution, and only nonblood fed female mosquitoes (6 d post-emergence for *Ae. albopictus* and 8 d post-emergence for *Ae. aegypti*) were used in this study. A CDC bottle bioassay [25] was conducted on a subset of each mosquito population (reported in a separate study) [17] to gather insecticide resistance/susceptibility data for permethrin.

Fabric treatment and washing

Figure 1 shows study parameters for both untreated and treated clothing. Permethrin-treated (125 μg/cm^2)

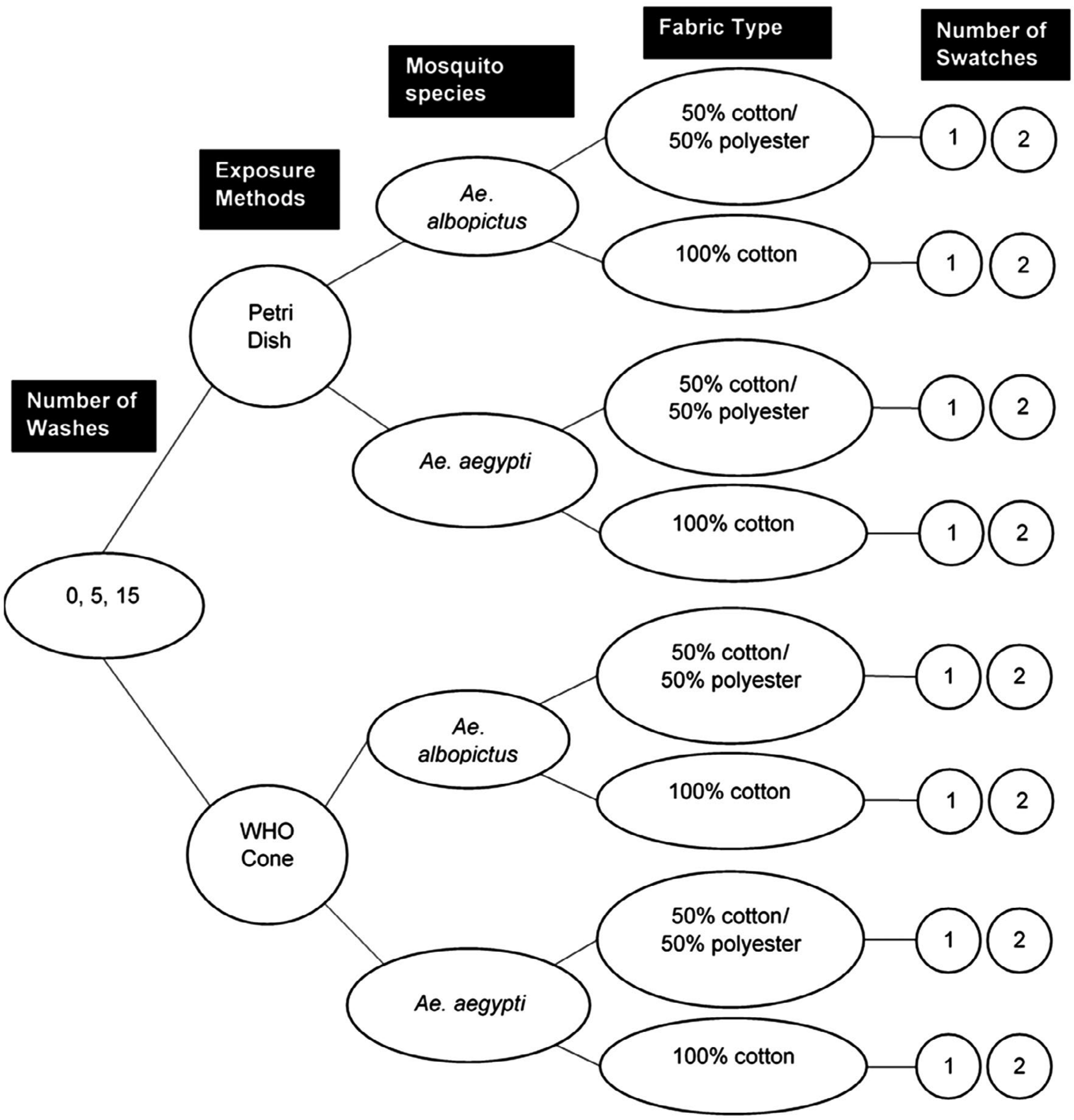

Figure 1. Flow chart of study parameters for both untreated and treated fabrics.

