Efficacy of Larvicidal Liquid Additive Against Mosquito Larvae (Diptera: Culicidae)

Alison Duke, Jeremy Feland, Jason Nguyen, Carlo Rodriguez, Matthew Snow, and Madeline Strickland

*Texas A&M University, Department of Entomology*

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**Abstract:** During the dry fall and winter months, mosquito prevalence tends to falter as the climate is cooler and water is sparse. However, once the rainy season begins and temperatures warm (March to September), mosquitoes become active and the risks associated with disease transmission heighten. Along with environmental factors, an increase in mosquito population is also influenced by urbanization and application of DDT. In efforts to control mosquito-borne diseases, such as West Nile Virus and yellow fever, we conducted a vector management experiment. For female oviposition to occur, a source of water, whether that be standing water or flood water, must be present. As eggs were laid on or near the surface of water and hatched into larvae, homemade mosquito dunks were utilized on three species: *Culex quinquefasciatus*, *Aedes* *aegypti*, and *Aedes albopictus*. This study aimed to determine whether common household chemicals would be effective in killing mosquito larvae. Our study challenges the stigma that Integrated Pest Management (IPM) and costly insecticides must be implemented in order to effectively combat the vectors for mosquito-borne diseases.

*Keywords: Mosquito larvae, Texas, prevention, cost-effective*

There are many factors that contribute to the rise and fall in mosquito-borne diseases in Texas. The variability of climate change is one of these major factors and has influence over the weather and vegetation. Specifically, vegetation is affected by temperature and precipitation patterns (Poh et al. 2019). More factors include uncontrolled mosquito populations and air travel due to the increase of urban areas. These aspects can be difficult to foresee, and thus, it can be tough to prevent the vector-borne diseases associated with mosquitoes.

Texas is home to numerous mosquito species, such as *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Mosquitoes of the Culex genus are standing water breeders. They lay their eggs directly into a non-turbulent body of water. As they develop, the *C. quinquefasciatus* adults are most commonly known to be the primary vector of West Nile Virus (WNV). Humans and horses are accidental hosts of this disease, while birds act as the amplifying host. In recent years, the incidence of WNV epidemics increased. For example, Texas has contributed to 12% of cases and 14% of deaths with WNV. Increased populations of *C. quinquefasciatus* can be due to favorable temperatures, high vegetation, and rainfall (Poh et al. 2019).

*Aedes aegypti* (L.) (Diptera: Culicidae) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) are also well-known species that transmit vector-borne mosquito diseases in Texas. *Aedes* mosquitoes are flood water breeders; they lay their eggs near a surface of water that is bound to overflow. As these eggs hatch and develop into adults, they are capable of transmitting the yellow fever virus, dengue fever, Mayaro virus, Oropouche fever, and Zika (Srinivasan et. al 2017).

Although there is an effective vaccine for yellow fever, it is costly, furthering the need for preventing the transmission of the disease (Barrows et. al 2018).

Dengue involves three distinctive phases that include the febrile phase, critical phase, and recovery phase. The critical phase is distinguished by systemic vascular leakage, which can lead to shock and vascular collapse if left untreated (Weaver et. al 2017). In 2013, there was a reemergence of dengue in southern Texan areas that resulted in a high hospitalization rate (Thomas et. al 2016.)

Mayaro fever causes abrupt symptoms, such as fever, arthralgia, and a rash. Although Mayaro virus is most common in rural areas, it may be able to appear in urban areas because of its ability to expand the range of its host and vector. Therefore, it has become a severe threat to North America. The abundance of *Aedes spp.* in Texas results in an increased risk of transmission (Izurieta et. al 2017).

Oropouche fever is caused by a bunyavirus that is characterized by two phases. Although fatality is rare and complete recovery is highly likely, recurrence can occur (Weaver et. al 2017). Like Mayaro fever, it has the potential to spread to North America. This expansion depends on favorable climate conditions. *Aedes spp.* have been known to vector the disease (Sakkas et. al 2018).

Zika virus is caused by a flavivirus that is primarily influenced by an urban transmission cycle. Contraction of Zika virus in pregnancy can result in birth defects in the fetus, such as microcephaly. Furthermore, it has been associated with an increased risk for Guillain-Barré syndrome (GBS). (Weaver et. al 2017).

In recent years, there has been a rise in mosquito-borne diseases. This increase has been linked to the growth in the mosquito population in Texas. Over the last 8 decades, the mosquito population has been primarily influenced by anthropogenic chemical use, such as DDT, and an increase in urbanization. As previously mentioned, the climate change has resulted in warmer temperatures, leading to expansion of mosquito populations. The subsequent population produced has an enormous potential for transmitting vector-borne disease (Rochlin et. al 2016).

