Novel Mosquito Control: A Natural Approach to Reducing and Repelling Mosquito Populations

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Mosquitoes are a major concern to human health because they serve as vectors to pass agents that cause diseases, such as malaria, the Zika virus, and the West Nile virus, which can have a multitude of harmful side effects, such as seizures, conjunctivitis, and death. The overuse of commercial mosquitocidal agents have caused mosquitoes to develop resistance and cause harm to humans and the environment. To prevent this, natural extracts could be used. The purpose of this experiment was to test various plant extracts for their effectiveness in acting as larvicides, adulticides, and repellents against *Culex quinquefasciatus* mosquitoes. It was hypothesized that if *Chrysanthemum coccineum* (Persian chrysanthemum), *Trachyspermum ammi* (ajwain), *Nymphaea odorata* (American water lily), and common commercial products such as VectoBac 12AS, permethrin, and DEET were tested as natural adulticides, larvicides, and repellents, the commercial products and *T. ammi* would be the most effective, followed by *C. coccineum* and *N. odorata*. For the purpose of this experiment, the essential oils from the leaves of *C. coccineum, T. ammi*, and *N. odorata* were extracted, and the extracts and commercial products were tested on larvae and adult *Culex* mosquitoes as larvicides, adulticides and repellents. Various statistical tests were conducted, and it was found that for the larvicide and adulticide, *T. ammi*, VectoBac 12AS, and permethrin were the most effective, and for the repellent, all the treatments were effective, thus supporting the hypothesis. While the chrysanthemum and lily work only as repellents, ajwain works as effectively as commercial larvicides, adulticides and repellents, with the advantage of being a single product that reduces and repels two life stages of the mosquito. Since these plants are found in third world countries, these natural extracts could be used by natives or refined by local companies to create an inexpensive mosquitocide and repellent.

Introduction

Vector-borne diseases account for over 17% of all infectious diseases, causing more than 1 million deaths annually. With an increase in globalization of travel and trade, these diseases are being transmitted faster than ever, emerging in countries where they previously did not exist. Mosquitoes are one of the major factors for this because when the female mosquitoes feed on mammals with a vector-borne disease, the mosquitoes can contract the vector and pass it on to other mammals. Because of this, mosquitoes are considered one of the most dangerous insects in the world, causing nearly 725,000 deaths globally per year by passing agents that cause malaria, the Zika virus, the West Nile virus, and many other communicable diseases. These Neglected Tropical Diseases (NTDs) are prolific in third world countries, where they are responsible for major economic burdens, such as disability, death, and missed educational opportunities. The diseases help contribute to the ongoing cycle of poverty, and it is often the poorest countries that are most affected (World Health Organization, 2016).

These vector-borne diseases are very threatening, and one virus that has recently received a lot of media attention is the Zika virus. It is a virus transmitted primarily by *Aedes* mosquitoes. While it was first isolated in the Zika Forest of Uganda in 1947, it has since spread rapidly. In the USA itself, 3,936 cases have been reported. Unfortunately, no vaccines or medications exist to prevent Zika. While it can be treated, it can cause conjunctivitis, malaise, and many neurological complications. A major concern is that Zika causes the Guillain-Barre syndrome and microcephaly, a congenital defect in the brains of newborns that usually causes death (Mosquito-borne diseases, n.d.). Many of these vector-borne diseases are contracted from mosquitoes.

Mosquitoes are small, ectoparasitic, midge-like flies that are in the family of *Culicidae*. The name "mosquito" originates from the Spanish word "mosca", meaning "little fly." There are many species of mosquitoes all over the world, all of which fall under the two subfamilies *Anophelinae* and *Culicinae* and three genera, Aedes, Culex and Anopheles. The most common mosquito found in the Southern U.S. is the *Culex quinquefasciatus,* which was used as a test subject in this experiment. More commonly known as southern house mosquito, it is a medium sized brown mosquito that lives in tropic areas and acts as a primary vector for encephalitis, filariasis, and West Nile virus and a possible vector for the Zika virus. Though it prefers to feed on the blood of birds, it also bites humans. These mosquitoes breed in dirty water collections, like septic tanks and stagnant water and have a life cycle that takes about seven days (Hill & Connelly, 2016).

The life cycle of mosquitoes includes 4 major stages: Egg, Larvae, Pupae, and Adult. *Culex* eggs are laid on the surface of nutrient-rich water and attach together to form "rafts." The eggs hatch into larvae in about 48 hours, but if it is winter, they remain as eggs until winter has passed. Larvae live in the water and come to the surface to breathe through their siphon tubes. The larvae molt 4 times, growing larger after each molt (thus the stages L1, L2, L3, and L4). After the 4th molt, they turn into pupae. In this stage, a pupae rests and does not feed, but it moves by flipping its tail. The pupae metamorphosizes into an adult, splits the pupal skin, and emerges as an adult. The adult remains on the surface of the water for a short time in order to dry its wings and body. Then, the mosquito begins feeding and mating (Mosquito info, n.d.).