and untreated clothing was supplied by Insect Shield (Greensboro, NC): (1) Men's High Vis SS Tee containing no SPF, short sleeved, 50% cotton/50% polyester, and (2) Men's Chambray Work Shirt containing no SPF, long sleeved, 100% cotton. Circular swatches (8.5 cm diameter; 2 replicates for each group) were cut from both treated and untreated clothing and used for mosquito experiments for a total of 48 swatches. A priori power analysis indicated that this sample size would allow us to detect changes (medium [$w = 0.5–0.6$] to large effect size) with approximately 95% power (G Power: http://www.psycho.uniduesseldorf.de/app/projects/gpower). These calculations are based on X^2 analyses that showed differences in knockdown and mortality rates between treatments. A General Electric (GE) washer (model WCSR2090DBWW) and dryer (model DJXR433EC3WW) were used. The washing machine was adjusted for delicate textiles at a warm temperature of 27 °C for a washing time of 39 min/wash. A commercially available fragrance-free Tide detergent was used for laundering with 59 mL of detergent used for each wash cycle. After washing, garments were dried in the dryer for 30 min at a setting of optimum dry with a temperature of 50 °C. These washing/drying procedures were used as these conditions are used in a home setting (where workers, such as foresters, may wash their clothing). No commercial washing/drying equipment was used in this study. Fabric swatches were cut from each garment (replicates from front to back of garments) before washing and after 5 and 15 washing/drying cycles. Swatches were stored in separate Ziploc bags in a closed drawer (no light) until further processing. To avoid any cross contamination from potential permethrin residue in the washer/dryer, the control group (untreated) fabrics were washed first, followed by the permethrin-treated group. Each fabric swatch was used for the mosquito knockdown/ mortality experiment prior to gas chromatography analysis of permethrin content.

Mosquito bioassays

The petri dish exposure method was adapted from an archived EPA document that commented on data from an outside entity conducting a bioassay for insecticidal activity of treated fabric swatches against adult mosquitoes [23]. For both control and treated fabrics, each mosquito species was exposed to two swatches for each fabric swatch, wash frequency, and exposure method (Figure 1). Each swatch was placed into a petri dish prior to mosquito exposure. Adult female mosquitoes ($N = 3–10$/dish) were immobilized with cold for 45 s before being transferred to the petri dish. While we attempted to standardize the amount of mosquitoes exposed to each swatch, in some cases, a few mosquitoes escaped or were squashed (human error) during the transfer and, hence, were excluded from analyses. After a 2 min exposure period, the petri dish (containing mosquitoes) was chilled for 45 s to immobilize mosquitoes. Mosquitoes from each petri dish were transferred into separate 0.25 L cardboard cages with mesh screening, provided 20% sucrose solution, and transferred to an incubator at 28 °C with approximately 80% humidity and 14:10 h day: night cycle. At 2 and 24 h post-exposure to swatches, the knockdown (i.e. lying on back or side and unable to fly) and mortality rates (i.e. the number of dead mosquitoes) were recorded. The same procedure was done using the WHO cone method adapted from WHO Pesticide Evaluation Scheme (WHOPES) [23]. Once the mosquitoes were immobilized with cold, they were exposed to fabrics for 2 min, i.e. placed onto the fabric (in the bottom of a petri dish) and covered with a plastic cone (8.5 cm in diameter at the base, 5.5 cm high), before being chilled and transferred to cardboard cages in incubators and provided sugar. After each mosquito experiment, the same fabric swatches were stored in separate Ziploc bags in a dark drawer until analyzed for permethrin residue by gas chromatography.

