CGCI Environmental Laboratory
Phase I to 2 evaluation Vector Control Research project number #10072019
Botanical FIFRA (25b) Big Shot repel – made with organic ingredients.
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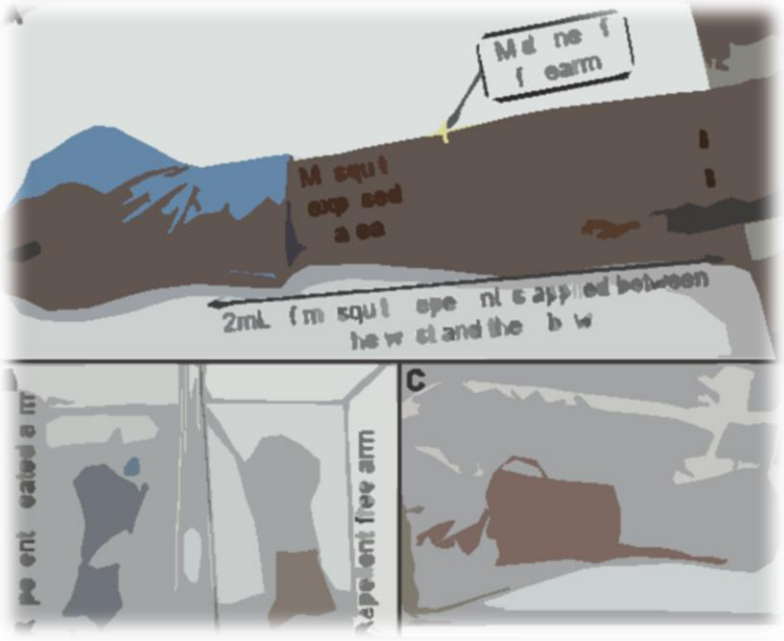
Evaluation of standard field and laboratory methods to Compare protection times of topical repellents

Plant Based Bigshot repellant and DEET. (WHO Modified)



Challenge – testing efficacy of Bigshot Repel a plant based made from organic ingredients (FIFRA 25(b) to DEET

Promising developments in plant-based repellents

The field of plant-based repellents is moving forward as consumers demand means of protection from mosquito bites that are safe, pleasant to use and environmentally sustainable. Perhaps the most important consideration is improving the longevity of those repellents. This study looked at a plant-based repellent of plant oils (Bigshot repel made from organic FIFRA 25(b) GRAS- to determine reliability as accurately as possible to DEET.

Topical repellents are typically tested either in the arm-in-cage (AIC) test under laboratory conditions or in the field, but not often under both conditions. We, therefore, investigated how two topical repellents, 15% BigShot repel and 15% N,N-diethyl-3-methylbenzamide (DEET) compare against each other both in the AIC test against three species recommended by the World Health Organization (i.e. Aedes, Anopheles, and Culex) and at two field sites in north east Georgia, while using the same study participant in all experiments. In the field, the median complete protection time (CPT) was at least 6 hours for both Bigshot and DEET, while in the AIC test DEET very only slightly performed better. CPTs for DEET in the AIC test were 0.5, 2 and 2 hours against Aedes, Anopheles and Culux, respectively, and the corresponding median CPTs for PMD were 0.5, 1 and 0.5 hours. In conclusion, DEET slightly outperformed PMD in the AIC test, while the observed landing rates suggest the AIC test to underestimate efficacy of topical repellents in areas with lower landing pressure.

INTRODUCTION

Biting mosquitoes are important vectors of several diseases. For most of the mosquito-borne diseases neither vaccines nor specific treatments exist and, therefore, travelers to disease endemic countries are advised to avoid mosquito bites, primarily by wearing appropriate clothing and by applying topical repellents on the bare skin. Topical repellents may provide good protection against mosquito bites over several hours depending on the active ingredient and its concentration.

The most widely used active ingredient in commercially available mosquito repellents is the synthetic compound *N*,*N*-diethyl-3-methylbenzamide (DEET) which is generally regarded as the "standard" due to its high efficacy against a broad range of insects. Although DEET is deemed nontoxic if used correctly, concerns have been raised about its safety. Moreover, DEET has plasticizing properties, a strong smell and may even cause discomfort, particularly when applied at higher dosages. Users may, therefore, wish to buy repellents containing alternative actives. An alternative compound which has been shown to be effective against a range of mosquito species is *plant-based repellants. Bigshot Repel is a made from organic formulated mosquito repellent.*

Several studies have compared the efficacy of plant based repellents against DEET, yet the existing data are somewhat difficult to read because the formulations tested containing questionable additional compounds that potentially also influence the efficacy of the repellent, or the concentrations of the plant based repellent and DEET formulations applied within the same study differed in percentages of actives present, or both.