Mosquitoes derive their food source from nectar or honeydew of plants, which provides sugar for males and females. However, females need protein to create eggs for reproduction. For this, they get blood from humans and other animals. The mosquito does this by sticking long, tube-like mouthparts called the proboscis into the skin. Using this, the female searches for a blood vessel. Once one is found, the mosquito releases its saliva into the wound, which works as an anticoagulant to keep the blood flowing. The saliva also causes histamine to be released as an immune reaction, which causes blood vessels in the area to swell and nerves in the area to become irritated. Since females attain blood, they act as vectors for vector borne disease, which is why only female mosquitoes were used in this experiment. When they feed on mammals with a disease/virus, they contract disease agents, and when the infected mosquito bites the human, these disease agents can be transmitted to the human (Mosquito info, n.d.).

To prevent mosquitoes from biting humans, many methods are used to kill or repel mosquitoes. The major methods for killing mosquitoes are by using ovicides (for eggs), larvicides (for larvae), pupacides (for pupae), or mosquitocides (for adults). Repellents are used to repel the mosquitoes. However, these mosquitocides and repellents pose major concerns to people and the environment. Recently, in September 2016, there was an insecticide spraying in order to kill the high mosquito populations in Summerville, South Carolina. While it reduced mosquito populations, it had a devastating effect on the honeybee populations. Millions of bees died from acute pesticide poisoning, which is a major problem because these bees play a huge role in pollination. A recent study by the University of Maryland found that many agricultural pesticides and mosquitocides are linked to honeybee deaths, even though many of these were regarded as "bee-safe" (University of Maryland, 2016). In addition, these mosquitocides and repellents have an adverse affect on humans, as exposure to the mosquitocides and repellents have been found to be carcinogenic or toxic. Unfortunately, mosquitoes can grow resistant to these methods, rendering them ineffective.

One of these harmful adulticides is permethrin. Permethrin is part of the pyrethroid family, a group of synthetic chemicals that act similar to the natural extracts from the flowers of *Chrysanthemum* genera plants. It was registered with the US EPA in 1979 and is now used to treat crops, lawns, animals, buildings, or clothes. Permethrin adversely affects insects' nervous systems because the pyrethrins bind to the sodium channels of the nerve cells, disturbing nerve signal transmission and causing muscle spasms, paralysis, and death. It is more toxic to insects than to humans because insects can't break it down quickly, (but) it is still harmful to humans. The International Agency for Research on Cancer stated that permethrin is likely to be carcinogenic to humans if consumed. Moreover, researchers are also finding that mosquitoes can adapt to become immune to the effects of permethrin (National Pesticide Information Center, 2009).

While mosquitoes can be killed in the adult stage, larvicides are used to kill mosquitoes in the larval stages. VectoBac 12AS is a common commercial larvicide for mosquitoes. It is an aqueous suspension of *Bacillus thuringiensis* subsp. *israelensis* (strain AM65-52) that is used to kill mosquito and black fly larvae. The *B. thuringiensis*, when consumed by the larvae, forms delta endotoxins, which are also known as crystal proteins. The crystals are denatured in the digestive tract, and toxin is released. The toxin paralyzes the digestive tract, forms a pore, and prevents the larvae from eating, essentially starving the larvae. It can be poisonous to humans before dilution, and if absorbed through the skin or ingested, toxic, yet it is used as a staple larvicide (Valent BioSciences, 2014).

Another technique for mosquito borne disease prevention is by repelling mosquitoes. *N*,*N*-Diethyl-*meta*-toluamide, also known as DEET, is the most common active ingredient found in insect repellents. Every year, about 1/3 of the US population uses DEET to protect themselves from mosquito-borne illnesses. DEET repels mosquitoes by making it difficult for them to smell humans. When applied, about 56% of DEET is absorbed through the skin, and 17% is absorbed into the bloodstream, something that is concerning after finding that DEET has adverse effects on the central nervous system (US Environmental Protection Agency, 2016).

While many chemical mosquitocides and repellents appear harmful, many plants show promise for serving as effective natural mosquitocides or repellents. In fact, permethrin is a chemical adulticide derived from pyrethrin. Pyrethrins are ethers that are found in the extract from the flowerhead of the *Chrysanthemum* plant. *Chrysanthemum coccineum* is a perennial plant native to Caucasus and is commonly known as the Persian chrysanthemum. It is known to contain some pyrethrum substances, but is a poor source compared to other plants in the *Chrysanthemum* genus, like the *Chrysanthemum cinerariifolium*. Still, *C. coccineum* is a common plant found in gardens as it is easy to care for, non-aggressive, and non-invasive (Ecological Agriculture Projects, n.d.).

Another promising plant is *Trachyspermum ammi*, commonly known as ajwain or carom. It is an annual herb originating in India that grows up to two to three feet in height and has tiny white-petaled flowers that eventually develop into small, oval-shaped seeds which are harvested early spring. Traditionally used as a remedial agent for diarrhea, abdominal tumours, and bronchial problems, ajwain has been proven to be antifungal, antimicrobial, and antihypertensive, among many other benefits. In addition, it has many constituents, most notably the volatile oils thymol, γ-terpinene, para-cymene, and α- and β-pinene (Bairwa, Sodha, & Rajawat, 2012).

Nymphaea odorata, also known as the American white water lily, is another plant that shows potential as a natural mosquitocide and repellent. It is an aquatic, perennial plant native to the northeastern U.S. and is commonly found in shallow lakes, ponds, and slow moving water. It usually has one 2-6 inch white flower per stem, which floats on top of the water. The lily has been used to treat diarrhea, kidney troubles, inflamed gums, and many other problems and contains many compounds, including quinolizidine alkaloids (Zhang et al., 2003).

