

Efficacy and Repellency Evaluation of Mosquito Free against *Anopheles quadrimaculatus* mosquitoes

Investigator:

Ameya D. Gondhalekar

Research Associate Professor

Department of Entomology, Purdue University

Direct spray bioassays with Tick Free/ Mosquito Free product
against *Anopheles quadrimaculatus* mosquitoes

Description of direct spray bioassay procedures

- The product was diluted in tap water as per label directions (1 oz. of Mosquito Free in 3 gallons of water)
- Adult mosquitoes (males and females) were held in a pint sized (473 ml) Mason jar with meshed top (Fig. 1)
- The internal surface area of the Mason jar was 72 square inches
- The application rate of 1 gallon per 1000 square feet was used
- This rate translated to 1.9 ml of diluted Mosquito Free product per Mason jar or 72 square inches
- A 200 ml mist sprayer was used to spray diluted Mosquito Free product directly on 15 adult mosquitoes that were held in a mesh-top Mason jar (Fig. 2)
- Control mosquitoes were sprayed with 1.9 ml of tap water
- Three replicates with 15 mosquitoes per replicate were conducted for Mosquito Free and Control treatments
- Mortality observations were recorded 5 min, 30 min, 60 min, 2 hours, 4 hours and 24 hours after treatment



Figure 1. Mesh-top mason jars used for direct spray treatment of *Anopheles quadrimaculatus* with Tick Free/ Mosquito Free product