The efficacy of topical mosquito repellents is usually tested following various national or international guidelines. Under laboratory conditions, the efficacy of mosquito repellents is typically evaluated in the so-called "arm-in-cage" (AIC) test. In the AIC test, according to the guidelines, a forearm of a study participant is treated with a defined amount of the repellent formulation (e.g. 1 ml per 600 cm^2). Then the participant exposes the treated forearm at regular intervals (e.g. every half hour) to a number (e.g. 200) of host-seeking female mosquitoes for a defined exposure period (e.g. 3 minutes) in a cubic cage (e.g. 64,000 cm^3). The endpoint is usually the complete protection time (CPT) or the relative protection *(%p)*. CPT corresponds to the time from the application of the formulation until its failure, while the relative protection is the percentage protection provided as compared to an untreated forearm. In the field, similar endpoints may be determined on the basis of mosquitoes landing or biting on an exposed skin area, usually the lower leg, while the mosquitoes are collected by aspiration allowing for the identification of the mosquito species in a laboratory.

While the guidelines recommend conducting efficacy studies both under laboratory and field conditions, mosquito repellents are hardly being compared side-by-side in both settings. This raises the question as to how protection efficacy measured under laboratory conditions compares to the efficacy under real conditions of use. In order to shed more light on the relationship between the protection provided by a topical repellent against mosquito bites in laboratory and field settings, and to evaluate the efficacy of the active ingredient in Bigshot repel against DEET under equal conditions, CGCI Environmental laboratory conducted an experimental study that compares the protection times of 15% actives and DEET by using similar parameters both in the field and in the laboratory.

STUDY DESIGN

This is a comparative study of laboratory and field methods to determine the efficacy of topical mosquito repellents. Two solutions, one of 15% DEET and 15% Bigshot Repel FIFRA 25(b) Natural mosquito repellant tested on a human subject using the repellents in the testing process. The same volunteer tested the same formulations both under laboratory and field conditions on different days. In order to generate comparable data, the parameters in the field and in the laboratory, experiments were kept as identical as possible, while having consideration of the World Health Organization (WHO) and the United States Environmental Protection Agency (US EPA) guidelines. The primary outcome was the complete protection time (CPT) and relative protection (*%p*) as compared over 6 hours post application of the repellent for information considerations.

STUDY PARTICIPANT

In this study as a preliminary test, the lab director showing low or no skin reactions against mosquito bites volunteered as the test subject. The age of the study participant 59 years. the study participant signed the informed consent form. To avoid unwanted bias the participant avoided alcohol and products such as cologne and lotions for at least 12 hours before and during the experiments. During the experiments the participant avoided rubbing, touching or wetting the repellent-treated area as well as any activity that might lead to increased perspiration.

TEST FORMULATIONS

The test formulations consisted of ethanolic solutions of 15% DEET (leg applied) (m/m) and 15% Bigshot repel (leg applied). DEET was chosen because it represents a standard and Bigshot (Plant Based Repellant as a FIFRA 25(b)) was chosen as these active ingredients for the test to determine its compared efficacy to DEET as a plant-based mosquito repellant.

FIELD EXPERIMENTS USING THE HUMAN LANDING CATCH METHOD

In the field, the repellents were evaluated at two locations in Georgia that differ in their ecology; a low-lying water wooded area in the town of Flowery Branch Georgia, and a drainage area in a semi industrial wooded warehouse area in oakwood Georgia. Both areas usually display a relatively high abundance of mosquitoes, these areas exhibited mosquito species diversity. During the study, both areas were neither treated with insecticides nor were they subject to any other vector control measure.

The efficacy of the repellent formulations was assessed in the Oakwood area between 20 July and 12 August 2019 and then in Flowery Branch between 17 and 30 August 2019. Using the human landing catch (HLC) method, observations were made hourly for 30 minutes over 6 hours, starting 1-hour post application of the formulations at 3:00 pm 8:40 respectively.

In the experiment, the study participant tested the 2 formulations, RTU (ready to use) BigShot Plant Based Repellant and 15% DEET. To avoid carryover effects of residual repellents the arms were treated on subsequent days.

In preparation of the field experiments, the test surface (i.e. the bare lower leg) was washed with neutral soap, rinsed, dried. One of the lower legs was then treated with either one of the two repellent formulations at a rate of 1 ml per 90 square inches. The application volume was estimated on the basis of the surface area, calculated as the average of the circumferences just below the knee, the calf and the ankle, multiplied by the length of the lower leg, measured from below the knee to the ankle.