Bermudez-Torres et al. examined the effects of the quinolizidine alkaloids from *L. montanus, L. stipulatus,* and *L. aschenbornii* against the *Spodoptera frugiperda*, a type of crop pest. They found that the alkaloid extracts from the *L. montanus* and *L. aschenbornii* were effective, but the extract from the *L. stipulatus* was found to be the most toxic against the larvae of the *S. frugiperda* (Bermudez-Torres et al., 2008). Deepa, Palanisamy, Krishnappa and Elumalai studied the ovicidal, larvicidal, and repellent effects of the extract of *Polygala arvensis* against three species of mosquitoes: *Aedes aegypti, Anopheles stephensi,* and *Culex quinquefasciatus*. It was found that the *P. arvensis* had strong ovicidal properties and the methanol extract had strong repellent action, providing 100% protection from mosquitoes (Deepa, Palanisamy, Krishnappa, & Elumalai, 2014). Govindarajan et al., studied the ovicidal and repellent effects of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against *Culex quinquefasciatus, Aedes aegypti*, and *Anopheles stephensi*. The *E. coronaria* and *C. pulcherrima* were both found to be effective ovicides, but the *E. coronaria* extract was the more repellent of the two (Govindarajan et al., 2011).

The purpose of this experiment was to find an effective, natural method to reduce pollution and mosquito borne disease by reducing and repelling mosquito populations. Although DEET, permethrin and VectoBac 12AS are commonly used, it has been found that they are harmful to humans, and insects can build immunity to them. The increase in the use of these chemicals or the increase in mosquito populations could lead to the increase in health problems and disease in humans and the environment. Therefore, other methods for controlling mosquito populations such as natural repellents and mosquitocides must be looked into in order to control the spread of mosquito-borne illnesses.

It was hypothesized that if *Chrysanthemum coccineum, Trachyspermum ammi*, and *Nymphaea odorata* are tested as adulticides, larvicides, and repellents, *T. ammi* would be the most naturally effective as an adulticide, larvicide and repellent, followed by *C. coccineum* and *N. odorata*. It was also hypothesized that the VectoBac 12AS, DEET and permethrin would be the most effective overall as larvicides, repellents, and adulticides, respectively.

To test this, female *Culex quinquefasciatus* mosquitoes were raised from the egg stage. Leaves of *Chrysanthemum coccineum, Trachyspermum ammi,* and *Nymphaea odorata* were distilled to make the plant extracts. To test the larvicides, 20 L4 larvae were placed in 10 mL of water, and 1 uL of the extract was added to the water. Mortality was observed. To test the adulticides, the 250 mL bottles were coated with a stock solution of ethanol and the extract. Mosquitoes were introduced and mortality observed. To test the repellents, mosquitoes were introduced to a tube with filter paper on one end and filter paper soaked in an extract on the other end. Movement of the mosquitoes was observed. After the data were collected, statistical analysis was conducted.

Materials

Extraction	Collecting Eggs	Testing Larvicide
Nymphaea odorata (water lilies) *Trachyspermum ammi* (ajwain) *Chrysanthemum coccineum* (chrysanthemums) Steam distillation apparatus Distilled H_2O Tap Water Electric Hot Plate	6 containers (1 6L, 5 500 mL) H_2O Malted milk powder *Saccharomyces cerevisiae* (Baker's yeast) Rearing Chamber	5 20 mL containers H_2O *C. quinquefasciatus* L4 larvae *N. odorata*, *T. ammi*, *C. coccineum* extracts VectoBac 12AS
	Testing Adulticide	**Testing Repellent**
	250 mL bottles Ethanol *N. odorata*, *T. ammi*, *C. coccineum* extracts Permethrin Female *C. quinquefasciatus* adults	3 foot clear tube Filter Paper *N. odorata*, *T. ammi*, *C. coccineum* extracts *C. quinquefasciatus* female adults DEET

Methods

Making Plant Extracts:
Dry steam distillation was used to create the plant extracts. The apparatus used is shown below in Figure 2. This process was used for the water lily, ajwain and chrysanthemum. The apparatus was constructed. Distilled water was boiled over a hot plate, producing steam that traveled through the biomass flask, which contained the plant matter. The water vapor burst the cell walls and released volatile oils and water, which traveled through the still head into the condenser, where it was condensed. These oils and water passed into the receiver, where they were divided based on density as denser-than-water oils, water, and lighter-than-water oils. The water was drained using the spout seen in Figure 2. The oils were removed from the receiver using a tip with a buret stopcock.

Figure 2. The steam distillation apparatus used for extraction.

Collecting Mosquito Eggs:
A black, 6 liter tray was filled with 5 liters of water, 10 grams of malted milk powder, and 10 grams of Baker's yeast. This tray was set outside under a bush for 5 days, after which the pupae, larvae and eggs were collected and put in containers in a rearing chamber. They were organized based on the age of the mosquitoes (eggs, L1, L2/3, L4, pupae) and mosquito type. Multiple mosquito species were caught, but only female *C. quinquefasciatus* mosquitoes were used.

Testing the Larvicide:
Five 20 mL containers were set up with 10 mL of water in each one. Twenty L4 larvae were placed in each of the containers. Then, 1 uL of the treatment (water lily, ajwain, chrysanthemum, VectoBac 12AS, or water/control) was placed in the containers. The mortality rate of the larvae was observed for 4 hours.