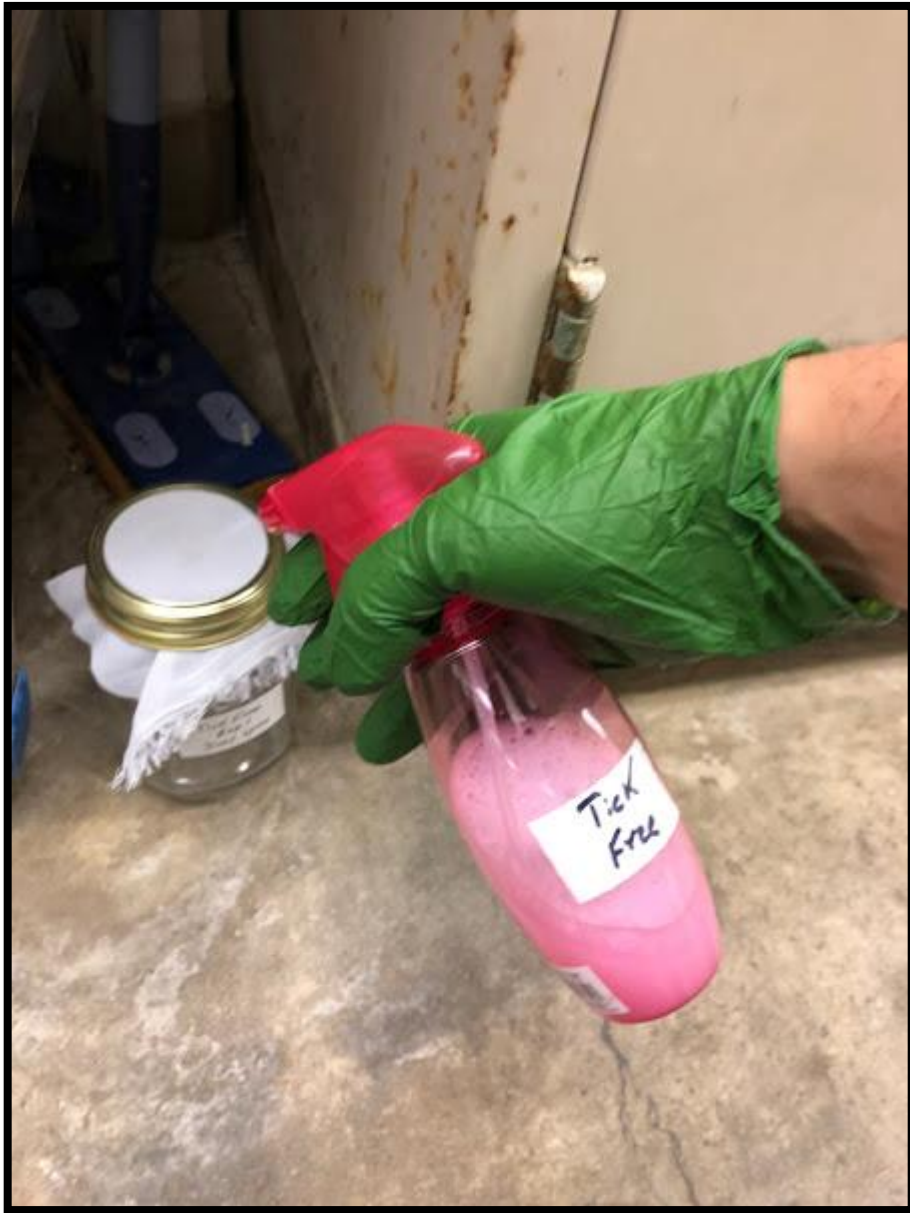


Figure 2. A 200 ml mist sprayer was used to directly spray the water-diluted Mosquito Free product on 15 mosquitoes held in the mesh top Mason jar. Application rate was 1 gallon per 1000 sq. feet or 1.9 ml per Mason jar and dilution rate was 1 oz. of Mosquito Free in 3 gallons of tap water.