Sixty minutes after application of the treatment, the study participant situated to one position location. With the exception of the treated lower leg the whole body was fully protected from mosquito bites by a tyvek suit, a bee keeper's hat and latex gloves through which mosquitoes could not bite. During an exposure period of 30 minutes the study participant sat on a stool and collected any mosquito alighting on the exposed lower leg using a mouth aspirator (Fig. 1). Collected insects were transferred to 250 ml jar with netted cover mesh. When natural light conditions were insufficient to carry out collections the study participants used a head lamp (LED).

Figure 1.

The human landing catch (HLC) method for measuring the efficacy of topical repellents under field conditions. Mosquitoes lighting on the treated and exposed lower leg were aspirated by a mouth aspirator and kept in collection jar for species identification in the laboratory.

At the end of the 30 minutes exposure period following the exposure, the study participant moved from the position for 30 minutes before moving to the next position, repeating the process above until 6 exposures over 6 hours was completed. As with the treatment allocation, the sequence of rotation between the positions followed by randomly selected areas at the beginning of each session. In addition to the mosquito collections by the study participant, mosquito traps were set in the test location areas to measure overall mosquito pressure. The traps were set during the same 6 hours the study participant tested the repellent. The traps were set at least 50 feet away in order to avoid unwanted attraction of mosquitoes away from, or to the study participant.

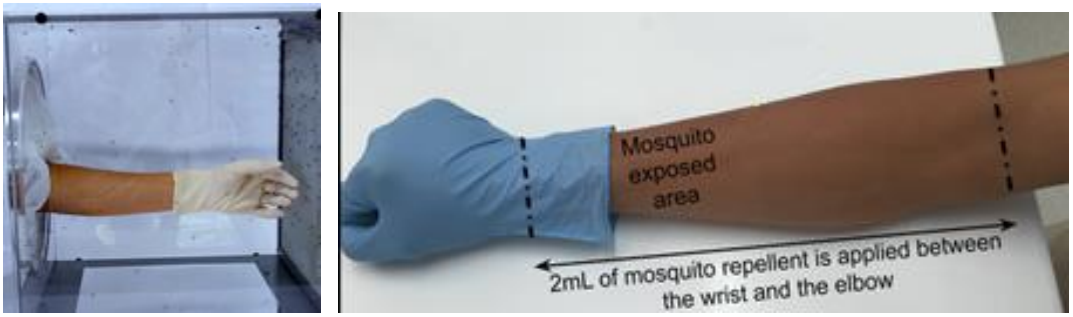
The trapping was accomplished with one (CDC) Miniature Light Trap equipped with dry ice but with the light bulb removed. All collected mosquitoes were identified with the aid of a stereo microscope using the *identification keys of Mosquito Genera* (Army PHC) If it was not possible to identify a mosquito specimen on the basis of morphological characteristics, for example when it was damaged or a member of a species complex, the specimen was processed and sent to university for molecular identification.

During the experiments, additional physical parameters were recorded, including wind speed, temperature and weather conditions. Experiments were only carried out as long as the weather was dry. Average wind speeds recorded in both field trials were 3-5 mph. and the mean temperature was 80^0F to 91^0F.

LABORATORY EXPERIMENTS USING THE ARM-IN-CAGE TEST (AIC)

The laboratory experiments were conducted at CGCI environmental lab in Oakwood Georgia between 9-1 and 10-2 2019, following the WHO guidelines for the arm-in-cage (AIC) test. In the AIC test, the protection time of a repellent is assessed by exposing a treated fore-arm to hungry mosquitoes at regular intervals (grown from larvae and not fed for 12 hours before the test). The cages measured 16" × 16" × 14" and were made of clear glass with an opening on the front side. At the bottom of the cage was a mirror positioned allowing for observation of mosquitoes landing on the lower side of the arm. The back side of the cage was made of a fine metal grid to ensure air supply during the experiments (Fig. 2).

Figure 2.



The arm-in-cage (AIC) test for measuring the efficacy of topical mosquito repellents under laboratory conditions. Hungry female mosquitoes are contained in a test cage and the repellent is applied to the forearm between the wrist and elbow, while the hand is protected by a latex glove through which the mosquitoes cannot bite.