Testing the Adulticide:
The CDC Bottle Bioessay procedure was used to test the extracts as adulticides. 100 uL of each sample treatment (water lily, ajwain, chrysanthemum, permethrin, and ethanol/control) was added to 100 mL of ethanol to create stock solutions. Then, 1 mL of each stock solution was placed in each of five 250 mL bottles. Once the inside of the bottle was fully coated and dried, twenty mosquitoes were removed from the rearing chamber with an aspirator and introduced to the bottles. The mortality rate of the mosquitoes was observed for 4 hours.

Testing the Repellent:
Twenty mosquitoes were introduced to a tube that has filter paper (control) on one end and filter paper soaked in an extract on the other end. The movement of the mosquitoes was observed after 1 minute as either towards the control, towards the extract or no movement.

Table 1. The experimental design diagram for the larvicide testing. It gives a brief view of how the experiment was designed, including the independent and dependent variables, hypothesis, control, number of trials, and treatments.

Independent Variable: Type of Larvicide				
DI H2O (Control)	VectoBac 12AS	Water Lily Extract	Ajwain Extract	Chrysanthemum Extract
1 trial	1 trial	1 trial	1 trial	1 trial
Dependent Variable: Mortality rate of the mosquito larvae				
Constants: containers used, environment of the container, mosquito species used, age of mosquitoes, gender of mosquitoes, concentration of extract, quantity of extract used				

Table 2. experimental design diagram for the adulticide testing. It gives a brief view of how the experiment was designed, including the independent and dependent variables, hypothesis, control, number of trials, and treatments.

Independent Variable: Type of Adulticide				
DI H2O (Control)	Permethrin	Water Lily Extract	Ajwain Extract	Chrysanthemum Extract
1 trial	1 trial	1 trial	1 trial	1 trial
Dependent Variable: Mortality rate of the mosquito adults				
Constants: containers used, environment of the container, mosquito species used, age of mosquitoes, gender of mosquitoes, concentration of extract, quantity of extract used				

Table 3. Experimental design diagram for the repellent testing. It gives a brief view of how the experiment was designed, including the independent and dependent variables, hypothesis, control, number of trials, and treatments. Repellence Testing

Independent Variable: Type of Repellent				
DI H2O (Control)	DEET	Water Lily Extract	Ajwain Extract	Chrysanthemum Extract
1 trial	1 trial	1 trial	1 trial	1 trial
Dependent Variable: repellence to the repellents				
Constants: containers used, environment of the container, mosquito species used, age of mosquitoes, gender of mosquitoes, concentration of extract, quantity of extract used				

Results

Larvicide

The treatment groups for the larvicide consisted of ajwain, chrysanthemum, lily, the control, and VectoBac 12AS. Table 4 shows the raw data of the number of live larvae when exposed to the different treatments at various time intervals for a total time of four hours, and Figure 3 shows the survivorship curves for the various treatments. As seen, the number of live larvae decreases over time. While there was little mortality in the first 30 minutes, VectoBac 12AS killed the most larvae after 4 hours, followed by ajwain, lily, chrysanthemum and the control.

Table 5 is a Repeated Measures ANOVA for the larval mortality data. The null hypothesis is that there is no significant difference between the various treatments. The alternative hypothesis is that there is a significant difference between the treatments. At an alpha value of 0.05 and a p-value of <0.001, the null hypothesis that there is no significant difference between the various treatments was rejected ($F(10,1085)=18.21$, $F(4,1085)=75.70$, $p<0.001$), suggesting that there was a significant difference between the various treatments.

Figure 4 displays the interaction plot for the larval mortality. Treatments 1, 2, 3, 4, and 5 correspond to the ajwain, chrysanthemum, lily, control and VectoBac 12AS treatments, respectively. No crossover interactions occurred, so it was concluded that there was no correlation between time and treatment.

Table 6 displays a One Way ANOVA for the larval mortality data. At an alpha value of 0.05 and a p-value of <0.001, it was concluded that there was a significant difference between two or more of the treatments ($F(4,95)=15.34$, $p<0.001$). To find where the significant difference lies, a post-hoc Tukey test was conducted. A significant difference in mortality was found between the ajwain and chrysanthemum, ajwain and lily, ajwain and control, chrysanthemum and VectoBac 12AS, lily and VectoBac 12AS, and the VectoBac 12AS and the control.

Table 4. Mortality when the live larvae were exposed to different treatments. In the first 30 minutes, there was little mortality, but after four hours, VectoBac 12AS showed the greatest mortality, followed by the ajwain, lily, chrysanthemum and control.

Time (mins)	Control	Chrysanthemum	Lily	Ajwain	VectoBac 12AS
0	20	20	20	20	20
15	20	20	20	20	19
30	20	20	20	19	17
45	20	20	20	16	13
60	20	20	19	13	13
90	20	19	17	13	9
120	20	19	17	13	9
150	20	19	17	10	7
180	20	19	17	9	6
210	19	17	16	8	5
240	19	17	13	6	3