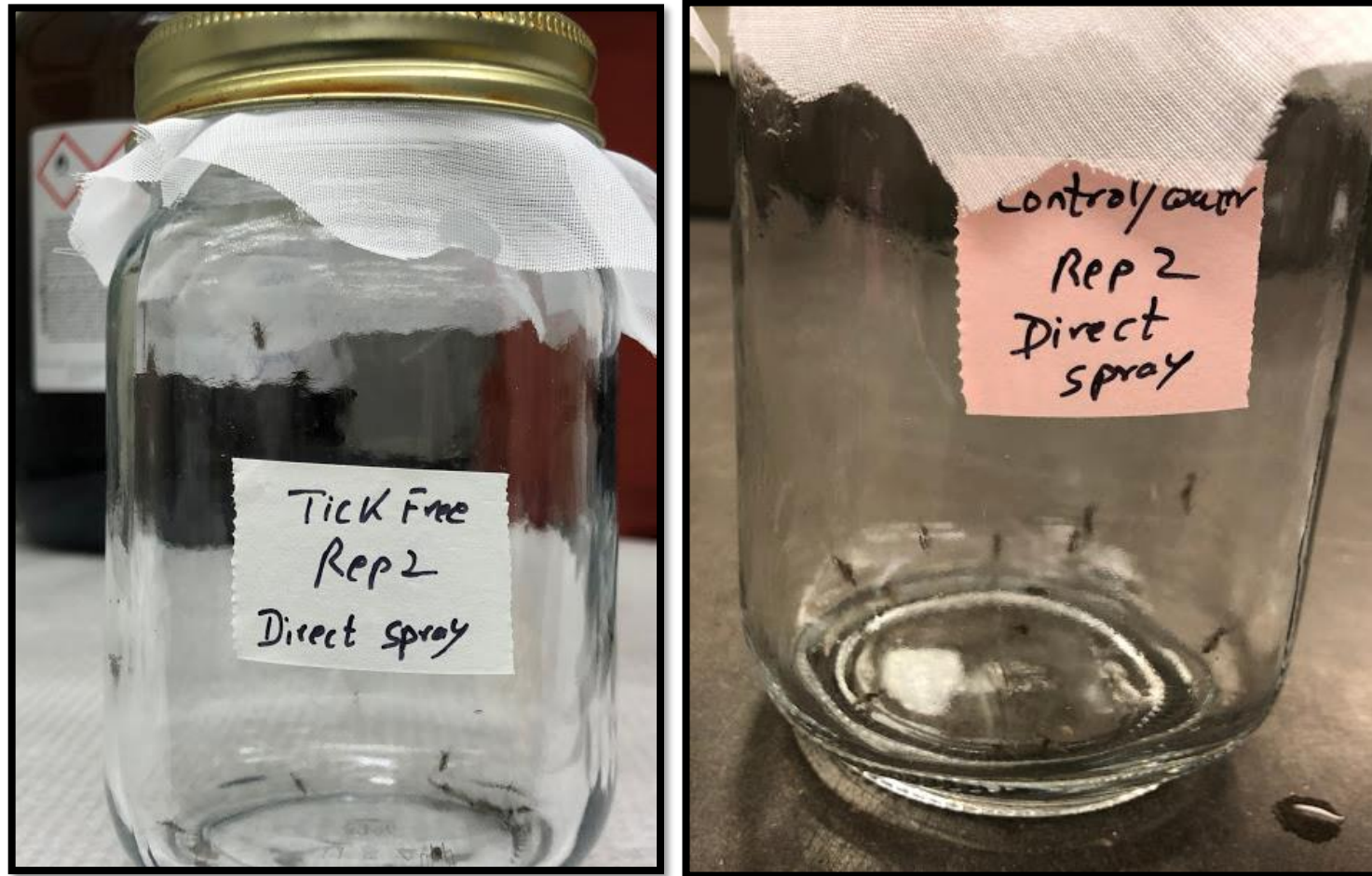


Figure 3. Five minutes after the direct spray treatment, ca. 60% of *Anopheles* adults were dead in the Mosquito Free/ Tick Free treatment, whereas a majority of mosquitoes (>90%) were alive in the control or water treatment.