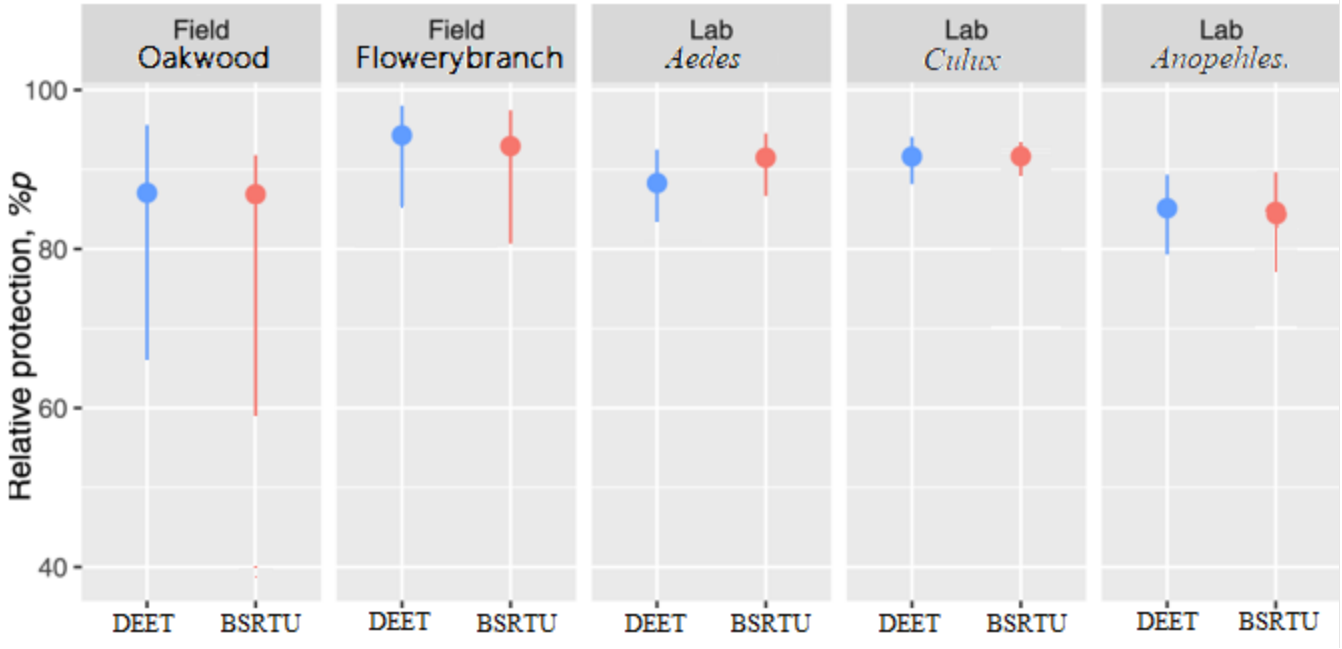
The test cages contained 200 to 220 host seeking 5 to 10-day-old females of one of the 3 main mosquito species; *Aedes, Culex* and Anopheles. The three mosquito species are among WHO recommended model organisms. Adult mosquitoes were fed with 10% sucrose solution and water *as needed*. Testing and rearing conditions for all mosquito colonies were 80°F ± 1.2and 64.8% ± 2.% relative humidity) and a 12:12 (light/dark) photoperiod. Male and female mosquitoes were kept in the same rearing cages to allow mating to occur.

Aedes experiments were performed under artificial light at an intensity of 630 Lux, while tests with *Anopehles.* and *Culux* were conducted under subdued light at an intensity of 50 Lux to mimic the conditions according to the mosquito species diurnal biting patterns. Twelve hours before the experiment, the sugar water was removed from the cage and the mosquitoes had only access to water.

Before exposure to the mosquitoes the forearm was washed with odorless soap, dried with a towel, swabbed with an 70% isopropanol wipe and then dried again. Then, to assess the readiness of the mosquitoes to land, the forearm of a study participant was exposed in the experimental cage for 60 seconds or until 10-15 landings were counted. A landing was defined as a mosquito lighting on the skin and remaining for at least 2 seconds. After measuring the landing activity with the untreated forearm, the forearm was treated from wrist to elbow with either Bigshot rtu or 15% DEET at an application rate of 1 ml per 600 cm^2. In order to estimate the application volume, the surface area of the forearm was calculated as the average circumference of the elbow, wrist and middle of the forearm multiplied by the distance between the wrist and the elbow. The volunteer tested only one repellent per day. Thirty minutes after application of the repellent the participant exposed the treated forearm in the test cage for 3 minutes or until 10 mosquitoes landed. The procedure was then repeated every 30 minutes over 6 hours. The duration until the first, second and tenth landing of a mosquito on the treated forearm was noted. During the exposure time the mosquitoes were shake off before they started biting, preventing an excessive number of bites. At the end of the experiment the arm was again washed and dried as before, and a second control measurement of the mosquitoes' landing activity was taken.