Quantification of permethrin residue

After the designated number of wash cycles and each mosquito experiment, the treated swatches were tested for permethrin residue using a gas chromatograph (GC). For the controls, three swatches from each fabric type and each number of washes were tested, and the same method was followed for the treated samples. We adapted the method used to analyze permethrin content from a previous study [26]. After mosquito bioassays were completed, the fabric swatches were transferred to separate 60 mL amber glass vials containing 40 mL acetone and soaked for one hour to elute permethrin in a water-filled Sonicator (Ultrasonic Bath, Fisher Scientific, Kennesaw, GA) with settings at a temperature of 75°F (23.8 °C) and timer for 60 min. An ultrasonic bath was also used in previous experiments [11,15,21] to extract permethrin from factory-based treated (polymer coated) clothing; however, the length of time and sonicator settings were not indicated in the previous studies. Eluent samples (1.5 ml) from three swatches were transferred to 1.5 mL amber GC vials. A portion of the eluent (1 μL) was analyzed directly by capillary GC with flame ionization detector (GC-FID) using an Agilent GC 6850 (Agilent Technologies, Alpharette, GA). The capillary column used was DB-5MS (5% phenyl-methylpolysiloxane), 15 mm × 0.25 (i.d.) mm, 0.25 μm (film thickness) (Agilent Technologies, Alpharette, GA). The injector and detector temperatures were set at 250 and 260 °C, respectively. The oven temperature was programmed from 200 to 250 °C (adapted from previous study) [27] at 10 °C/min and held for 7 min, with a total run time of 12 min. Nitrogen was used as both carrier (32.6 mL/min) and make-up (10 mL/min) gas, and hydrogen were used as the detector gas (30 mL/min). A permethrin stock solution was prepared by dissolving 0.01 g permethrin standard in 40 mL acetone, and was used to prepare the calibration standards. Five-point calibration curves were used to generate the calibration curve for quantification. The linearity of the detector response was checked before conducting analysis by using these calibration curves. The range of standards used for the calibration curve was between 5 and 35 ug/mL and results varied within the curve with a few results at both ends of the tails (low and high), but most falling within the center of the curve. The average permethrin content ($N = 3$) for each fabric type and number of washes was determined. To confirm that there were no erroneous peaks, a blank vial filled with 1.5 mL of acetone was run in between sample runs.

Statistical analysis

Analysis of variance (ANOVA) was used to evaluate differences in permethrin content between fabric types and number of washes. Permethrin quantities were log-transformed ($x + 1$) prior to using ANOVA to improve normality and ensure normal distribution among all values. A Duncan test was used to determine differences in means and to determine the extent to which differences were observe in permethrin concentrations between fabric types and/or numbers of washes. P-values < 0.05 were considered significant. Cross tabulation and Pearson X^2 tests were performed to analyze the proportions of mosquitoes knocked down or dead at 2 h post-exposure and 24 h post-exposure against several independent variables (i.e. fabric type, number of washes, mosquito population, and exposure method) (SPSS Institute, Chicago, IL). P values for treated clothing were determined by using X^2 values under asymptotic significance (2 sided).

Results

Permethrin content of fabrics

The treated 100% cotton fabric showed higher initial permethrin content at 0 washes (mean ± standard deviation)

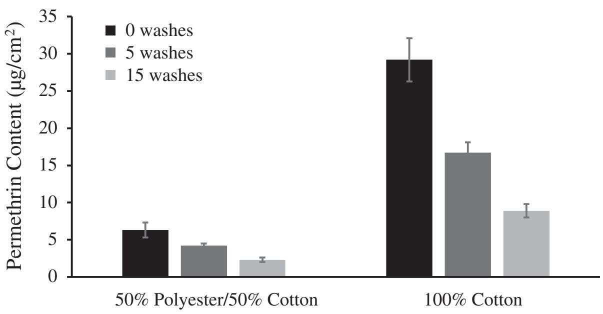

Figure 2. Permethrin content by fabric type and number of washes (mean ± standard deviation). The average permethrin content ($N = 3$) for each fabric type and number of washes was determined.

(29.2 ± 2.9 µg/cm²) compared to the 50% polyester/50% cotton fabric (6.3 ± 1.0 µg/cm²) (Figure 2).