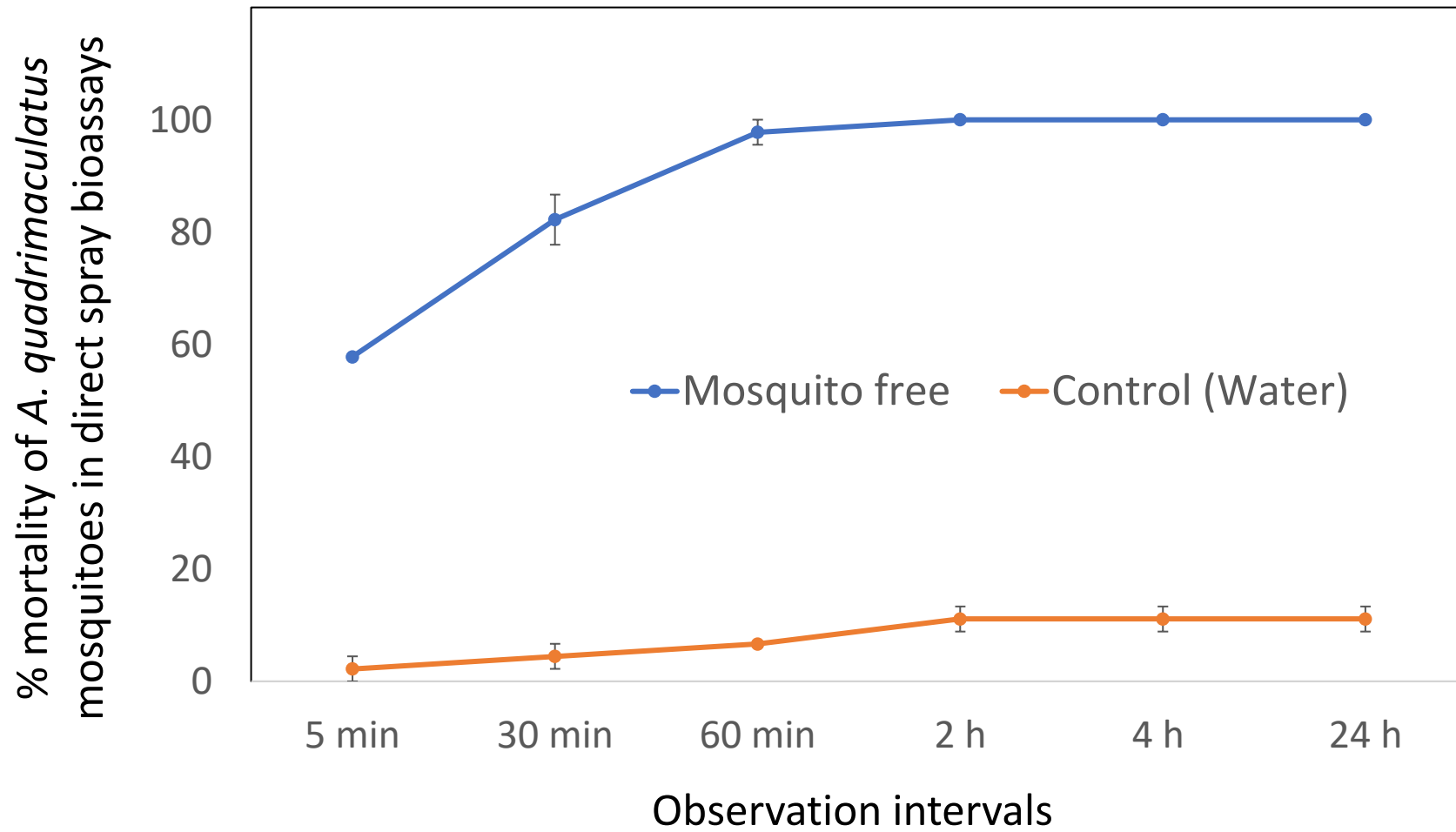


Figure 4. The results of direct spray bioassays with Tick Free/ Mosquito Free product @ 1 gallon per 1000 sq. ft. and dilution rate of 1 oz. Mosquito Free in 3 gallons of water. Blue line graphs show mortality observed in the Mosquito Free treatment and orange line indicates results from control (water treatment) bioassays. Error bars represent \pm standard error values.

Overview of direct spray bioassay results for *Anopheles quadrimaculatus* adults

- Within five minutes after direct spray, ca. 50–60% of adult mosquitoes were dead in the direct spray treatment (Fig. 3 and Fig. 4)
- By 2 hours after direct spray treatment, 100% mortality was observed (Fig. 4)
- Mortality levels were the same at 24 hours after treatment (Fig. 4)
- Control mortality varied from 2 to 11% during the 24-hour bioassay period (Fig. 3 and 4)
- Given these promising results in laboratory tests with Mosquito Free/ Tick Free, future testing on indoor and outdoor plants and porous substrates is justified

Residual (dry residue) bioassays with Tick Free/ Mosquito Free product against *Anopheles quadrimaculatus* mosquitoes

Description of residual or dry residue bioassay procedures

- The product was diluted in tap water as per label directions (1 oz. of Mosquito Free in 3 gallons of water)
- Bioassays were conducted in 100 x 15 mm plastic Petri dishes (Fig. 5). The internal surface area of the Petri dish base and lid was ca. 25 square inches
- The application rate of 1 gallon per 1000 square feet was used
- This rate translated to 0.67 ml of diluted Mosquito Free product per Petri dish (base and lid combined)
- A 200 ml mist sprayer was used to treat the Petri dish lid and base with the diluted Mosquito Free product
- The treated Petri dishes were allowed to dry for 24 h at room temperature
- Control Petri dishes were treated with 0.67 ml of tap water and were allowed to dry for 24 h
- After 24 h, 15 adult Anopheles mosquitoes that were anesthetized with carbon dioxide were introduced to control and treated Petri dishes
- Three replicates with 15 mosquitoes per replicate were conducted for Mosquito Free and Control treatments
- Petri dishes were provisioned with a piece of cotton soaked in 10% sugar solution
- Mortality observations were recorded, 4 hours and 24 hours after introduction of mosquitoes to treated and control Petri dishes



Figure 5. Photos of residual bioassays conducted with the Mosquito Free/ Tick Free product against *Anopheles quadrimaculatus* adults. Three replicates were conducted for Mosquito Free and control (water) treatments. Photos of water treatments are not shown. Each Petri dish was provisioned with cotton ball soaked in 10% sugar solution.

