Effectiveness of DEET & Non-DEET Repellents on Culex quinquefasciatus

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ABSTRACT
In this experiment the effectiveness of various mosquito repellents were tested. Mosquitoes are known to be carriers of numerous diseases, making repellents an important means of protection. Because of with the growing “anti-GMO” and “organic” trends this experiment tested the most common chemical repellent as well as “natural” repellents. The tested species of mosquito were in the family Culicidae (Culex quinquefasciatus). The C. quinquefasciatus used were reared in the lab to be test subjects for this feeding experiment. Out of the three repellents, only the one with diethyltoluamide, or DEET, appeared to have any noticeable effect on the feeding of mosquitoes. Non-DEET repellent appeared to have a significantly less effect on the feeding habits of C. quinquefasciatus.

Keywords: Diethyltoluamide, Culex quinquefasciatus, Feeding, Repellent.

Introduction:
Vector-borne transmitted diseases are currently a major source of many deaths and illnesses worldwide. Typically, mosquitoes transmit numerous types of diseases to more than 700 million individuals yearly. The tropical and subtropical areas seem to poise a bigger health problem because mosquitoes flourish in those certain climates. Even though it is more prevalent in areas closer to the equator, no area in the world is immune to the risks of mosquitoes. Many reports over the past years show that transmitted diseases by mosquitoes are continually increasing and the death rates are also increasing. Mosquitoes can spread very fatal diseases such as Malaria, West Nile Virus, and encephalitis.

It is very crucial to keep in mind, especially when traveling to different countries or being outdoors in areas mosquitoes like to inhabit to watch out for them, as they could potentially be carrying diseases which could be transmitted to humans. Protection from mosquito bites is best attainable by avoiding certain infested habitat, having on protect clothing, and also the use of insect repellent. In a practical and most effective situation, applying mosquito repellent to different areas of the skin may be the only reasonable way to protect individuals against mosquito bites. A single bite from an infected mosquito could potentially result in the transmission of a disease, so it is very important to know which type of repellent can be sufficient and dependable on to provide the best prolonged protection from mosquito bites.

There are two main types of insect repellent that is made readily for the public consumers; synthetic chemicals and plant based essential oils. The best known chemical in most insect repellers is N,N-diethyl-m-toluamide which is now, N,N-diethyl-3-methylbenzamide or DEET.

![Figure 1. DEET Structure](image1.png)

The main objective of this experiment is to discover which type of mosquito repellent will work best to avoid mosquito bites. Three different types of mosquito repellents were used and applied separately to a mosquito blood
feeding device which included 8 mL of rabbit’s blood. The three different types of mosquito repellent that will be used is Coleman DEET Free Repellent, Cutter 25% DEET, and Citronella Oil. One trial will consist of no repellent, this will be considered the control. The mosquitoes that were used in this experiment were the species of Culex quinquefasciatus. There were about 100 of the mosquitoes and they were divided into four groups each containing about 25 mosquitoes. The hypothesis of the outcome was developed and discussed that Cutter 25% DEET will be most effective of the experiment and will have prolonged effectiveness from mosquito bites.

Figure 2. Larval stage C. quinquefasciatus

Figure 3. Adult stage C. quinquefasciatus

Materials:

1. One Mosquito Blood Feeding device
2. 4 square sheets of parafilm
3. 8 mL of rabbit blood
4. 3 different types of repellent
5. Approximately 100 Culex quinquefasciatus mosquitoes
6. Water heater

Methods of Experimentation:

1. Turn on water heater and heat to 37.5 degrees Celsius in order to warm blood.
2. Once the blood is the desired temperature, a piece of parafilm was applied to the feeding device. The parafilm will serve as the membrane (skin) that the mosquitoes will attempt to feed on for a blood meal.
3. Water tubes were then attached to the feeding device and a machine that will pump warm blood into the feeding device was initiated. (Be sure that tubes are secure and unable to slip)
4. Once the machine was functioning properly, a cage was placed beneath the device, exposing the membrane (parafilm).
5. Next, 2 mL of blood was injected into the device and the mosquitoes were allowed to feed for 10 minutes, counting the amount of mosquitoes feeding at 1 minute, 4 minutes, 7 minutes, and 10 minutes.
6. After the trial was completed, the device was thoroughly cleaned to ensure that no contaminants would tamper with the following trials with other repellants.
7. The procedure was repeated for each type of repellent. (total of four trials)
8. Once all the data was collected, the materials were cleaned and disposed or put away properly

Figure 4. Mosquito Experimental Instruments

Experimental Design:
This experiment was designed to be as fair and accurate as possible. All 100 mosquitoes were the same species, Culex quinquefasciatus, and originated from the same lab source to ensure blood feeding habits were consistent throughout. One caveat presented was the sexes of the
mosquitoes in their group, and even though each cage was prepared with the same number of mosquitoes per cage, there is no efficient or reliable way to have an even number of male and female mosquitoes. Since only females blood feed, the data needs to take into account this notion. Regardless, a general trend can be analyzed and used to accurately depict the effectiveness of the mosquito repellants being tested.