Regardless of the fabric type, the permethrin content decreased as the number of washes increased, such that 0 washes had the highest permethrin concentration while 15 washes had the lowest. For the treated clothes, 50% polyester/50% cotton started at 6.3 ± 1.0 µg/cm² and decreased to 2.3 ± 0.3 µg/cm² (63% reduction) after 15 washes, while the 100% cotton fabric started at 29.2 ± 2.9 µg/cm² and decreased to 8.9 ± 0.9 µg/cm² (70% reduction) after 15 washes (Figure 2). Permethrin quantity was significantly highest in the 100% cotton fabric ($p = 0.001$, df = 1, $F = 698.3$) and for the 0 wash group ($p = 0.007$, df = 2, $F = 137.2$).

Knockdown and mortality of mosquitoes

No mortality or knockdown was shown in either population of mosquitoes exposed to untreated (control) fabrics (Tables 1 and 2).

The *Ae. albopictus* population used here was known to be susceptible to permethrin as determined by a CDC bottle bioassay (data not shown) [17]. Figure 3 shows knockdown and mortality of *Ae. albopictus* by fabric type, wash frequency, and exposure method.

For the petri dish evaluation, no significant differences were seen in *Ae. albopictus* knockdown or mortality knockdown for different fabric types between wash frequencies (Table 1, Figure 3).

Similarly, for the cone evaluation, no significant differences were seen in *Ae. albopictus* knockdown or mortality knockdown for different fabric types between wash frequencies (Table 3, Figure 4).

Regardless of exposure method, unwashed treated fabric (0 washes – both types of fabrics averaged) resulted in the highest *Ae. albopictus* knockdown (mean ± standard error) (37 ± 0.04%) and mortality (46 ± 0.09%) rates, while the 15 wash group of fabrics had the lowest knockdown (9 ± 0.02%) and mortality (14 ± 0.03%) rates for this mosquito species. Significant differences were observed in knockdown ($p = 0.002$, $X^2 = 12.720$; df = 2) and mortality ($p = 0.002$; $X^2 = 12.912$; df = 2) rates between the fabrics washed at different frequencies (Figures 3–4).

The cone method did not result in knockdown or mortality of *Ae. aegypti* (previously classified as resistant to permethrin); however, the petri dish method showed a knockdown and mortality rate of 2% in this mosquito population ($p = 0.178$; $X^2 = 1.811$; df = 1) (Figure 3). *Aedes aegypti* exposed to unwashed treated 50% cotton/50% polyester fabrics using the petri dish method showed knockdown and mortality rates of (mean ± standard error) 2 ± 0.09%, while fabrics washed five times showed respective rates of 2 ± 0.07%, and fabrics washed 15 times did not result in any knockdown or mortality for *Ae. aegypti* ($p = 0.650$; $X^2 = 0.862$; df = 2). The 100% cotton fabric did not knock down or kill any *Ae. aegypti*, while

Table 1. Knockdown (2-h post-exposure) and mortality (24-h post-exposure) rates by exposure method (*Ae. albopictus*).

		Knockdown						Mortality					
Sample type	Exposure method	*n*	Total	%	X^2	df	*P*-Value	*n*	Total	%	X^2	df	*P*-Value
Control	Cone	0	71	0.00	–	–	–	0	71	0.00	–	–	–
	Petri Dish	0	95	0.00				0	95	0.00			
Treated	Cone	13	75	17.3	3.304	1	**0.069**	19	75	25.3	3.230	1	**0.072**
	Petri Dish	23	77	29.9				30	77	39.0			

Table 2. Knockdown (2-h post-exposure) and mortality (24-h post-exposure) rates by exposure method (*Ae. aegypti*).

		Knockdown						Mortality					
Sample type	Exposure method	*n*	Total	%	X^2	df	*P*-value	*n*	Total	%	X^2	df	*P*-value
Control	Cone	0	69	0.00	–	–	–	0	69	0.00	–	–	–
	Petri Dish	0	98	0.00				0	98	0.00			
Treated	Cone	0	76	0.0	1.811	1	**0.178**	0	76	0.0	1.811	1	**0.178**
	Petri Dish	2	85	2.4				2	85	2.4			

