FINAL REPORT

Outdoor Screen House Tests of Packaging Service Co., Inc. Tiki Fuel Against *Aedes aegypti*

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Submitted to:

Mark Vorderbruggen, Ph.D. | R&D Manager
Packaging Service Co., Inc. | SolvChem, Inc.
1904 Mykawa Rd., Pearland, TX 77581
Direct: 832.300.4072 | Main: 281.485.1458, ext. 333
Mark_Vorderbruggen@packserv.com

Submitted from:

John P. Smith, Ph.D., B.C.E.
Principal Investigator
Medical/Veterinary Entomologist
Public Health Entomology Services (PHES)
8205 Grand Palm Blvd.
Panama City Beach, Florida 32408
FEIN: 65-1301715
(850) 319-9475
docmx8@gmail.com

Assisted by:
Ann Smith, Lab Manager and Research Technician

Abstract

Burning Tiki torch fuel composed of a citronella and cedar oil mixture prepared by Packaging Service Co., Inc. resulted in significantly ($p<0.05$) fewer *Aedes aegypti* bites than non-treated controls in outdoor screen house studies. Repellency was calculated to be 66%, 75%, and 70% at 0.25, 1.25, and 2.25 hrs. post mosquito release, respectively. Overall repellency was 71%.

Study Objective

Determine the repellency of Packaging Service Co., Inc. Tiki torch fuel (citronella and cedar oil mixture) compared to negative control in repelling *Aedes aegypti* mosquitoes.

Materials and Methods

1. This study was conducted at the principal investigator's property located 3 miles south and east of Ebro, FL on Pine Log Creek, a swamp ecosystem dominated by slash pines, laurel oaks, cypress, and sweet bay trees.

2. Three to five-day old female *Aedes aegypti* were lab reared for the study. The mosquitoes were starved approximately 12 hrs. prior to testing to insure avid biting activity.

3. Two 12' W X 24' D X 5.8' Wall & 8' Peak; (290 $ft.^2$; 1,926 $ft.^3$) outdoor screen houses fitted with 20/20 0.013 diameter mesh screen and positioned 60 ft. apart were employed…one for the experimental treatment (i.e., torches fueled with a citronella & cedar oil mixture) and one for the negative control (i.e., no torch). **(Fig. 1)**

Fig. 1. Screen houses used for torch tests. Note location of torches in near house.

4. Torches were arranged in a rectangular pattern inside one of the two screen houses with two positioned at 3 ft. to the immediate left and right of the evaluators and two set 3 ft. directly in front of the first two. The other screen house served as the control and was not supplied with torches. The nearest torches were about 18 to 20 ft from the mosquito release points. **(Fig. 2).**

Fig. 2. Torch location relative to mosquito release from small cage on far side of screen house.

5. Torches were burned for 15 minutes prior to mosquito release and continued to burn throughout the 2-hour+ evaluation period.

6. 100 mosquitoes were stocked into each of two 1 ft^3 holding cages placed at one end of each screen house. The mosquitoes were released by opening the cage tops to allow the mosquitoes to acclimate for 15 minutes prior to taking biting counts.

7. Evaluators conducted 1-minute biting counts at 0.25 hrs., 1.25 hrs., and 2.25 hrs. post mosquito release by inserting a hand through zipper ports installed at the opposite end from where the mosquito were released (**Fig. 3**).

Fig. 3. Biting counts taken by placing hand inside screen house through zipper port.

8. A 2X2 Latin square design was employed whereby both treatments were tested separately in each of the two screen houses.

9. The tests were repeated three times for a total of 6 tests (2 tests/day X 3 days).

10. Mosquitoes were removed using vacuum aspirators and a fresh cohort of 100 was supplied when treatments were switched to the opposing screen house. The screen house containing the experimental treatment was ventilated with a fan for 15 minutes to eliminate any lingering vapors (**Figs. 4 & 5**).

Fig. 4. Mosquito aspiration

Fig. 5. Fan used to exhaust vapors between tests.

11. Ambient environmental conditions (temperature, humidity, wind speed and direction) were recorded throughout the study with an Onset Hobo® data logger and Kestrel 5500AG weather data logger positioned between the two screen houses. **(Fig. 6)**

Fig. 6. Kestrel weather data logger.
Hobo data logger not pictured.

Data Analysis and Reporting:

Mean biting counts were calculated by test, evaluator, screen house location, time post release, and treatment, and the variances were analyzed by Proc ANOVA and Tukey's Studentized Range Test for significant differences at p=0.05 using SAS PC 9.4. The interactive effect of time X treatment was analyzed with Proc GLM and Least Square Means. Normality was checked with the Shapiro-Wilk test using Proc Univariate. Percent repellency was calculated by subtracting treatment means from control means, dividing by the control means, and multiplying by 100. Biting count means were charted with error variance using Microsoft Excel 2019.

