

Duration of Effectiveness of Permethrin-Treated Clothing Against *Aedes albopictus* and *Aedes aegypti* Mosquitoes After Repeated Washing

Background

- Mosquitoes are vectors of pathogens that cause many mosquito-borne diseases (e.g. malaria, West Nile encephalitis, dengue, and Zika) that may affect outdoor workers (e.g. forestry workers) and individuals participating in recreational activities, (e.g. camping, hiking, hunting, or gardening).
- Measures to stop the impact of mosquito bites are vital to protect public health.
- People can protect themselves from mosquito bites and mosquito-borne disease through personal protection methods, such as insecticide-treated clothing.

Purpose of the Study

- To evaluate biological and chemical aspects of permethrin-treated fabrics
- To determine the amount of permethrin in two fabric types after repeated washing
- To investigate the knockdown/mortality of different *Aedes* populations (*Ae. aegypti* and *Ae. albopictus*) to permethrin-treated clothing
- To examine the difference in knockdown/mortality rate between two different methods of mosquito exposure (EPA petri dish and WHO cone testing methods).

Significance of Study

- Findings in this study will help us understand the extent to which fabrics retain permethrin and provide protection against mosquito bites.

Methods

Treatment and Laundering of Fabrics

- 2 fabric types: 1) 50% cotton/50% polyester; 2) 100% cotton
- Permethrin-treated and untreated shirts were supplied by InsectShield™.
- Shirts went through 15 cycles of washing and drying.
- After 0, 5, and 15 launderings, fabric swatches were cut from each garment and stored in Ziploc bags in a closed drawer until mosquito testing.

Mosquito Knockdown/Mortality Experiment

- Mosquitoes were exposed to fabric swatches using 2 methods: WHO cone method and EPA petri dish methods (Figure 1).
- Each swatch was placed into a petri dish base.
- 8 female mosquitoes were chilled in ice for 45 s so they could be transferred onto fabric without escaping (Figure 2).
- Petri dish was covered (with either a petri dish cover or a clear plastic cone) and mosquitoes were observed for 2 min.
- After 2 min, mosquitoes were chilled in a -20°C freezer for 45s to 1 min, and then transferred to 0.25 L cardboard cage (Figure 3), provided 20% sucrose solution and placed into an 28°C incubator.