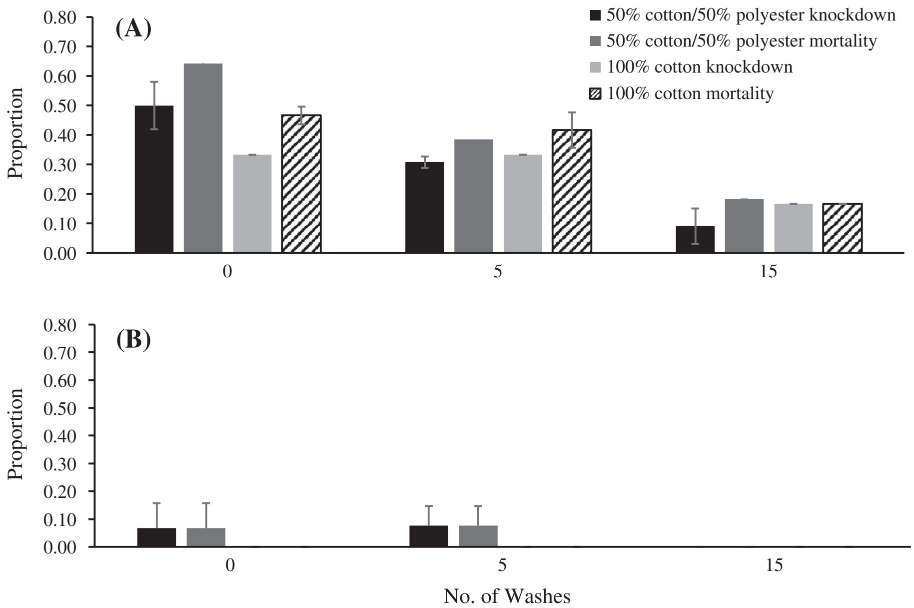

Figure 3. 2-h Knockdown and 24-h Mortality (mean ± standard error) of A) *Ae.albopictus* and B) *Ae. aegypti* using Petri Dish exposure method.

50% cotton/50% polyester had a knockdown and mortality rate of 2% ($p = 0.168$; $X^2 = 1.903$; $df = 1$).

Discussion

As expected, the efficacy of permethrin-treated fabrics for knocking down and/or killing mosquitoes varied between the (susceptible and resistant) mosquito populations and testing conditions (petri dish and cone) evaluated here. When evaluating the efficacy of permethrin treated clothing, resistance status of mosquito populations should be evaluated. Furthermore, when comparing results between different studies, the testing conditions and procedures should be considered. Here, the petri dish method produced higher knockdown and mortality than the cone method. However, it is understood that laboratory exposure procedures such as the petri dish (forces constant contact of mosquitoes with fabric) or cone (allows mosquitoes to fly away from the fabric for short periods) methods are only a proxy for field conditions and this should be considered when interpreting the results. Future studies should consider testing mosquito repellency to permethrin treated fabric, in addition to knockdown and mortality, as this is also an important consideration for disease prevention. If a mosquito is merely repelled (not knocked down or killed) by permethrin-treated clothing so that it does not land on the fabric (or the person wearing the clothing), protection against mosquito bites is still provided. The findings of this study contribute to current research on permethrin-treated clothing as personal protection for reducing risk to vector-borne diseases.

The unwashed treated 100% cotton fabric showed 29.2 ± 2.9 μg/cm^2 permethrin and the unwashed treated 50% polyester/50% cotton fabric showed 6.3 ± 1.0 μg/cm^2. However, the amount of permethrin (reported by Insect Shield) applied to the fabric was 125 μg/cm^2. This may be the amount of permethrin initially applied to

Table 3. Knockdown and mortality rates of mosquito populations by number of washes under different fabric types and exposure methods.

				Knockdown				Mortality			
Species	Exposure method	Fabric type	No. of washes	%	X^2	df	*p*-value	%	X^2	df	*p*-value
Ae. albopictus	Cone	100	0	33.3	3.902	2	0.142	40.0	3.385	2	0.184
			5	20.0				30.0			
			15	5.9				11.8			
		50/50	0	33.3	5.186	2	0.075	33.3	1.699	2	0.428
			5	9.1				27.3			
			15	0.0				10.0			
	Petri Dish	100	0	33.3	1.140	2	0.566	46.7	2.858	2	0.240
			5	33.3				41.7			
			15	16.7				16.7			
		50/50	0	50.0	4.777	2	0.092	64.3	5.479	2	0.065
			5	30.8				38.5			
			15	9.1				18.2			
Ae. aegypti	Cone	100	0	0.0	–	–	–	0.0	–	–	–
			5	0.0				0.0			
			15	0.0				0.0			
		50/50	0	0.0	–	–	–	0.0	–	–	–
			5	0.0				0.0			
			15	0.0				0.0			
	Petri Dish	100	0	0.0	–	–	–	0.0	–	–	–
			5	0.0				0.0			
			15	0.0				0.0			
		50/50	0	6.7	0.918	2	0.632	6.7	0.918	2	0.632
			5	7.7				7.7			
			15	9.9				0.0			