Results

Environmental Conditions

Temperature ranged from 73°-98°F and humidity from 97-46% at the beginning and end of the first set of tests completed on September 11, 2019 (**Fig. 7**). The first biting counts were performed between 7:22 A.M. and 9:22 A.M. The second biting counts were performed between 10:44 A.M. and 12:44 P.M. Wind ranged 0-3 mph from the SW to S at 272° to 186°.

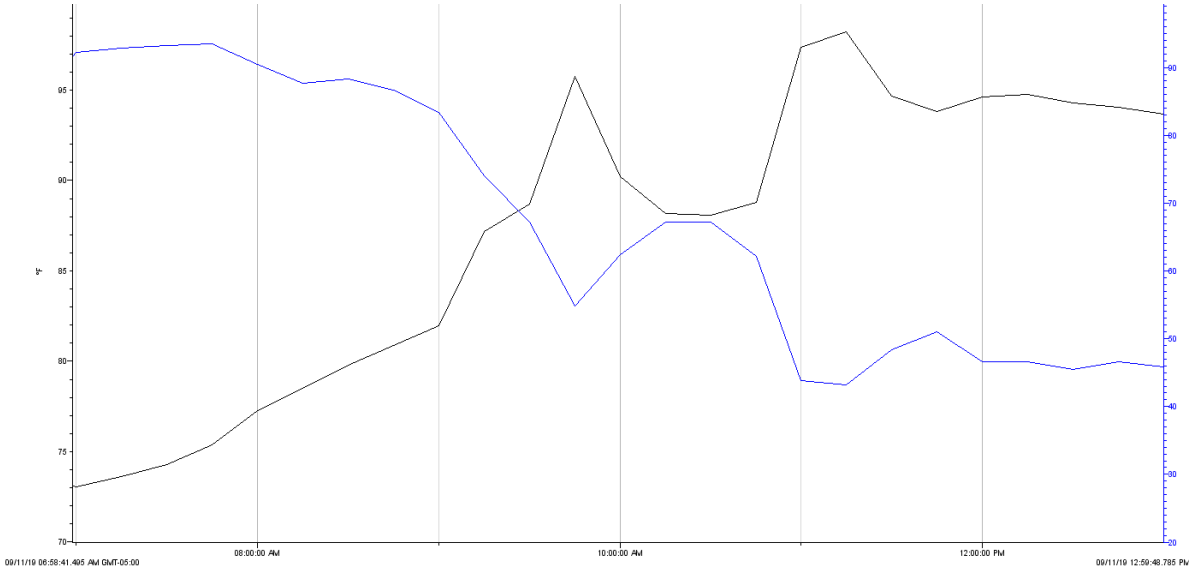

Fig. 7. Temperature and humidity records during September 11, 2019 tests.

Temperature ranged from 73°-93°F and humidity from 97-46% at the beginning and end of the second set of tests completed on September 13, 2019 **(Fig. 8)**. The first biting counts were performed between 7:03 A.M. and 9:03 A.M. There was no wind between 7:03 A.M. and 8:03 A.M. and 0-3 mph wind from S187° at 9:03 A.M. The second biting counts were performed between 10:25 A.M. and 12:25 P.M. The wind increased to 1-6 mph from the NE at 045°-054° at 10:25 A.M. and 12:25 P.M. and NW at 314° at 11:25 A.M.

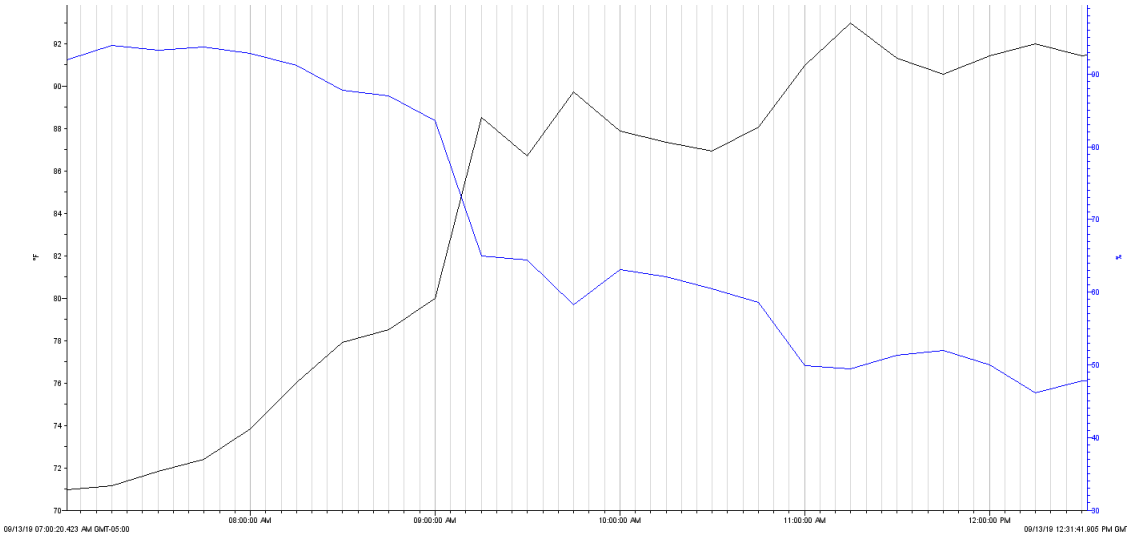

Fig. 8. Temperature and humidity records during September 13, 2019 tests.