One option for controlling mosquitoes is implementing dunks. These dunks utilize *Bacillus thuringiensis* subspecies *israelensis* bacteria, suspended in a dried block, to eradicate the larvae and eggs without posing a danger to humans (Derua et al. 2019). They only last a few months before the bacteria are ineffective, so they must be purchased often (Fansiri et al. 2006). Having to continually purchase these bacterial larvicide dunks can put an economic strain on areas implementing this method, especially rural communities (Derua et al. 2019). While in some settings bacterial larvicides are cost-effective, in other settings, they are not. Current mosquito control methods are not feasible for communities that need them, so there is a high demand to find new, more affordable strategies.

Integrated Pest Management (IPM) is an approach implemented to limit pest populations from reaching economic injury levels in a given area. Application of IPM practices brings many advantages: reduced impact on humans and the environment and reduction in cost. However, IPM has not yet gained traction in some areas, and the current dilemma facing the adoption of IPM in these areas is behavioral avoidance (Alwang et al. 2019). Therefore, a more simplified approach is desired because of IPM’s complexity.

One way to combat cost restrictions and familiarity is by implementing a simplified chemical control method to prevent larval growth using familiar household products (Cheng 2003). Regardless if it is a standing water breeder or flood water breeder, water is imperative to the beginning of a mosquitoes life cycle. The addition of bleach, dish soap, vinegar, plant-based essential oils, and canola oil to larvae infested water was investigated to measure the effectiveness of preventing larvae growth. Some chemicals are easy to obtain, such as soaps, but these items also contaminate water sources making them uninhabitable for aquatic ecosystems. Organic oils or vinegar may be a more accessible, more cost-effective, and much safer choice. An effective product would potentially provide convenient usage in rural areas.

**Materials and Methods**

**Mosquito Collection Procedures**

Mosquito eggs were collected using a mosquito dipper at ponds located on Texas A&M University’s campus in College Station, Texas. Eggs were placed in mosquito breeders (Bioquip) labeled A, B, C, D, E, and F. Eggs were left alone until they hatched into larvae between the dates of April 10 and April 17. Each container held 20 larvae.

**Application of Test Materials**

Each of the six larvae pools were treated with one ounce of the following household chemicals: Clorox Bleach (Procter & Gamble Company, Oakland, CA) in container A, Dawn Ultra Dishwashing Liquid Dish Soap (Procter & Gamble Company, Oakland, CA) in container B, Heinz Distilled White Vinegar (H. J. Heinz Company, Pittsburg, PA) in container C, citronella essential oil (NOW Health Group, Bloomingdale, IL) in container D, and canola oil (H-E-B, San Antonio, TX) in container E. The sixth container, container F, was used as the control. The time it took for larvae to be eradicated by the chemicals and if applicable, the number of remaining, live larvae were noted.

Using the information gathered, the most cost-effective treatment was determined based on spreadsheets.

 **Results**

Clorox bleach proved to be the most effective product in regards to time. The first larval death occurred in 30 minutes. Dish soap was also effective, but the yield for time taken for first larval death was double that of Clorox Bleach (Table 1; Fig. 1). Both of these materials were successful in complete eradication of larvae present.

Vinegar, on the other hand, took 12 hours to kill its first larva, and by the end of the test, was unable to eliminate all larvae. Vinegar is the most cost effective, but it has low efficacy (Table 1; Fig. 1).

The two oil products killed the larvae in 60 minutes each (Table 1; Fig. 1). Citronella oil was the most expensive test materials used while canola oil was one of the cheapest.

A total of 120 mosquitoes were collected from three mosquito species: *Culex quinquefasciatus*, *Aedes* *aegypti*, and *Aedes albopictus*. Each container had a different number of species (Table 2). Application of the test materials proved effective for these species.

Table 1. Number of larvae death after application of the test materials and their respective costs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **A: Clorox Bleach** | **B: Dawn Dish Soap** | **C: Vinegar** | **D: Citronella Oil** | **E: Canola Oil** | **F: Control** |
| **Initial Number of Larvae** | 20 | 20 | 20 | 20 | 20 | 20 |
| **Time for First Larva to Die (min)** | 30 | 60 | 720 | 60 | 60 | N/A |
| **Number remaining alive** | 0 | 0 | 10 | 0 | 0 | 20 |
| **Cost per ounce** | $0.10 | $0.13 | $0.05 | $6.16 | $0.06 | N/A |



 **Fig. 1.** Time for first larval death after application of test materials.

 Table 2. Number of respective species in each test material.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Clorox Bleach** | **Dawn Dish Soap** | **Vinegar** | **Citronella Oil** | **Canola Oil** | **Control** |
| *Culex quinquefasciatus* | 6 | 8 | 10 | 7 | 6 | 6 |
| *Aedes* *aegypti* | 7 | 6 | 5 | 7 | 6 | 7 |
| *Aedes albopictus* | 7 | 6 | 5 | 6 | 8 | 7 |

**Discussion**

Although Clorox bleach is poisonous to the larvae, it can also have a detrimental effect on the environment. Clorox bleach can be used in small amounts and still leave a drinkable water source, but in larger amounts and over time, it can affect humans. Therefore, bleach can be a possible tool for clearing larvae from bodies of water that are not needed for consumption. However, bleach will eliminate almost all forms of wildlife as well, including animals who drink from such sources.