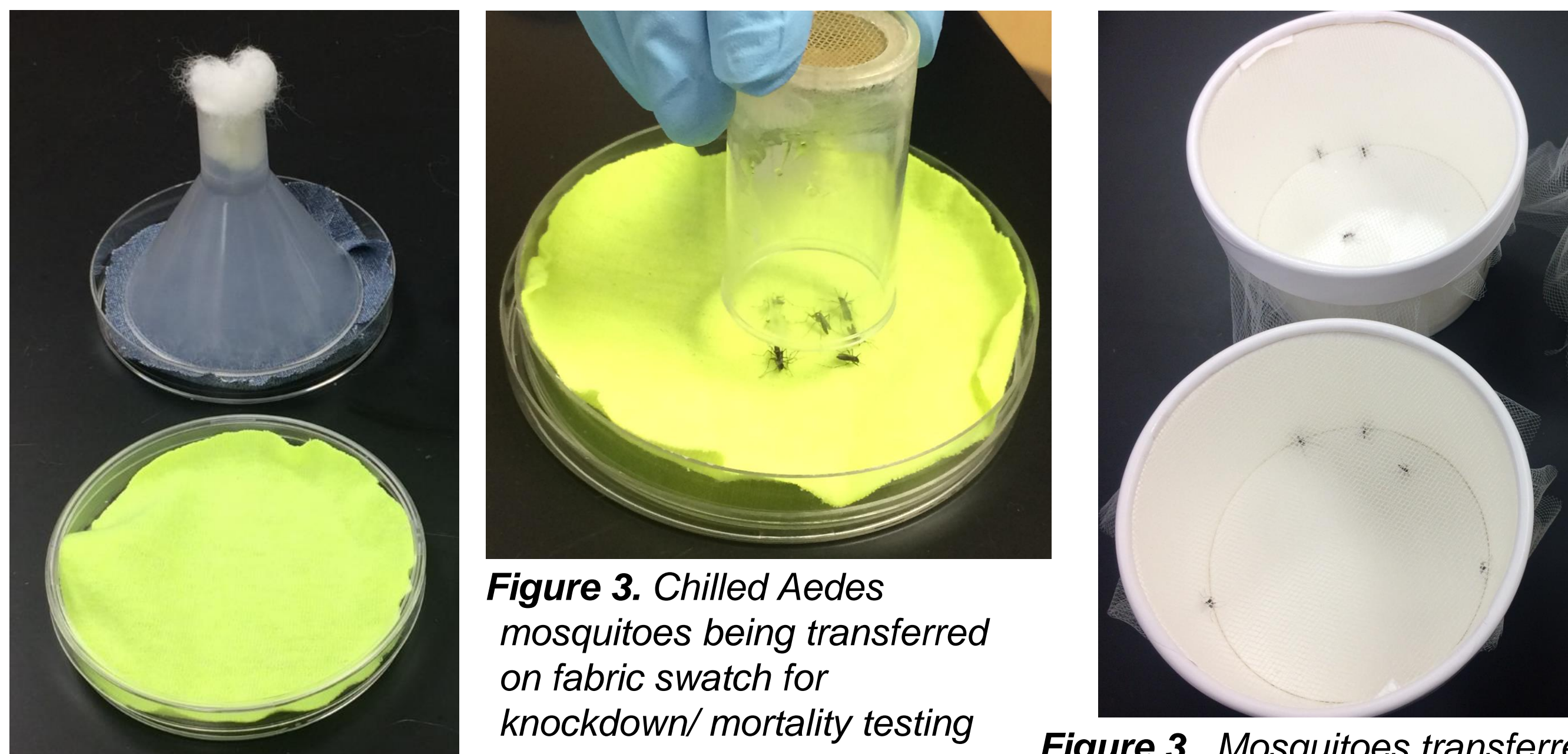


Figure 1. WHO Cone Method (above) and EPA Petri dish method (below)

Figure 3. Chilled *Aedes* mosquitoes being transferred on fabric swatch for knockdown/ mortality testing

Figure 3. Mosquitoes transferred in cardboard cages after exposure to fabric swatches

Methods, cont'd

- At 2 h and 24 h post-exposure to fabric swatches, knockdown rate (i.e. lying on back or side and unable to fly) and mortality rate (i.e. the number of dead mosquitoes) were recorded.

Permethrin Content Analysis in Fabrics

- Two swatches each from 0, 5 and 15 wash cycles were analyzed for permethrin content.
- Each swatch was transferred to a 60-mL amber glass vial containing 40mL acetone and soaked for 1 hour to elute permethrin in a water-filled Sonicator.
- Portions (1 μ L) of the eluent samples from each swatch were analyzed by gas chromatography with flame ionization detector (Agilent GC 6850).