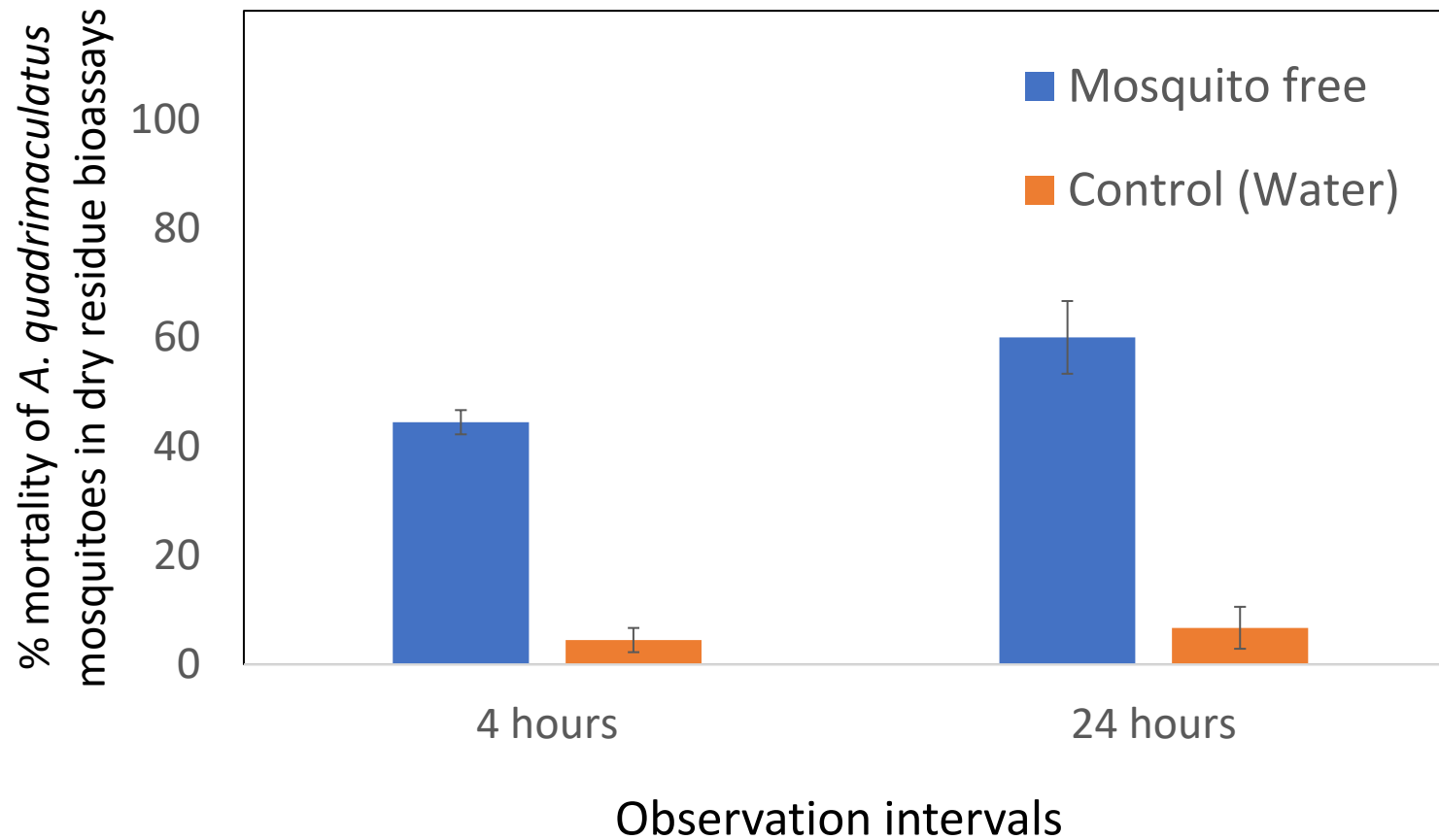


Figure 6. Results of residual or dry residue bioassays with Tick Free/ Mosquito Free product @ 1 gallon per 1000 sq. ft. and dilution rate of 1 oz. Mosquito Free in 3 gallons of water. Blue bars show mortality observed in the Mosquito Free treatment and orange bars indicate results from control (water treatment) bioassays. Error bars represent \pm standard error values.

Overview of residual bioassay results for *Anopheles quadrimaculatus* adults

- Average mortality caused by dry residues of Mosquito Free/ Tick Free was 40% at 4 h after exposure and 60% at 24 h after exposure (Fig. 6)
- Mortality in control (water) treatment was under 10% up to 24 h after exposure (Fig. 6)
- Bioassays were not continued beyond 24 h because at 48 h control mortality at that point >30%, which is unacceptable (data not shown)
- At 48 h, mortality in the Mosquito Free/ Tick Free treatment was still 60-70%
- Given these promising results, residual bioassays on plants and porous substrates treated with Mosquito Free/ Tick Free are justified

Repellency bioassays with fresh (wet residues) of Tick Free/
Mosquito Free product against *Anopheles quadrimaculatus*
mosquitoes

