Evaluating dye compounds as adulticides via toxic sugar bait and assessing the role sex plays in lethal toxic dosing

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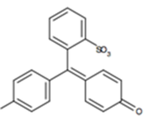
Abstract

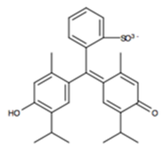
e increased resistance of certain mosquito species to the insecticides on the market and the vast tbreak of Zika virus diagnoses has called the need to develop more efficient insecticides. Answering that ll, many researchers have been experimenting with different methods and chemicals to come up with a w set of insecticides that can effectively eliminate adult mosquitoes. This study focuses on one type of ethod that can be used to control the population of adult *Aedes aegypti* mosquitoes. That method is own as a toxic feeding assay, wherein a sugar solution is made toxic by the addition of pesticide. Utilizing is assay method, we evaluate the possibility of using dye compounds as adulticides and assess the pact that sex may plays in the lethal dosing. The feeding assays are conducted to see the toxicity and cretion rate of different anionic dye compounds. In this study, we show that (1) a feeding assay is an fective method to evaluate dye compounds toxicity, (2) dye compounds with sulfonate groups are creted quickly, (3) the changes in chemical structure can affect mortality rate, (4) female mosquitoes are ore tolerant to dye toxins than males, and (5) dye compounds can be used as adulticides.

ntroduction

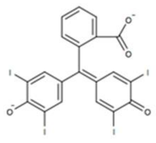
des aegypti are associated with many of the mosquito-borne diseases. *Aedes aegypti* transmit viruses at cause diseases such as yellow fever, west Nile, Zika fever, dengue fever, and many more. The ability of osquitoes to becomes resistance to insecticides has increased the outbreak of mosquito-borne diseases the past few years. With no readily available vaccinations for mosquito transmitted diseases, there is an gent need for the development of a new set of insecticides that can specifically target the solute nsport systems of mosquitoes and decrease their overall population. The need for new insecticides have d to many research studies that involve chemical compounds such as dyes. Dye compounds have been oven effective and has increased our knowledge of the mosquito physiological systems. Using dye mpounds is also an affordable method of decreasing mosquito populations and the diseases they cause. e propose that the feeding assay of mosquitoes via toxic sugar bait can lead a more promising secticide. We will evaluate the toxicity and effects of dyes compounds based on their chemical structures ing the feeding assays methods and assess the role sex plays in the lethal toxic dosing.



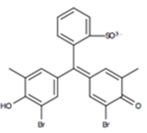
Phenol Red



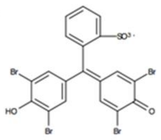
Thymol Blue



FD&C Red 3
Erythrosine



Bromocresol Purple

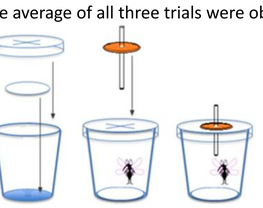


Bromophenol Blue

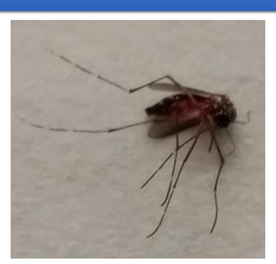
Method

ionic dyes mainly containing the triarylmethyl functional group were studied in the feeding assay. Some e stocks were made using 100% DMSO and other dye stocks were made by dissolving the dyes in 100% hanol, 10% sucrose solution, 20 mM β-cyclodextrin. The choice of solvent used is determined by the lubility of each dye. In preparation for the feeding assays, each was dissolved in a combination of all lvents order to be consistent and have all the dyes in the same solution condition. The 100% ethanol as allowed to evaporate under vacuum to achieve a solution containing 95% sucrose, 4% dye (1 mM or 5 M), and 1% β-cyclodextrin (1 mM) in a total volume of 200 µl.

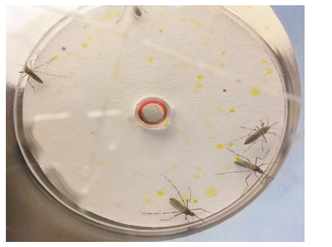
amond mini cups were used as the feeding chambers. The chambers consisted of a 42 mm Whatman ter paper, and capillary tubes. An "X" shape was cut into the lids of the cups to make it easier to place e mosquitoes and capillary tubes in the cups. 5 µl of white mineral oil was put in the capillary tube using pillary action and followed by 20 µl of the feeding assay solution. The experimental groups were fed dye lutions whilst the control fed on sucrose solution. To be consistent in the experiment, the adult osquitoes were separated by sex. 5 adults of the same sex were placed into each cup and incubated at ˚C. The mosquitoes were observed every 24 hours and were provided with new sets of capillary tubes ntaining new solutions daily. The survival and mortality rate of each group were recorded and analyzed determine the lethal dose response. Three experimental trials were conducted for each hypothesis and e average of all three trials were obtained and analyzed in a bar graph.