Results

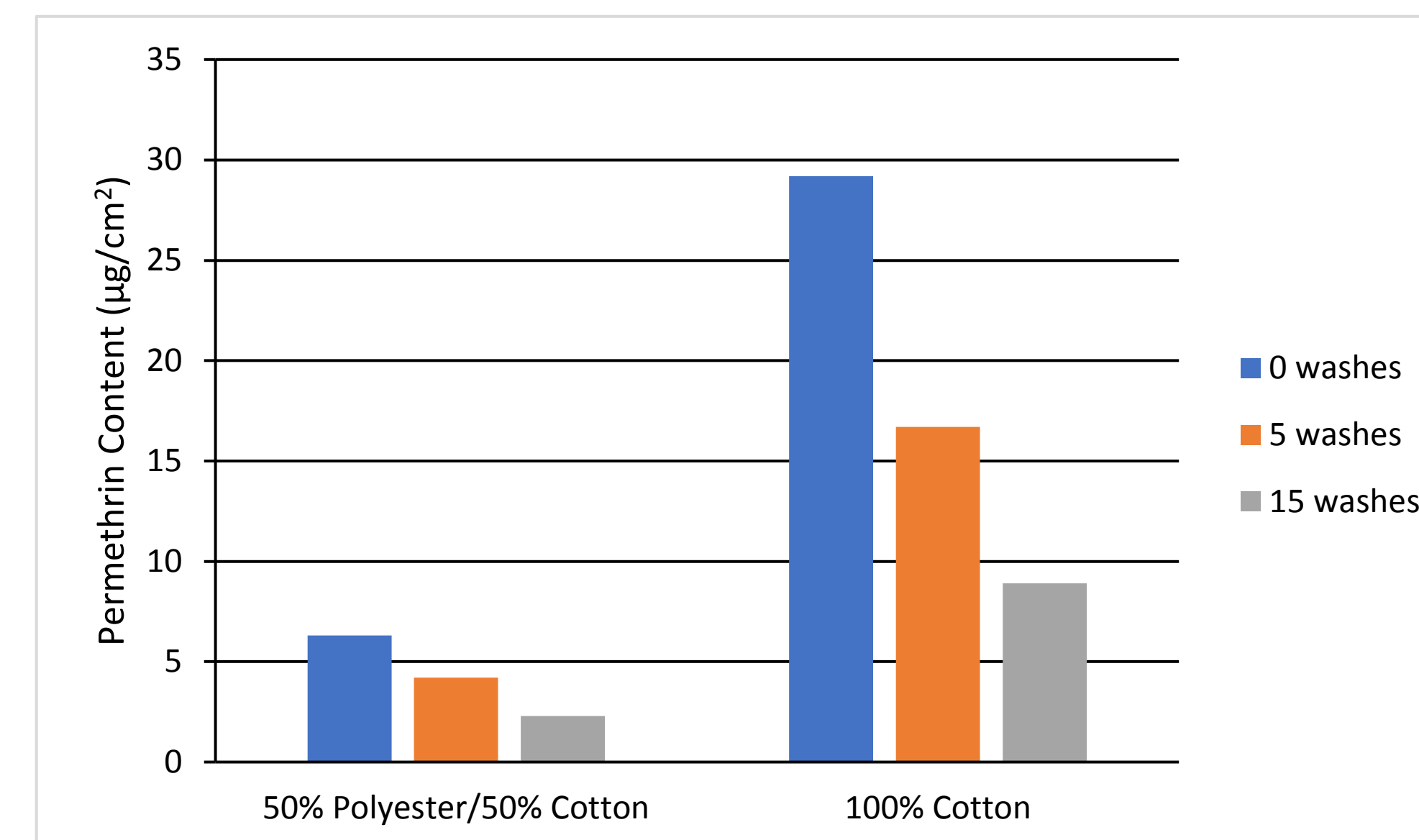


Figure 4. Permethrin Content by Fabric Type and Number of Washes