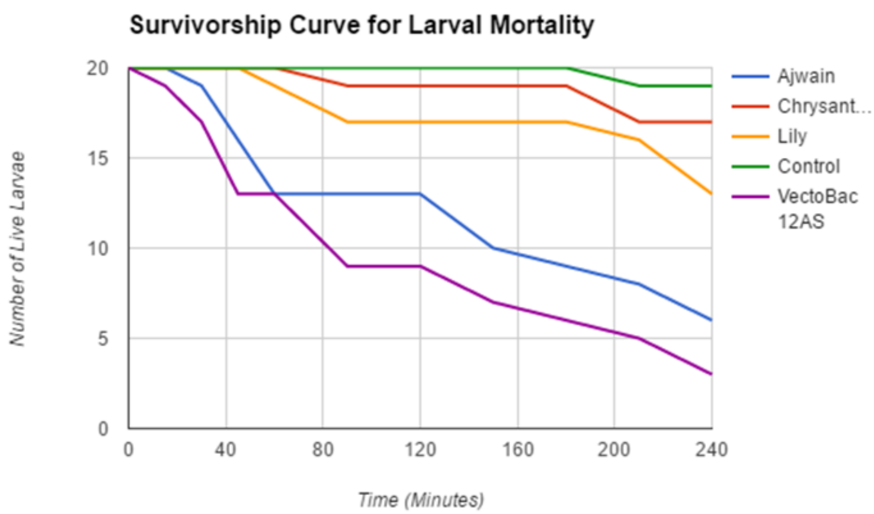

Figure 3. The number of live larvae alive after exposed to different treatments over time. VectoBac 12AS was the most effective, as only 3 larvae were alive after 2 hours. The ajwain treatment also showed similar mortality rates, with only 6 live after 2 hours. While the chrysanthemum and lily also killed the larvae, they were not as effective.

Table 5. The results from a Repeated Measures ANOVA on the larval mortality data. At an alpha value of 0.05, the null hypothesis was rejected ($F(10,1085)=18.21$, $F(4,1085)=75.70$, $p<0.001$).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	10	19.5691	19.5691	1.9569	18.21	<0.001
Treatment	4	32.5327	32.5327	8.1332	75.70	<0.001
Error	1085	116.5673	116.5673	0.1074		
Total	1099	168.6691				

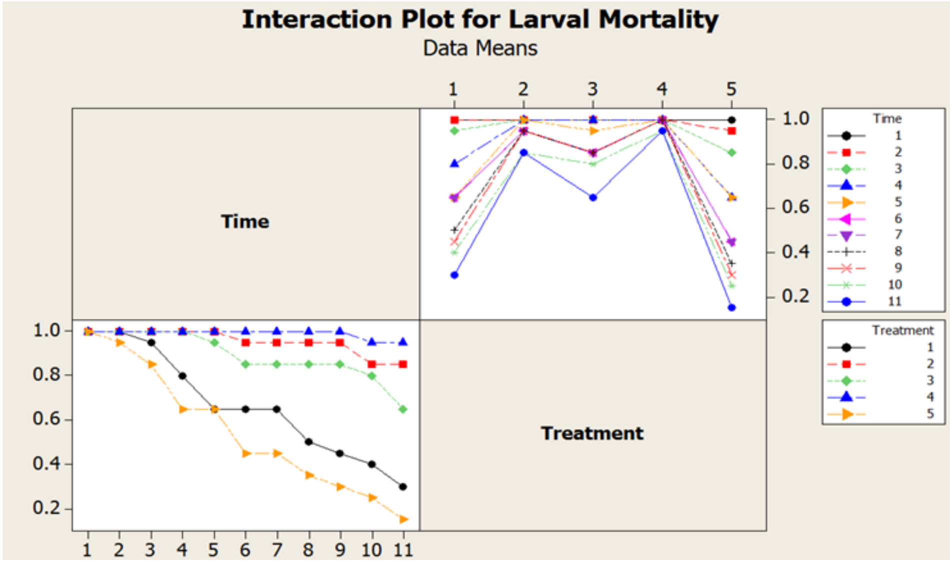

Figure 4. An interaction plot for the larval mortality. Since there were no crossover interactions, no correlation exists between time and treatment.

Table 6. The results of a One-Way ANOVA on the larval mortality data. At an alpha value of 0.05, significant difference was found in the data (F(4,95)=15.34, p<0.001).

Source	DF	SS	MS	F	P
Treatment	4	9.560	2.390	15.34	<0.001
Error	95	14.800	0.156		
Total	99	24.360			

Adulticide

The treatment groups for the adulticide consisted of ajwain, chrysanthemum, lily, the control, and permethrin. Table 7 contains the raw data of the number of live mosquitoes at various time intervals for a total time of four hours, and Figure 5 is the survivorship curves for the various treatments. As shown, the number of live mosquitoes decrease over time for all the treatments, but the permethrin and ajwain treatments resulted in greater mortality than the chrysanthemum, lily and control treatments.

Table 8 is a Repeated Measures ANOVA for the adult mosquito mortality data. The null hypothesis is that there is no significant difference between the various treatments. The alternative hypothesis is that there is a significant difference between the treatments. At an alpha value of 0.05 and a p-value of <0.001, the null hypothesis was rejected, suggesting a significant difference between the treatments (F(10,1085)=18.44, F(4,1085) =76.83, p<0.001).

Figure 6 displays the interaction plot for the adult mosquito mortality. Treatments 1, 2, 3, 4, and 5 correspond to the ajwain, chrysanthemum, lily, control and permethrin treatments, respectively. No crossover interactions occurred, so it was concluded that there was no correlation between time and treatment.