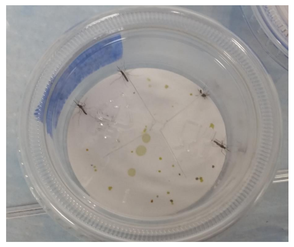


esults

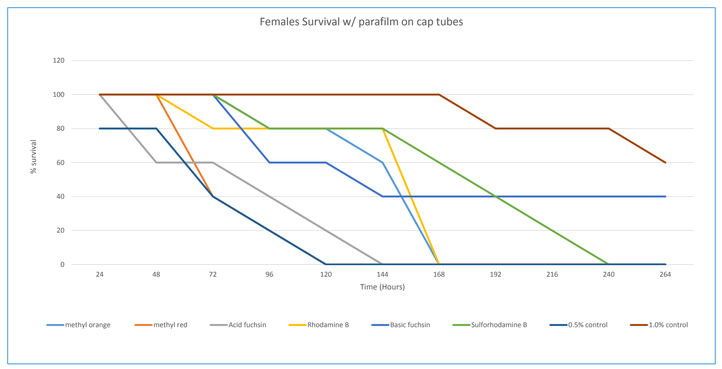


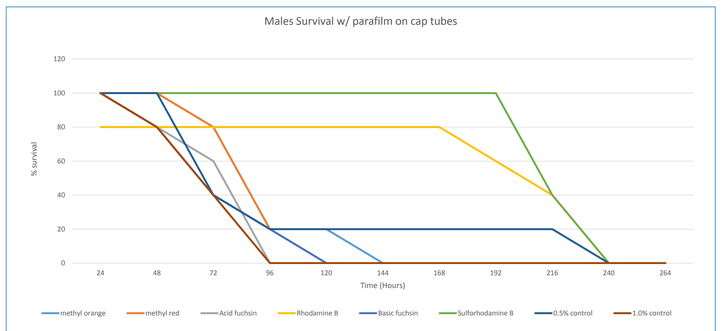
dence of Feeding. Left: Toxic sugar bait was put in a capillary tube using capillary action. The mosquitoes were ovided with fresh solutions daily. Adult male *mosquito feeding from a capillary tube containing sugar in the ntrol group.* Right: Adult female mosquitoes were allowed to feed on toxic sugar bait. The abdomen is stain by e color of the toxins.



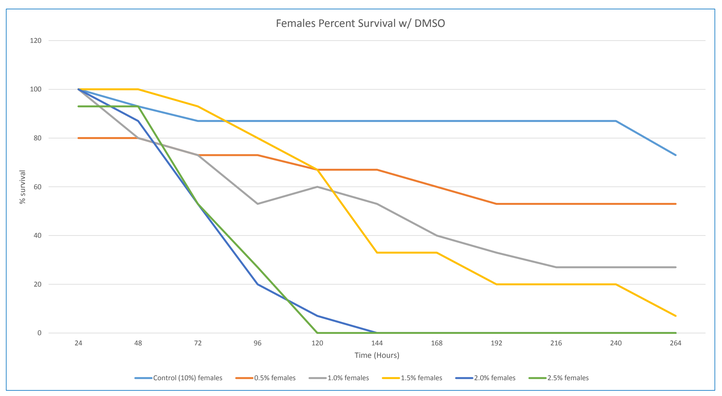


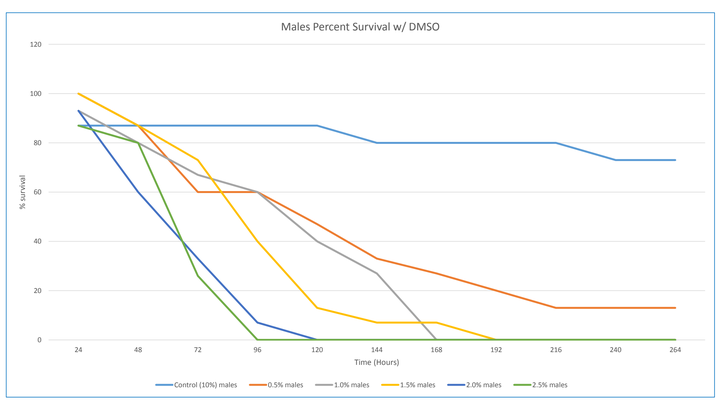
Excretion. Left: Excretion of phenol red after 24 hours of toxic sugar bait feeding assay. The dye was consumed and excreted very quickly possibily due to the fact that it contains one sulfonate group. Right: Excretion of bromocresol purple after 24 hours of toxic sugar bait feeding assay.