DATA ANALYSIS

Raw data recorded on paper forms. Each entry was double-checked and the records were inspected for outliers and inconsistencies. The endpoint measured in the experiments was the number of mosquitoes landing on the bare skin during each exposure period. Based on the number of landings and exposure times two outcome measures were estimated following the WHO guidelines: the complete protection time (CPT) and the percentage protection *(%p)* over time. Here, the percentage protection *(%p)* over time is defined as the time elapsed between the application of the repellent and the first mosquito landing. Average CPTs (median and 95% confidence interval; For the field data, landing rates were shown as a function of treatment, time post application and location. Landing rates in the AIC test were demonstrated as a function of treatment, time post application and mosquitos. In both models an offset term with the log of the exposure time was introduced to capture the differences in exposure times between tests. For example, the study participant was allowed to conclude an exposure after 10 landings in the AIC test, leading to different exposure times. As for the landing rates average *%p* over the 6 hours test period was estimated using the total number of landings per person per second and then compared between Bigshot rtu and 15% DEET. The same approach was also used to compare the control landing rates in the AIC test before and after the experiments.



DISCUSSION AND RESULTS

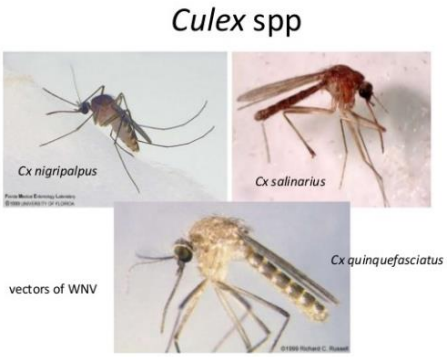
The results from the comparative study between the AIC test and the HLC method in the field revealed comparable efficacy for 15% PMD and 15% DEET. The median CPTs in the AIC tests ranged between 0.5 and 2 hours for BigShot rtu and between 0.5 and 2 hours for 15% DEET, while the CPTs measured in the field were at least 6 hours for both BigShot rtu and 15% DEET. In contrast to the CPTs, *%p* did not vary greatly between the laboratory and field experiments. The repellent efficacy is estimated by CPT or *%p* the relative outcome between BigShot rtu and 15% DEET remains the same.

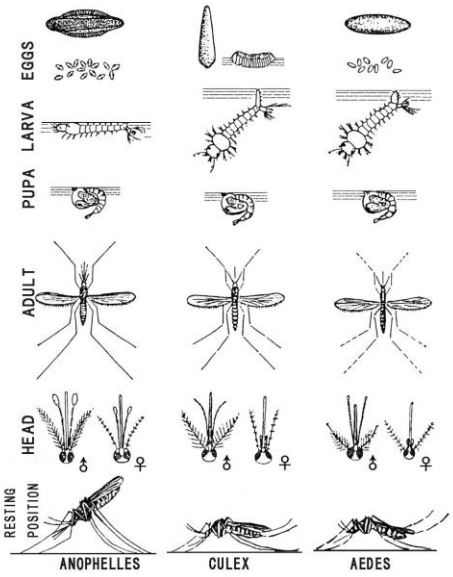
Many previous studies are present in meta-analysis and have compared the duration of protection provided by Plant based repellents compared to DEET in biting mosquitoes using human subjects, both in the laboratory and in the field, and found that Plant based repellents shows comparable efficacy to DEET. A caveat of those studies is, however, that the formulations were all commercially available products, also containing additional compounds that may influence the efficacy outcome in one or the other way. In the research studies the amount contained in the formulations differed between the plant based and DEET products, making a side-by-side comparison difficult. This study took these weaknesses of the earlier studies into consideration and compared the Bigshot Maxim and DEET formulations that differed only in the active ingredient itself. That is both formulations evaluated consisted of solutions at the same concentration of either Plant based or DEET; hence the present study provides important information to the debate of alternative repellents to DEET and confirms that Bigshot repellant provides a high level of protection that is comparable to DEET.

RESULTS

Bigshot repel Plant based repellant and DEET show similar efficacy over 6 hours against outdoor biting mosquitoes in northeast Georgia, irrespective of the ecological setting. Both CPT and *%p* over time provide a similar picture with reference to comparative protection efficacy between Bigshot and DEET.

CDC Light Trap





Mil-ID-Key

Mosquito Breeder Jars

Aspirator

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