Table 9 displays a One-Way ANOVA for the adult mosquito mortality. At an alpha value of 0.05 and a p-value of <0.001, it was concluded that there was a significant difference between two or more of the treatments (F(4,95)=19.61, p<0.001). A post-hoc Tukey test was conducted to find where the significant differences lay, and it was found that there was a significant difference between the ajwain and chrysanthemum, ajwain and lily, ajwain and control, chrysanthemum and permethrin, lily and permethrin, and the control and permethrin.

Repellent

The treatment groups for the repellent consisted of ajwain, chrysanthemum, lily, the control, and DEET. Table 10 consists of the raw data of the number of mosquitoes attracted or repelled, and Figure 7 is a bar graph of mosquito movement after various treatments. As seen, DEET repelled the greatest number of mosquitoes, the chrysanthemum attracted the most, and the control resulted in the least movement in the mosquitoes.

Table 11 displays the results for a One-Way ANOVA for the adult mosquito repellency. At an alpha value of 0.05 and a p-value of 0.002, it was concluded that there was a significant difference between two or more of the treatments (F(4,95)=4.65, p=0.002). A post-hoc Tukey test was conducted, and it was found that there was a significant difference between the ajwain and control, chrysanthemum and control, lily and control, and DEET and control.

Table 7. Raw data for the adult mosquito mortality when exposed to various treatments for four hours. Permethrin had the greatest mortality rate whereas the control had no mortality.

Time	Control	Chrysanthemum	Lily	Ajwain	Permethrin
0	20	20	20	20	20
15	20	20	20	20	19
30	20	20	20	19	17
45	20	19	20	16	13
60	20	19	20	14	13
90	20	18	19	13	9
120	20	17	19	13	9
150	20	17	18	10	7
180	20	17	17	8	6
210	20	16	17	7	5
240	20	15	16	5	3

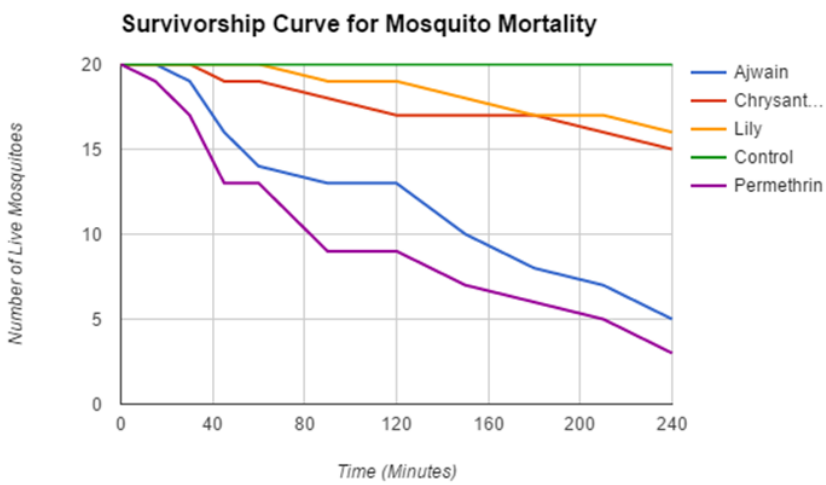

Figure 5. Survivorship curve for the adult mosquitoes. It depicts the number of live mosquitoes after they are exposed to various treatments for a certain amount of time. The permethrin and ajwain treatments showed the greatest mortality, followed by the chrysanthemum, lily, and control respectively.

Table 8. Results for a Repeat Measures ANOVA on adult mosquito mortality. At an alpha value of 0.05, the null hypothesis was rejected ($F(10,1085)=18.44$, $F(4,1085)=76.83$, $p<0.001$).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	10	19.8691	19.8691	1.9869	18.44	<0.001
Treatment	4	33.1182	33.1182	8.2795	76.83	
Error	1085	116.9218	0.1078			
Total	1099	169.9091				

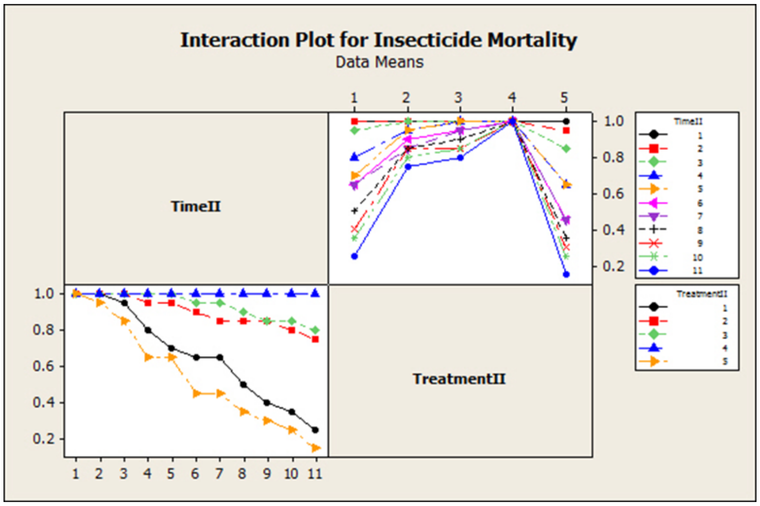

Figure 6. Interaction plot for the adult mosquito mortality data. Since no crossover interactions occurred, no correlation exists between time and treatments.

Table 9. One-Way ANOVA for the adult mosquito mortality. At a p-value less than 0.001, it was concluded that there was a significant difference between two or more of the treatments (F(4,95)=19.61, p<0.001).