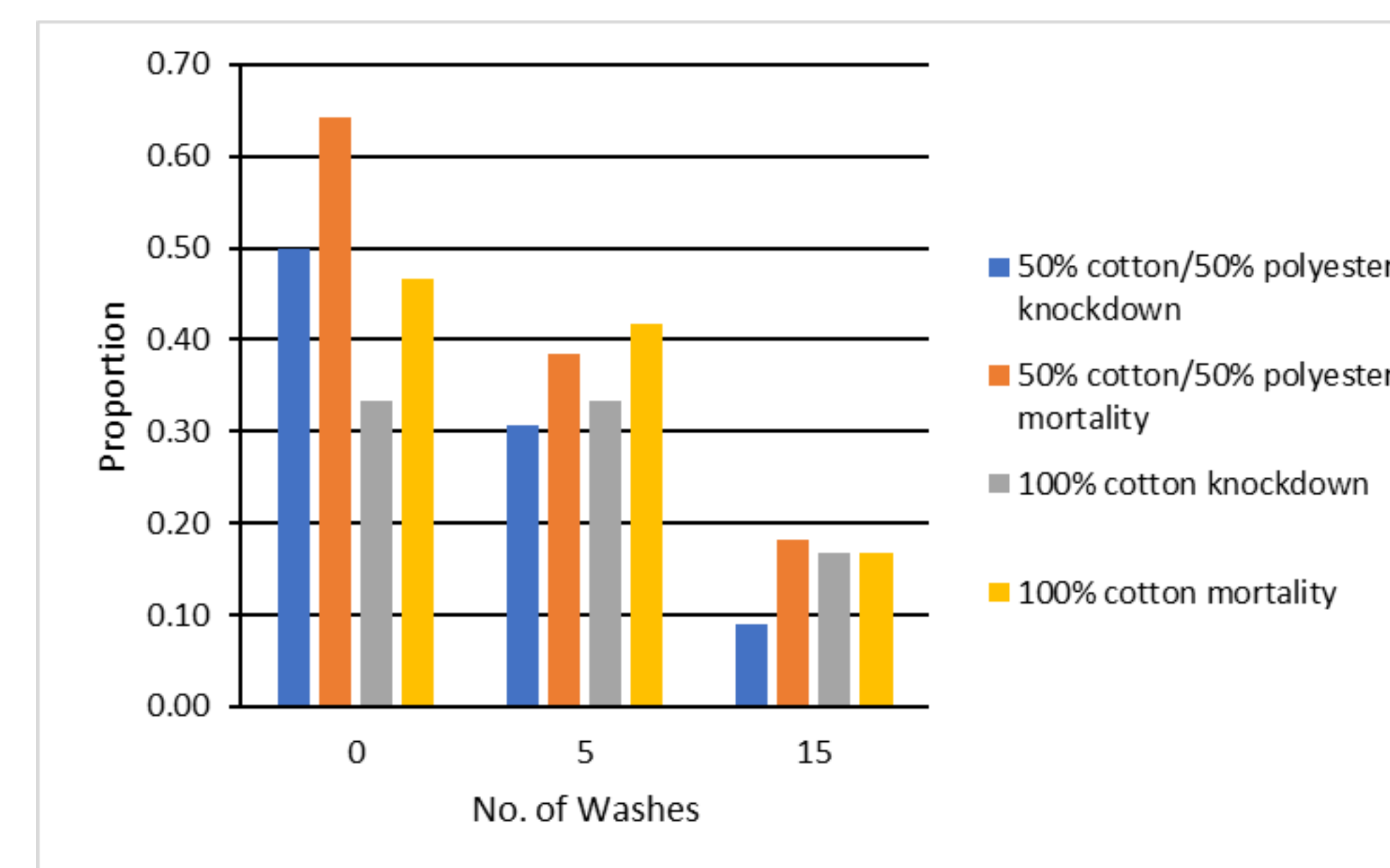


Figure 5. 2-hr Knockdown and 24-hr Mortality Proportion of *Ae. albopictus* using Petri Dish Exposure Method