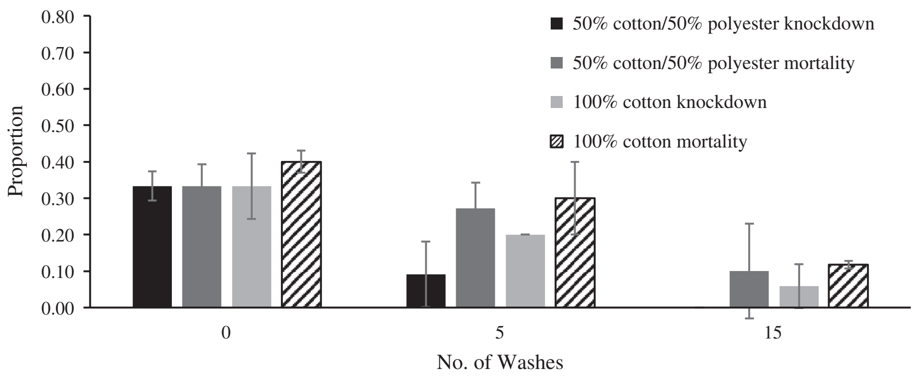

Figure 4. 2-h Knockdown and 24-h Mortality (mean ± standard error) of *Ae. albopictus* using Cone exposure method. *Aedes aegypti* experience no knockdown or mortality using this method.

the fabric; however, it is possible that not all of the permethrin applied was bound to the fabric. Our permethrin extraction methods are similar to other research that has extracted permethrin from fabrics. Other studies used an ultrasonic bath (as we did here) to extract permethrin from factory-treated clothing (polymer coated), but the length of time and sonicator settings were not indicated [11,15,21]. Furthermore, we used acetone for elution rather than toluene [11,15,21]. Another study used a sonicator to elute permethrin (35 kHz for 45 min at room temperature) and used methanol as the solvent [28]. However, the aforementioned study used self-treated (not factory-treated) fabrics so may not be directly compared to our study.

Others tested *Ae. albopictus* against permethrin treated clothing for 5 min and showed knockdown rates of 78, 16, 10 and 4% for the first four washes, respectively, and 100% mortality rates after 24 h for the same first four washes [14]. However, approximately 50% of permethrin was lost after clothing had been washed five times, producing no knockdown (15 min post-exposure) and an 88% mortality rate after 24 h. In the current study, *Ae. albopictus* experienced a range of 30–50% (both fabric types at 0 washes) knockdown rate after 2 h and 45–65% mortality (both fabric types at 0 washes) rate after 24 h. Since we used two different fabric types and a shorter exposure time than the aforementioned study, care is advised when comparing our results to other studies that may have used a different fabric type and exposure time. Another study found that polymer coated treated clothing was effective for up to 30 washes but saw a decrease in mosquito knockdown/mortality between 30 and 50 washes [12].