Temperature ranged from 75°-92°F and humidity from 95-56% at the beginning and end of the third set of tests completed on September 15, 2019 **(Fig. 9)**. The first biting counts were performed between 7:15 A.M. and 9:15 A.M. The wind ranged between 0-2 mph from the NNW339° at 7:15 A.M., 2-3 mph from the NNW331° at 8:15 A.M., and 1-4 mph from NNW340° at 9:15 A.M. The second biting counts were performed between 10:25 A.M. and 12:25 P.M. The wind increased to 3-7 mph from the NNE050° at 10:30 A.M. and 5-7 mph from ENE075° at 11:30 A.M. and 1-6.5 mph from ENE067° at 12:30 P.M. The wind was constantly shifting from the north and east.

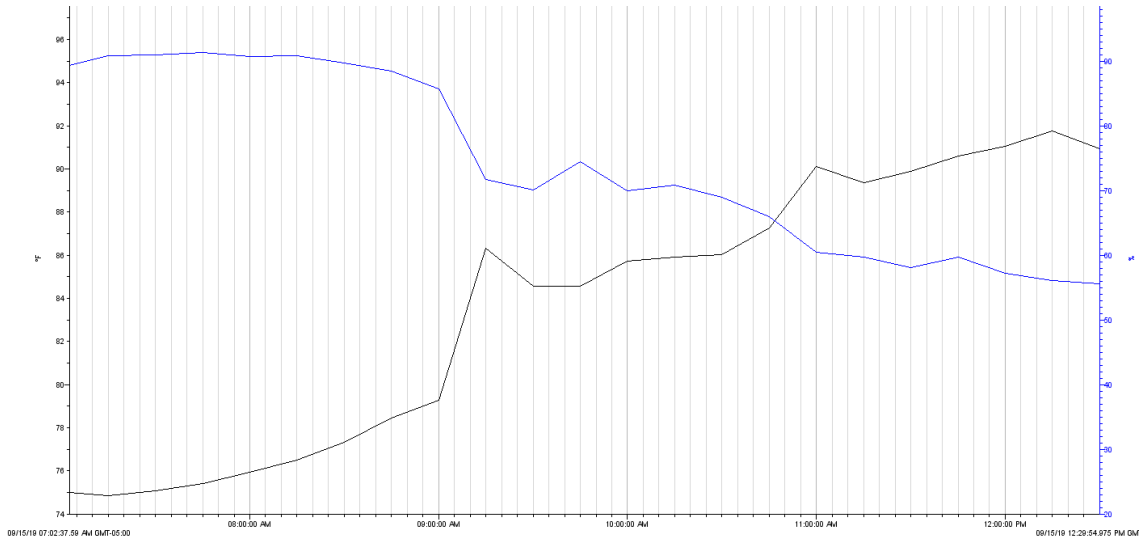

Fig. 9. Temperature and humidity records during September 15, 2019 tests.

Biting Counts

Biting count data fit a normal distribution (Shapiro-Wilks Pr<W =0.34). A total of 267 mosquito bites were recorded between the two evaluators in the torch and negative control treatments. No significant difference ($p>0.05$) was observed in mean biting counts between evaluators ($\mu=7.3$ and 7.5). There were no significant differences ($p>0.05$) in biting counts among tests, screen house locations, and times post mosquito release. However, biting counts were significantly ($p<0.05$) greater in the control than the experimental treatments (i.e., torch) **(Fig. 10).**