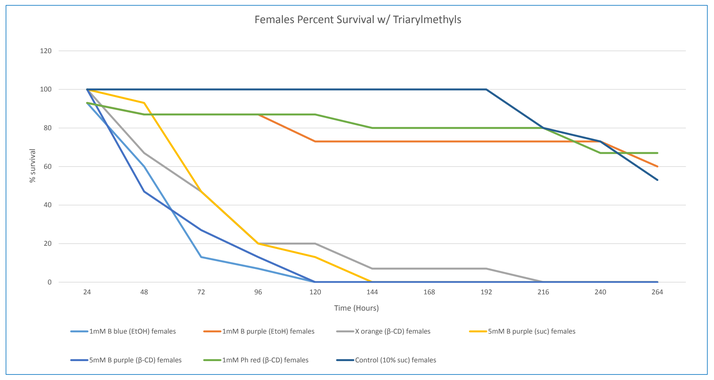


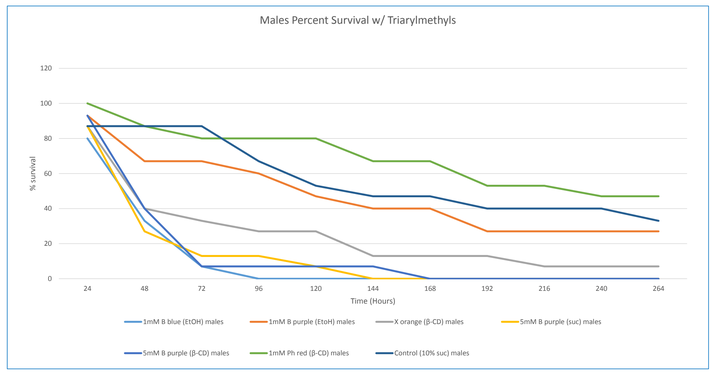
Chemical structure. The slight differences in the chemical structure of the dyes affect the mortality rate of both female and male mosquitoes. Mortality rate changes from one dye to the next in even the same solvents.



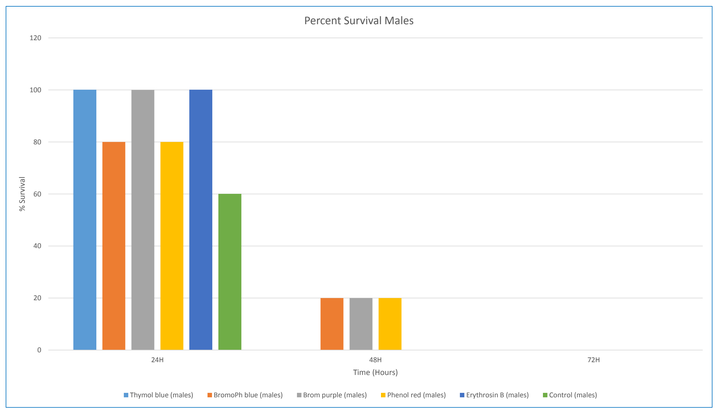


Role of sex. Females and males were tested for a tolerance level using 100% DMSO a toxin in the sugar bait. The solutions were changed daily and the mortality rate was recorded. Overall, the males had a higher mortality rate than the females.





Dyes as adulticides. Dyes were dissolved in a combination of 100% ethanol, 10% sucrose solution, and 20 mM β-cyclodextrin. The mortality rate was recorded every 24 hours. The population of the mosquitoes significantly decreased daily. The increase in mortality rate shows the toxicity of each dye even when in basic/neutral conditions.



Dyes as adulticides. Dyes were dissolved in 100% DMSO. The mortality rate was recorded every 24 hours. The experiment did not last for more than 72 hours.

Conclusions

- Feeding assay is an effective method to evaluate dye compounds toxicity
- Dye compounds with sulfonate groups such as phenol red are excreted quickly
- Changes in chemical structure can affect mortality rate
- Female mosquitoes are more tolerant to dye toxins than males
- Dye compounds can be used as adulticides
- Bromophenol blue is highly toxic when dissolved in the three combination of solvents
- Erythrosin B is highly toxic when dissolved in 100% DMSO

Acknowledgments

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