Description of wet residue repellency tests

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- The product was diluted in tap water as per label directions (1 oz. of Mosquito Free per 3 gallons of water)
- Bioassays were conducted in 12-inch nylon meshed cubicle cage purchased from Bioquip (Fig. 7)
- Of the six sides of the cubicle cage, each of which was 144 sq. inches in the area, three sides were treated with Mosquito Free and the remaining three sides were left untreated
- The application rate of 1 gallon per 1000 square feet was used
- This rate translated to 3.8 ml of diluted Mosquito Free product on each side of the cubicle cage
- A 200 ml mist sprayer was used to treat three sides of the cubicle net with diluted Mosquito Free product
- Three sides of the control cage were treated with tap water (3.8 ml per side)
- The treated and untreated sides of the Mosquito Free and Control nets were marked
- Immediately after treatment, 15 mosquitoes were released in each cage and the number of mosquitoes resting on treated vs. untreated sides of the cage were counted at 5, 10, 20 and 60 mins post release
- Repellency percentages were calculated using the formula listed on the next slide

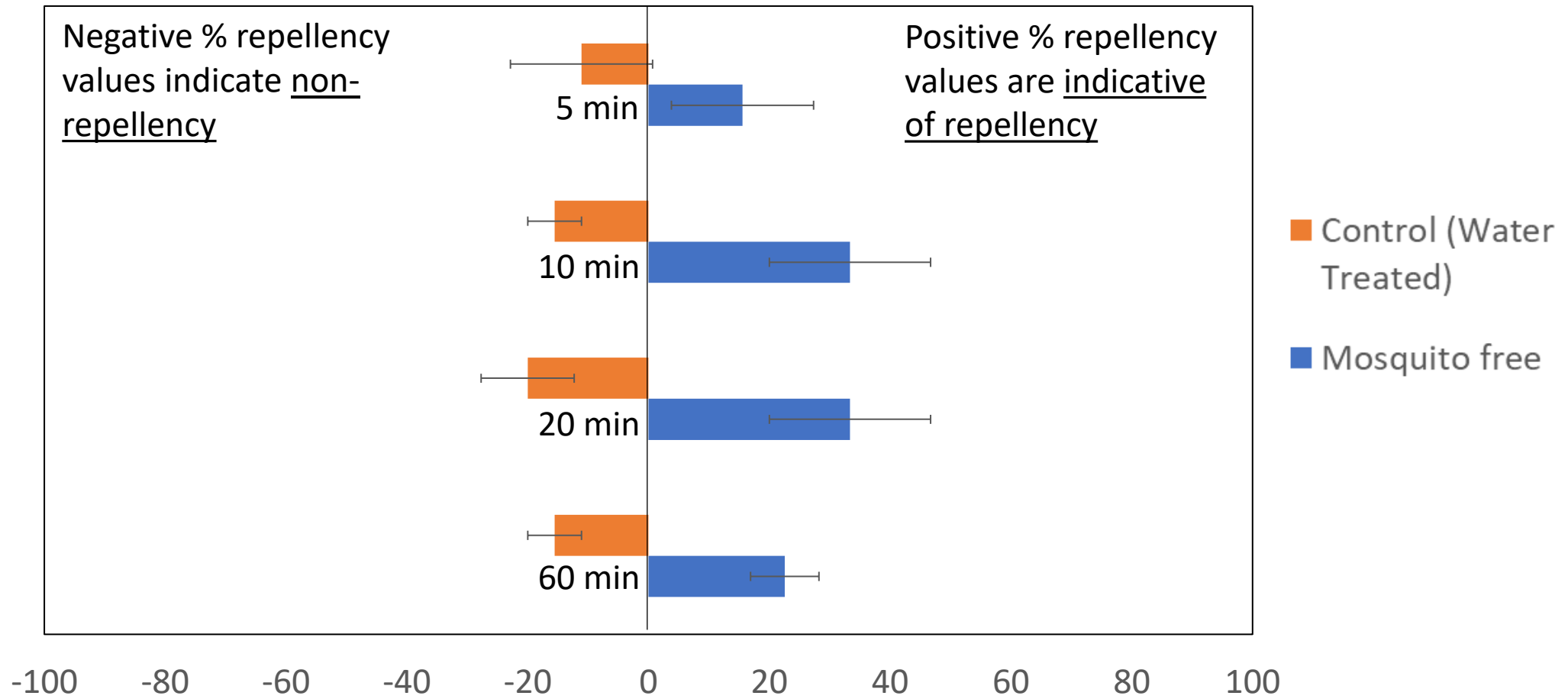


Figure 6. Photos of cages used for the fresh (wet) residue repellency test of the Mosquito Free/ Tick Free product. Each side of the cubical cage was 12 x 12 inches in size.