The *Ae. aegypti* mosquitoes used here were classified as resistant to permethrin according to the CDC bottle bioassay (data not shown). Results of the current study showed a difference in *Ae. aegypti* knockdown/mortality rates compared to other studies [12,13,15,22,30] in which *Ae. aegypti* were more susceptible to permethrin. However, caution is advised in comparing the results of our study to other studies, as a variety of different methods (i.e. exposure times, exposure methods, permethrin impregnation methods, fabric types, and other differences). A Thailand study exposed *Ae. aegypti* to permethrin treated school uniforms using the WHO cone method and found knockdown and mortality decreased as washing frequency increased, with a marked decrease (*ca.* 20% knockdown and mortality) after 20 washes compared to 0 washes (100% knockdown and mortality) [30]. However, the same study did not indicate the resistance status or origin of the *Ae. aegypti* population tested and also did not indicate the type of fabric or permethrin in the school uniforms, making the results difficult to compare to our findings. In a previous study [12], *Ae. aegypti* mosquitoes used were from Australia, were about 5–8 days old, and were exposed to treated swatches for about 10 min. For mosquitoes exposed to fabrics washed five times, the knockdown and mortality rates were 50% and 19%, which differs from our study where a 2% *Ae. aegypti* mortality rate was observed. In another study [15], *Ae aegypti* mosquitoes were 5–8 days old and achieved 100% knockdown when exposed to fabrics washed 15 times. For our study where *Ae. aegypti* were exposed to fabrics washed 15 times, no knockdown or mortality was observed in *Ae. aegypti*. Such differences between the current study and other studies could result from resistance/susceptibility status of the mosquito population, duration of exposure to fabrics, and other factors.

Others [22] observed variation in *Ae. aegypti* mosquito knockdown rate when comparing two washing methods. In the same study, for *Ae. aegypti*, 100% cotton fabrics undergoing a WHO washing technique (hand washing) achieved 90% mosquito knockdown for up to six washes, while the same fabrics undergoing a machine-washing technique maintained 90% knockdown for up to 10 washes. Mosquito knockdown decreased for both washing methods after 10 washes [22].

Another study [29] found that long-lasting permethrin impregnated clothes could retain tick-repellent activity for over 70 washes. Their study followed outdoor workers for two years and found that one year of wear reduced tick bites by > 80% but effectiveness significantly decreased in the second year. The same study suggested that after one year of wear, clothing should be retreated. It was also suggested that clothes treated using self-applied spray or dipping methods lose effectiveness unless reapplied every 3–5 washes [29]. Polymer coating lasts longer against washing, aging, rinsing, wearing, and weathering. Do-it-yourself impregnation methods (i.e. dipping or spraying) can expose individuals to permethrin through inhalation or skin contact [31]. Thus, factory treatments are expected to be safer for the end user.

Many studies use either a WHO plastic tube method [15], the cone method [14], or field exposure [32] to evaluate efficacy of permethrin-treated clothing at impacting mosquito exposure. No known published studies used the petri dish exposure method that we have used. Our findings indicate the petri dish method works better (although not significantly) than the cone method at inducing knockdown and mortality in the susceptible *Ae. albopictus* populations used here, but not the resistant *Ae. aegypti*. These experiments should be repeated using larger sample sizes and assessments of repellency should also be included as repellency can also reduce the possibility of pathogen exposure.

Comparing results obtained using different methods should be done with caution as studies may use different exposure methods. There are few published studies on the effect of permethrin-treated clothes against a variety of different mosquito species and populations. A previous study showed that *Aedes* mosquitoes were more susceptible to permethrin compared to *Culex* or *Anopheles* mosquitoes [22]. Another study showed that *Ae. aegypti* was more susceptible to permethrin-treated clothing compared to *An. farauti* [13]. However, resistance/susceptibility status of mosquito populations was not assessed in the aforementioned studies. The current study examined the results of permethrin exposure to both permethrin-susceptible and –resistant populations of mosquitoes. We bring attention to this important issue as insecticide resistance is a growing issue of global concern.

Conclusion

The protective effect of permethrin-treated clothing against potential vectors is impacted by many factors, e.g. wash frequency, fabric type, and the susceptibility/resistance status of local mosquito populations. If local mosquito populations are resistant to permethrin, another active ingredient could be considered for treatment of clothing, bed nets, and other items. Permethrin-treated clothing loses its effectiveness over time due to repeated washings, hence, retreatment or replacement of fabrics is necessary over time. We show that the efficacy of permethrin-treated fabrics for knocking down and/or killing mosquitoes varies between mosquito populations and this may be impacted by susceptibility/resistance status. Further larger scale studies are needed to evaluate differences observed in the petri dish and cone exposure methods. Permethrin-treated clothing has demonstrated potential for personal protection against mosquitoes. Mosquito and tick borne diseases should be recognized as occupational health risks and the use of permethrin-treated clothing should be included in standard prevention practices.

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