Source	DF	SS	MS	F	P
Treatment	4	10.940	2.735	19.61	<0.001
Error	95	13.250	0.139		
Total	99	24.190			

Table 10. Raw data for the mosquito movement after exposed to various treatments. The DEET treatment repelled the most mosquitoes, followed by the ajwain, lily, chrysanthemum and control, respectively. The chrysanthemum attracted the most, while the control caused the least movement.

	Control	Chrysanthemum	Lily	Ajwain	DEET
Attracted	1	6	3	2	0
Repelled	2	4	11	14	18
No Movement	17	10	6	4	2

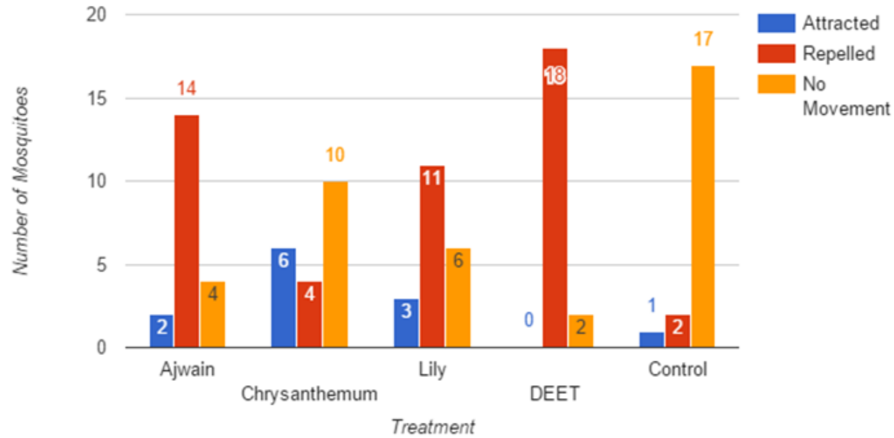

Figure 7. Movement of the mosquitoes after exposed to various treatments. The DEET treatment repelled the most mosquitoes, followed by the ajwain, lily, chrysanthemum and control, respectively. The chrysanthemum attracted the most, while the control caused the least movement.

Table 11. One Way ANOVA for adult mosquito repellency. Since p=0.002, there is a significant difference between 2 or more of the treatments (F(4,95)=4.65, p=0.002).

Source	DF	SS	MS	F	P
Treatment	4	7.160	1.790	4.65	0.002
Error	95	36.550	0.385		
Total	99	43.710			

Discussion

While many mosquitocidal and repellent agents are used, mosquitoes can develop resistance, and these agents have been proven to be harmful to humans. Natural extracts could be a viable alternative. In this experiment, various plant extracts were tested as larvicides, adulticides, and repellents against *Culex quinquefasciatus* with the purpose of finding an effective natural method to killing and repelling mosquitoes.

It was hypothesized that if the chrysanthemum, ajwain and water lily were tested as natural adulticides, larvicides, and repellents and VectoBac 12AS, permethrin, and DEET as commercial mosquitocides and repellents, VectoBac 12AS, DEET, and permethrin would be the most effective as larvicides, repellents, and adulticides, respectively. Ajwain would be the most naturally effective, followed by the chrysanthemum and water lily. This hypothesis was partially supported, as VectoBac 12AS, DEET, permethrin, and ajwain were the most effective as larvicides, repellents and adulticides, but the chrysanthemum was less effective than the water lily.

For the larvicide, the survivorship curve (Figure 3) showed that the VectoBac 12AS was most effective, and ajwain showed similar mortality rates, while the chrysanthemum, lily, and control had low mortality rates. A Repeated Measures ANOVA (Table 5) was conducted and it was found that the null hypothesis that there is no significant difference between the various treatments was rejected (F(10,1085)=18.21, F(4,1085)=75.70, p<0.001), which indicates that there was a significant difference between the treatments VectoBac 12AS, ajwain, chrysanthemum, lily, and the control. This also suggested that there was a significant difference in each time that mortality of the mosquitoes was taken. An interaction plot was looked at to see where the time and treatment interacted but there was no crossover interaction on the plot, which indicated that there was no correlation between time and treatment (Figure 4). Based on this, a One-Way ANOVA (Table 6) was conducted to see if there was a significant difference between two or more treatments and it was found that there was a difference between the treatments (F(4,95)=15.34, p<0.001). A post-hoc Tukey test was conducted to see which treatment was significantly different from another and it was found that there was a significant difference in mortality between the ajwain and chrysanthemum, ajwain and lily, ajwain and control, chrysanthemum and VectoBac 12AS, lily and VectoBac 12AS, and the VectoBac 12AS and the control (Table 7). Based on these statistical tests, it was found that the hypothesis was partially supported. Ajwain and VectoBac 12AS were the most effective, but the chrysanthemum and water lily were as equally effective as the control. The chrysanthemum may not have been as effective due to the type of species used in this experiment. The water lily has not been previously experimented on with researchers and was used in this experiment due to its availability and abundance in this part of the US.