Calculation of percent repellency values

$$\text{Percent repellency} = [(N_c - N_t) / (N_c + N_t)] \times 100$$

- N_c is number of mosquitoes resting on the untreated sides of the cage
- N_t is number of mosquitoes resting on the Mosquito Free or Water treated sides of the cage
- Positive percent repellency values are indicative of “REPELLENCY”
- Negative percent repellency values indicate “NON-REPELLENCY”



% repellency values indicating magnitude of repellency or non-repellency

Figure 8. Results of repellency bioassays conducted with fresh (wet) residues of Tick Free/ Mosquito Free product @ 1 gallon per 1000 sq. ft. and dilution rate of 1 oz. Repellency values of -100% indicate complete absence of repellency and values of +100% indicate complete repellency. Mosquito Free in 3 gallons of water. These bioassays with *Anopheles* adults were conducted in 12-inch cubicle nets. Three sides of the net were treated with insecticides and the other three sides were treated with water. Observations on repellency were recorded at 5, 10, 20 and 60 minutes after exposure. Error bars represent \pm standard error values.

Overview of results from the fresh (wet) residue repellency test

- Fresh (wet) residues of Mosquito Free were repellent to *Anopheles* mosquitoes (Fig. 8). However, the magnitude of repellency was low because repellency values were much lower than +100%.
- At various time points within the 1-hour experiment, repellency values for Mosquito Free ranged from 15 to 33% (Fig. 8)
- Repellency bioassays were not continued beyond 60 mins because by that time the treated sides of the nylon cage were completely dry
- In control (water) treatments, no repellency was observed as indicated by negative repellency values
- Overall, it can be said that wet residues of Mosquito Free are somewhat repellent to *Anopheles* mosquitoes.

Repellency bioassays with dry residues of Tick Free/ Mosquito Free product against *Anopheles quadrimaculatus* mosquitoes

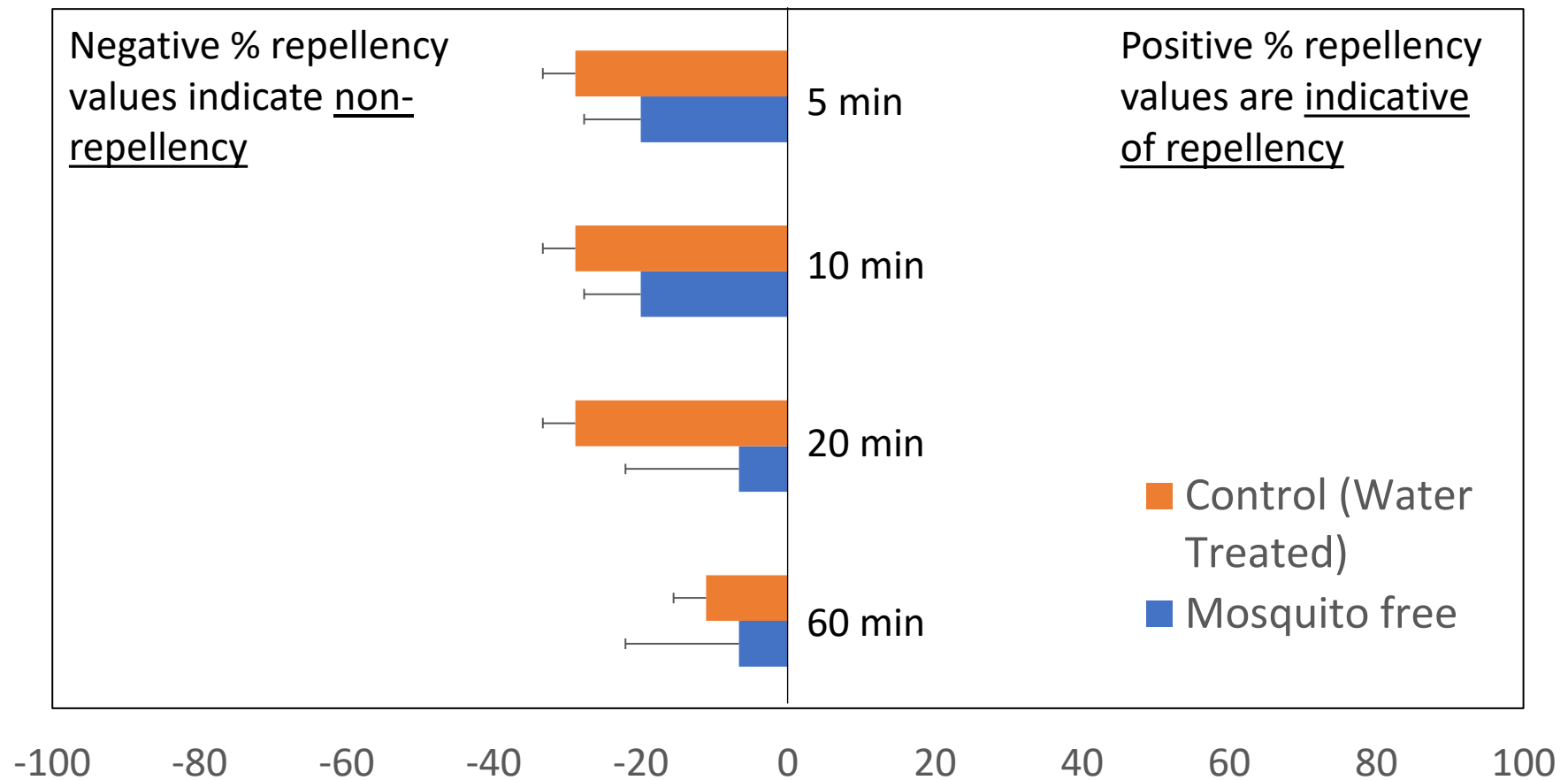
Description of wet residue repellency tests