Dish soap breaks the water surface tension, also causing the larvae to drown, but dish soap contaminates water, leaving it undrinkable. Like bleach, dish soap would be the prime candidate for bodies of water not being used for human consumption, as it would disrupt the surface tension with very little volume compared to the oil options which need to coat the surface to be effective.

Vinegar is the least effective and because of the results of this study, it is only recommended if cost is a factor and time is not. Vinegar is nontoxic, so it does not affect cleanliness of water. However, due to its time consuming effort to kill larvae, it may need to be applied more often and in greater amounts to be a viable option.

Both oils settle on top of the water’s surface and disrupt the ability of the larvae to use their siphons to float and breathe. The flat-like, hydrofuge lobes of the siphon use the surface tension of the water to float on its surface (Lee 2017). When oil is added to water with larva, the hydrofuge lobes of the siphons cannot float because the oil disrupts the surface tension of the water (Lee 2017). Citronella oil has the added bonus of being a repellent to insects, keeping the water clear of new eggs or larvae until the scent has faded. Death by asphyxiation is rapidly effective, but volume needed to cover the surface of bodies of water may influence effectiveness by cost restriction.

To summarize the qualities of the liquids used, and determine the best option, a consideration of the breeding grounds in Texas is in order. Texas has a developed economy and water filtration mechanisms, so the water treatments only need show concern for the damage they do to wildlife. If the treatments are used in holding ponds or cooling tanks that have no fish and no nearby wildlife in need of the water source, then bleach would be the best and most cost effective option per gallon of larvae breeding water. Soaps, a close second, as they will clear large areas effectively, but they do not sterilize as bleach does.

If wildlife is taken into consideration, the two oil treatments are the primary candidates. The citronella oil will have a marginally more long lasting effect, as it will discourage egg layers for a significant period, while also drowning larvae and preventing adults landing on the water’s surface. Citronella oil may cause wildlife to shy away from the water as well, but this should not be too great an issue, as animals should either ignore it if they need it badly enough, or find another source. Canola oil will have all the same effects as the citronella oil but without the scent deterrent. Canola oil will drown larvae and prevent adults from landing on the water’s surface tension. Because canola oil is cheaper per ounce than citronella oil, it is more readily available to rural families or businesses who need to treat their water. Some may even consider taking old oil from food businesses and repurposing it for larvicide.

This study proves that the effective test materials, canola oil, citronella oil, and in some cases, bleach and dish soap could be implemented instead of IPM. This mechanism would be sufficient for controlling mosquito populations in areas where IPM is thought of as too complex. Furthermore, these test materials would be less expensive when compared to the cost of executing IPM.

Each of these items can be purchased in various sizes at local grocery stores. The following prices were found at H-E-B in College Station, Texas. A 48 oz bottle of Hill Country Fare Canola Oil can be purchased for $2.41. This container could potentially be responsible for eradicating 960 larvae. NOW Citronella Essential Oil can only be purchased in one ounce bottles at a price of $6.16; this cost is only capable of killing 20 mosquito larvae. To compete with canola oil, 48 bottles of citronella oil would be required to kill 960 mosquito larvae with an associated cost of $295.68. The standard size of Clorox Bleach, 121 fl oz, can be purchased for $4.68, and it would have a potential yield of 2,420 larvae deaths. 40 oz of Dawn Ultra Original Scent Dishwashing Liquid Dish Soap is $4.99 and has a capability of killing 800 larvae. With comparative prices between bleach and canola oil, bleach would be the better option due to the potential for more deaths. However, since it causes ecological imbalance, canola oil is the best and most cost effective option for treating water.

While we do not know the exact number of larvae deaths per ounce of product, further research should be dedicated to precisely and accurately predicting the amount of product needed to effectively eradicate mosquito larvae populations. A focus on using as little product as necessary would allow for further cost deductions for consumers and an increase in the longevity of product.

The public needs to be informed of the mosquito breeding cycle, and the bodies of water that each species uses to lay their eggs. They should be aware of when mosquito season is most active, but if not, this information should be provided as well. If individual families each treated their water sources, there could be a massive reduction in mosquito populations. Rural areas would make it difficult to treat every water source, but the percentage of areas that could be treated should decrease the populations immensely.

This study measured the estimated reduction in mosquito population by using household products. Because the species collected were some of the most important species in Texas for transmitting vector-borne diseases, it also measured the approximate decrease in such diseases. Therefore, these household products utilized can be productive in reducing the vector-borne diseases associated with mosquitoes.

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