For the adulticide, the survivorship curve (Figure 5) showed that permethrin was the most effective, followed by ajwain, chrysanthemum, lily and the control, respectively. The Repeated Measures ANOVA (Table 9) was conducted and it was found that the null hypothesis that there is no significant difference between the various treatments was rejected(F(10,1085)=18.44, F(4,1085)=76.83, p<0.001) which indicates that there was a significant difference between the treatments permethrin, ajwain, chrysanthemum, lily, and the control. This also suggested that there was a significant difference in each time that mortality of the mosquitoes was taken. This also suggested that there was a significant difference in each time that mortality of the mosquitoes was taken. An interaction plot was looked at to see where the time and treatment interacted but there was no crossover interaction on the plot, which indicated that there was no correlation between time and treatment (Figure 6). Based on this, a One-Way ANOVA (Table 10) was conducted to see if there was a significant difference between two or more treatments (F(4,95)=19.61, p<0.001), and it was found that there was. A post-hoc Tukey test was conducted to see which treatments were significantly different, and it was concluded that there was a significant difference in mortality between the ajwain and chrysanthemum, ajwain and lily, ajwain and control, chrysanthemum and permethrin, lily and permethrin, and the permethrin and the control (Table 11). Based on these statistical tests, it was found that the hypothesis was partially supported, as the ajwain and permethrin were the most effective, but the chrysanthemum and water lily were as equally effective as the control. The chrysanthemum may not have been as effective due to the type of species used, and the water lily has not been previously experimented on with researchers and was used due to its availability in the southern US.

For the repellent, the bar graph (Figure 7) showed how the DEET treatment repelled the most amount of mosquitoes, followed by the ajwain, lily, chrysanthemum and control, respectively, and the control caused the least movement. A One Way ANOVA (Table 13) was conducted, and it was found that the null hypothesis that there is no significant difference between the various treatments was rejected ($F(4,95)=4.65$, $p=0.002$), which indicates that there was significant difference between the treatments of DEET, ajwain, lily and chrysanthemum. A post-hoc Tukey test was conducted to see which treatment was significantly different from the others, and it was concluded that there was a significant difference in repellence between the ajwain and control, chrysanthemum and control, lily and control, and DEET and control (Table 14). This partially supported the hypothesis, as the DEET and ajwain were effective, but the chrysanthemum and lily were just as effective as the DEET and ajwain. All the treatments may have been effective due to their chemical components. While no research has been conducted on the effectiveness of ajwain and water lily as mosquitocides or repellents, research has been conducted on their chemical constituents.

Bermudez-Torres et al. examined the effects of the quinolizidine alkaloids, found in the American water lily, from *L. montanus, L. stipulatus,* and *L. aschenbornii* against the Spodoptera frugiperda, a type of crop pest. They found that the alkaloid extracts from the *L. montanus* and *L. aschenbornii* were effective, but the extract from the *L. stipulatus* was found to be the most toxic against the larvae of the *S. frugiperda* (Bermudez-Torres et al., 2008). These findings are different than my project's findings because *Nymphaea odorata* contain quinolizidine alkaloids, but they did not show any larvicidal or insecticidal properties (Bermudez-Torres et al., 2008). Govindarajan et al. studied the ovicidal and repellent effects of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against *Culex quinquefasciatus, Aedes aegypti*, and *Anopheles stephensi*. They found that the *E. coronaria* and *C. pulcherrima* were both found to be effective ovicides, but the *E. coronaria* extract was the more repellent of the two. These findings were similar to mine in that their natural extracts were found to kill and repel the mosquitoes (Govindarajan et al., 2011).

These positive findings could be due to the chemical constituents of these natural extracts. Ajwain contains many volatile oils, including thymol, γ-terpinene, para-cymene, and α- and β-pinene. Thymol has been proven to work as an adulticide, and γ-terpinene has a strong aroma. The extracts contain these oils, which could explain why it is an effective larvicide, adulticide and repellent. *Chrysanthemum coccineum* was expected to work because flowers from *Chrysanthemum* plants have pyrethrins, which are effective adulticides. However, the species used in this experiment may have been ineffective because C. coccineum has a poor source of pyrethrins. The *Nymphaea odorata* showed repellent properties, which may be because it has quinolizidine alkaloids, which have been shown in other experiments to repel and kill insects.

Some sources of uncertainty include the method for applying the treatments and the health of the mosquito. When drying the bottles for the CDC bottle bioassay, the bottles may not have been evenly coated, so some areas of the bottle may have gotten a more concentrated coating. The health of the mosquitoes may have varied because even though food was provided for the mosquitoes, some mosquitoes may have eaten less food compared to other mosquitoes, having an adverse affect on their health. This principle can also be applied when looking at the rate of death for the mosquito larvae. Some mosquito larvae may have consumed more of the extract or commercial product than other larvae, which can cause them to die faster.

For future research, more trials could be conducted. Also, research can be conducted to look at the effect ajwain has on the mosquito eggs and pupae as a potential ovicide and pupacide. In addition, these extracts could be tested on various other mosquito species, such as the *Aedes*.

Since it was found that ajwain can be an effective larvicide, adulticide and repellent and the lily and chrysanthemum can be effective repellents, they can work as commercial products. However, ajwain would be the most effective because it can be used to kill and repel mosquitoes in multiple life stages. The plants used to make the extracts (*C. coccineum, N. odorata* and *T. ammi*) are found in third world countries, so people in third world countries could use these plants to repel or reduce mosquito populations. However, it may be difficult for them to use steam distillation to make extracts. Thus, these plants can be used by local factories in order to mass-produce cheap, natural products to reduce and repel mosquito populations.

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