The parafilm used to represent the membrane-skin stayed consistent in thickness throughout as well. Too thick of a film could defer female mosquitoes from feeding, while too thin of a film would allow a much easier blood meal. Additionally, all 100 mosquitoes were intentionally not fed the day before so that each individual would be readily hungry prior to testing. The mosquitoes were killed via freezer after all data was collected for further analysis.

After each cage’s mosquitoes fed with the assigned mosquito repellent, the feeding device was bleached and sanitized to ensure none of the other repellants being tested affected another group. This was emphasized to allow for an accurate understanding on the feeding habits of the mosquitoes when in contact with a specific repellant. Each cage was allowed to feed for 10 minutes, with measurements being recorded at 3 minute intervals following the first minute. The number of mosquitoes feeding at the time intervals were recorded to show how feeding habits changed over time.

Results/Graphs:

Table 1: Group A was identified as the control group and was not treated with repellent.

Table 2: Group B was treated with Coleman DEET-free repellent, producing noticeable results.

Table 3: Group C was treated with Cutter 25% DEET repellent and resulted in the most effective repellent tested.

Table 4: Group D was treated with Citronella Oil repellent and the most effective repellent tested, produced noticeable results.
Table 5: By comparing Groups A, B, C, and D, the repellent that produced the best results in proving most effective against the mosquito species, *Culex quinquefasciatus*, was concluded to be Cutter 25% DEET repellent.

**Discussion:**

In the experiment the mosquitoes were separated into four groups: Group A was the control group, Group B was tested with Coleman DEET-free repellent, Group C was tested with Cutter 25% DEET repellent, and Group D was tested with Citronella Oil repellent. Each group was tested over a 10 minute span that was divided into intervals of three beginning after one minute had passed. After a minute passed Group A had nine mosquitoes feeding on the blood pack. At the four minute mark there were twelve mosquitoes, at the seven minute mark there were fifteen. Finally after ten minutes the number of mosquitoes feeding on the blood pack peaked at sixteen. Group B had two mosquitoes feeding on the blood pack after one minute had passed. Once four minutes had gone by there were four mosquitoes feeding on the pack of blood, the number of mosquitoes feeding on the pack peaked at seven when the seven minute mark was reached. After ten minutes the mosquitoes feeding on the blood pack dropped down to three. Group C began with no mosquitoes feeding on the blood pack until around the four minute mark when two mosquitoes began to feed. However, this number dropped by the seven minute mark where only one mosquito was found feeding on the blood pack and again at the ten minute mark where only one was seen feeding. Group D had only one mosquito after the initial minute, but the amount gradually increased as the time passed. At four minutes, there were three mosquitoes, which then increased to five mosquitoes at minute seven. At ten minutes, the amount peaked at six mosquitoes before the time duration was ceased. From this experiment, it can be assumed that, DEET (N,N-diethyl-3-methylbenzamide), was the most efficient ingredient in combating mosquito feeding. The other repellents did not contain DEET, and thus this conclusion can be made. It should be noted, however, that DEET is toxic to both animals and the environment at high concentrations and should be used with caution. Although our results prove that DEET repellent is undoubtedly the best choice for mosquito protection, the negative effects should be taken into consideration when applied to global health issues related to mosquito-borne diseases. This dilemma is troubling, and the search continues for an active ingredient with similar qualities as DEET, but safe for the environment at any concentrations.

**Conclusion:** Based off the results, Cutter 25% DEET repellent was observed to be the most successful in deterring *Culex quinquefasciatus* from feeding on the experimental tissue, followed by Citronella Oil, and then Coleman DEET-free repellent. The control group’s results were expected, yielding significantly higher mosquito amounts than the tested repellents.

"The Effectiveness of Permethrin and Deet, Alone or in Combination, for Protection against Aedes Taeniorhynchus." *The Effectiveness of Permethrin and Deet, Alone or in Combination, for Protection against Aedes...* N.p., n.d. Web. 29 Apr. 2016.