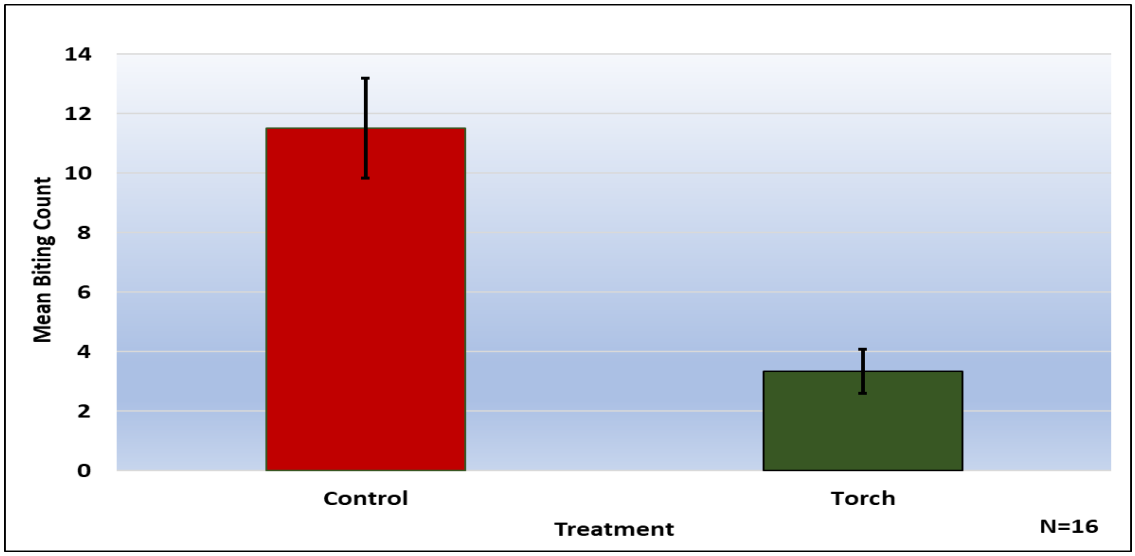

Fig. 10. Mean *Aedes aegypti* biting counts with standard errors for negative control (no torch) and torch treatments.

There were observable differences in biting count means calculated by time post-release; however, the only significant difference ($p=0.02$) was at 1.25 hrs. **(Fig. 11).**

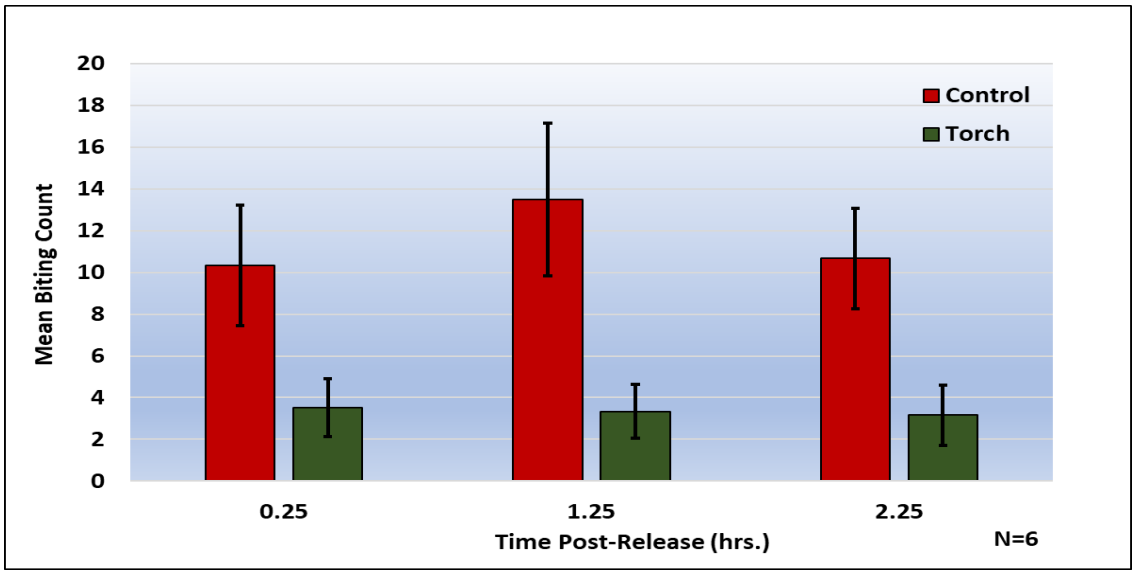

Fig. 11. Mean biting count by time post mosquito release.

Repellency

Overall, the torch provided 71% repellency compared to the control. At 0.25 hrs., 1.25 hrs., and 2.25 hrs. repellency was measured at 66%, 75%, and 70%, respectively.

Conclusions

The results of this study were quite encouraging considering citronella is generally not considered one of the most potent repellents. Addition of the cedar wax oil and positioning the torches in close proximity to the evaluators may have improved efficacy. This study was designed to test tiki torches burning citronella and cedar oil fuel outdoors where the product is labelled for application. Factors such as mosquito species, density, physiological age, and to some degree biting pressure were controlled in these semi-field experiments. Outdoor studies, although more realistic, introduce factors that cannot be controlled. Although adverse weather (i.e., rain and extreme wind events) could and were avoided, micro environmental conditions could only be monitored. The efficacy of spatial repellents will always be dependent on changing wind conditions and other meteorology. The results of this study may not be the same for other mosquito species and thus, additional experimentation may be warranted.