- The product was diluted in tap water as per label directions (1 oz. of Mosquito Free per 3 gallons of water)
- Bioassays were conducted in 12-inch nylon meshed cubicle cage purchased from Bioquip (Fig. 7)
- Of the six sides of the cubicle cage, each of which was 144 sq. inches in area, three sides were treated with Mosquito Free and the remaining three sides were left untreated
- The application rate of 1 gallon per 1000 square feet was used
- This rate translated to 3.8 ml of diluted Mosquito Free product on each side of the cubicle cage
- A 200 ml mist sprayer was used to treat three sides of the cubicle net with diluted Mosquito Free product
- Three sides of the control cage were treated with tap water (3.8 ml per side)
- The treated and untreated sides of the Mosquito Free and Control nets were marked
- Both Mosquito Free and water treated (control) cages were allowed to completely dry (~2 hours) before using them for dry residue repellency test
- To initiate the dry residue repellency tests, 15 mosquitoes were released in each cage and the number of mosquitoes resting on treated vs. untreated sides of the cage were counted at 5, 10, 20 and 60 mins post release
- Repellency percentages were calculated using the formula listed on the next slide

Calculation of percent repellency values

$$\text{Percent repellency} = [(N_c - N_t) / (N_c + N_t)] \times 100$$

- N_c is number of mosquitoes resting on the untreated sides of the cage
- N_t is number of mosquitoes resting on the Mosquito Free or Water treated sides of the cage
- Positive percent repellency values are indicative of “REPELLENCY”
- Negative percent repellency values indicate “NON-REPELLENCY”



% repellency values indicating magnitude of repellency or non-repellency

Figure 9. Results of repellency bioassays conducted with dry residues of Tick Free/ Mosquito Free product @ 1 gallon per 1000 sq. ft. and dilution rate of 1 oz. Mosquito Free in 3 gallons of water. Repellency values of -100% indicate complete absence of repellency and values of +100% indicate complete repellency. These bioassays with *Anopheles* adults were conducted in 12-inch cubicle nets. Three sides of the net were treated with insecticides and the other three sides were treated with water. Observations on repellency were recorded at 5, 10, 20 and 60 minutes after exposure. Error bars represent standard error values.

Overview of results from the dry residue repellency test

- Dry residues of Mosquito Free did not exhibit any repellent effect against *Anopheles* mosquitoes (Fig. 9) as indicated by negative repellency values ranging from -7 to -28%.
- Similarly, in control (water) treatments, no repellency was observed as indicated by negative repellency values ranging from -11 to -29% (Fig 9)
- Given the fact that dry residues of Mosquito Free are not repellent to *Anopheles* adults likely explains the residual toxicity exhibited by this product on non-porous substrates such as plastic Petri dishes