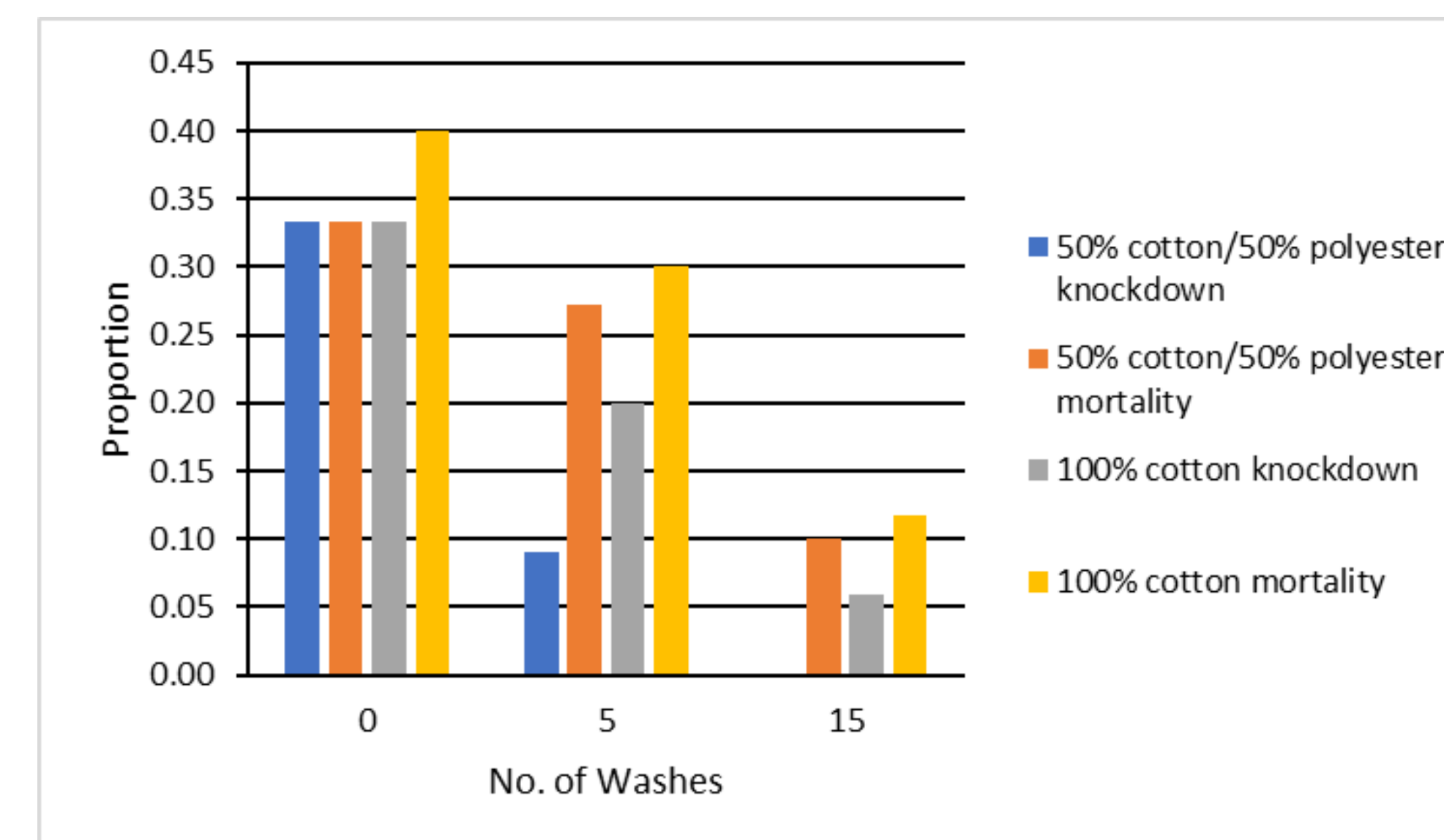


Figure 6. 2-hr Knockdown and 24-hr Mortality Proportion of *Ae. albopictus* using Cone Exposure Method

The treated 100% cotton fabric has significantly higher ($P=0.001$) initial permethrin content at 0 wash ($29.2 \pm 2.9 \mu\text{g}/\text{cm}^2$) compared to the treated 50% polyester/50% cotton fabric ($6.3 \pm 1.0 \mu\text{g}/\text{cm}^2$), and consequently has a higher average permethrin content after 15 washes ($9.0 \pm 0.9 \mu\text{g}/\text{cm}^2$) (Figure 4).

Aedes albopictus by Mosquito Exposure Method

- The petri dish method resulted in a higher knockdown (13.4%) and mortality rate (17.4%) compared to the cone method (8.9% and 13.0%, respectively), but the differences were not statistically significant for either knockdown ($P=0.210$) or mortality ($P=0.276$) (Figures 5 & 6).

Aedes albopictus by Fabric Type

- Overall, *Ae. albopictus* exposed to 100% cotton had a slightly higher knockdown (11.9%) and mortality (15.6%) rate compared to 50% cotton/50% polyester (10.8% and 15.2%, respectively), but the differences were not statistically significant for either knockdown ($P=0.754$) or mortality ($P=0.914$) (Figures 5 & 6).

Aedes albopictus by Number of Washes

- Ae. albopictus*, 0 washes had the highest knockdown (18.6%) and mortality (23.0%) rates while 15 washes had the lowest knockdown and mortality rates (3.8% and 6.7%, respectively). The differences were statistically significant for both knockdown ($P=0.003$) and mortality ($P=0.004$) (Figures 5 & 6).

Results, cont'd

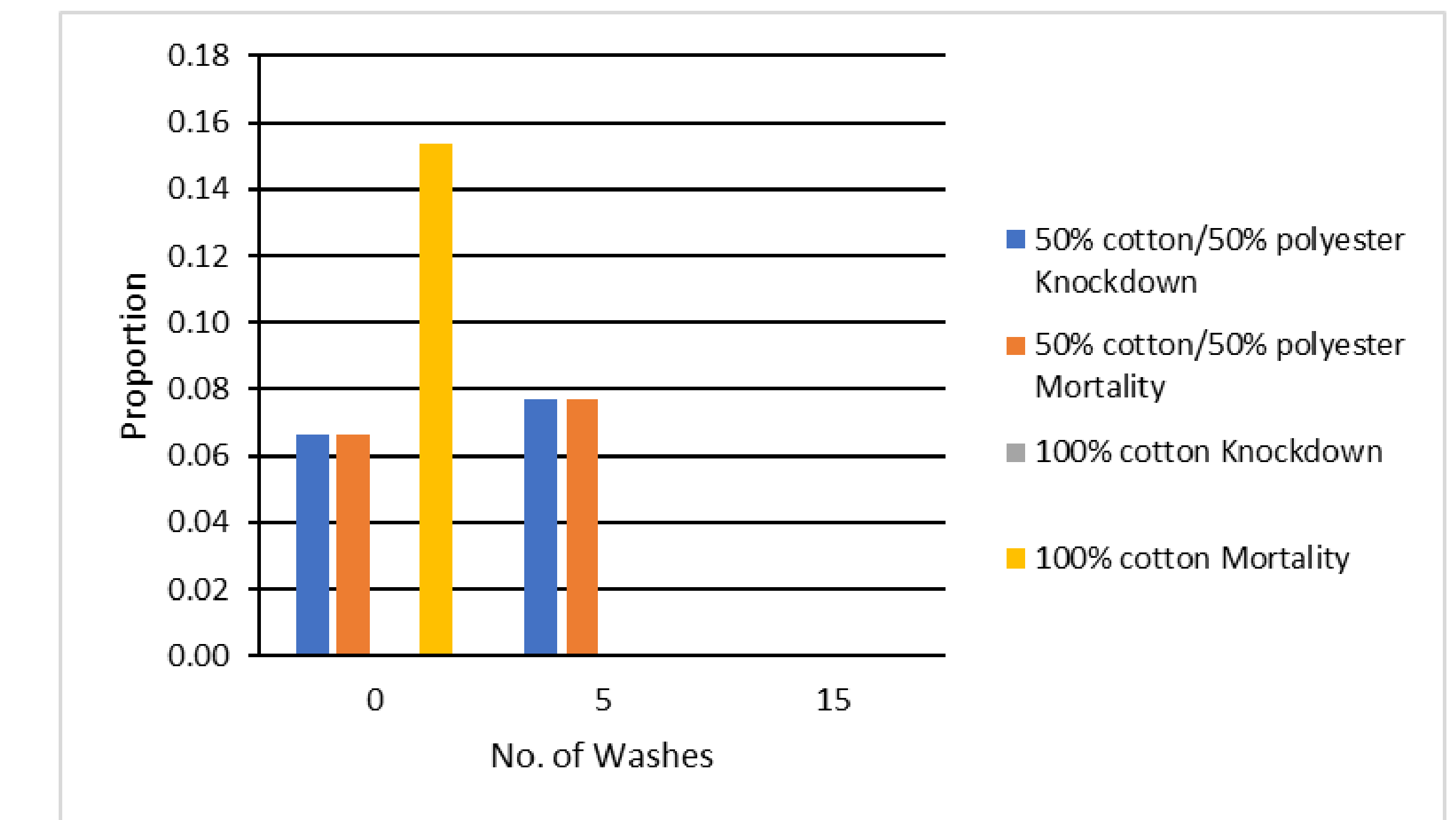


Figure 7. 2-hr Knockdown and 24-hr Mortality Proportion of *Ae. aegypti* using Petri Dish Exposure Method

Aedes aegypti by Mosquito Exposure Method

- The *Ae. aegypti* mosquitoes used in this experiment were highly resistant. The cone method was not successful in knocking down or killing any mosquitoes, but the petri dish had a knockdown and mortality rate of 1.1% ($P=0.207$).

Aedes aegypti by Fabric Type

- 100% cotton did not knockdown or kill any mosquitoes, while 50% cotton/50% polyester had a knockdown and mortality rate of 1.2% ($P=0.156$).

Aedes aegypti by Number of Washes

- 0 and 5 washes both had knockdown and mortality rates of 0.9% ($P=0.631$), while 15 washes had 0% for both knockdown and mortality rates (Figure 7).

Conclusion

- Permethrin-treated clothing was effective at killing *Aedes albopictus* mosquitoes in this lab experiment.
- Effectiveness of permethrin-treated clothing may depend on the mosquito species and/or populations due to permethrin resistance.
- Protection provided by permethrin-treated clothes against mosquito bites may be reduced over time due to repeated washing and, thus, there may be a need to re-treat or replace this protective clothing after certain period of use.

Recommendations

- Further studies on mosquito knockdown and mortality comparing both petri dish and cone methods with a larger sample size.
- Testing other genera (e.g. *Culex*) and *Aedes* spp. of mosquitoes that are abundant in certain areas to effectively assess the best use of permethrin-treated clothes in different locations
- Studies on dermal exposure of permethrin from treated clothes to understand and ensure the safe use of permethrin-treated clothes

Acknowledgment

- Special thanks to InsectShield™ for providing both treated and untreated clothing used in this study.