Efficacy of Essential Oils as a Larvicide Against *Aedes albopictus* (Skuse) (Diptera: Culicidae)

Lauren Krupa, Renne Holmes, Jakalynne Gosnell, and Adrienne Brundage

Texas A&M University

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Abstract: As a prominent vector of deadly diseases such as Dengue and Yellow Fever, the control of *Aedes albopictus* (Skuse) (Diptera: Culicidae) is a constant battle for the public. As winged adults, mosquitoes can be harder to control with pesticides and have grown resistant to commonly used chemicals. In many cases, essential oils have been used as a means to control various arthropods while minimizing the amounts of harmful chemicals in the environment. While certain essential oils have been shown as an effective method of controlling arthropods, none have compared lemongrass, nerolidol, and tea tree oils against various mosquito instars. This study tests lemongrass, nerolidol, and tea tree oils in the killing of both laboratory reared and wild caught mosquitoes located in College Station, Texas. Results show that nerolidol (P<0.001) and a ratio of 1:2 tea tree and nerolidol (P<0.001) are significantly effective as a larvicide in the amounts tested.

Key words: Dengue, essential, oils, *albopictus*, larvicide, instars

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Aedes albopictus can be found in abundance in the southern U.S., including College Station, Texas (Boothe 2015). Also known as the Asian tiger mosquito, this species is not only a nuisance to the public, but also a danger. This species has been known as a vector of Yellow Fever, Dengue, and Chikungunya. These with a combined annual infection rate of over 100 million, these diseases are a threat to public health (Kraemer UGM et al. 2015). Controlling *A. albopictus* is important in preventing further spread of diseases. Female *Aedes* mosquitoes lay their eggs in water, often near human activity. These breeding sites, if left untreated, can potentially cause a rise in populations if the hundreds of eggs hatch. Treating adults can be difficult since they can disperse quicker than larvae and have shown to grow resistant to certain pesticides (Vontas et al. 2012).

With the resistance to traditional pesticides from mosquitoes including *A. albopictus*, people have turned to more natural and organic methods of controlling these pests (Kimbaris et al. 2007). A wide variety of essential oils have been shown to deter and kill adult and larvae of various arthropods, including mosquitoes (Moore et. Al. 2007) (Campli et al. 2012). Essential oils have been used throughout Chinese, Greek and Indian history in a variety of ways, including as an insecticide. Modern insect repellents can contain traces of essential oils such as lemongrass, cedar, and pine oil (Maia and Moore 2011).

This experiment aims to test the efficacy of lemongrass, nerolidol, and a ratio of tea tree and nerolidol oil as an organic larvicide against *A. albopictus.* In a previous study, nerolidol, alone and in a 1:2 ratio with tea tree oil, has shown to be very efficient in killing human head lice in less than 30 minutes (Campli et al. 2012). Nerolidol and tea tree oil have not been tested together on *A. albopictus* larvae or compared to the currently used lemongrass oil in the water to oil ratios this experiment is testing. It is predicted as with the previous study on lice, the tea tree and nerolidol 1:2 mix will be most effective at killing the mosquito larvae.

Materials and Methods

Mosquito Larvae

For this experiment, both laboratory raised and wild caught mosquitoes were used for testing the efficacy of essential oils as a larvicide. For the lab raised mosquito larva, *A. albopictus* eggs were obtained and hatched in Dr. Hammer's laboratory at Texas A&M University. The eggs were allowed to hatch over the course of three days and were given powdered beef liver as a food source. The larva collected ranged from the first through fourth instar stage. Wild mosquito larva were caught from a puddle in College Station, TX, at Wolf Pen Creek Trails. The larvae were keyed out and identified as *A. albopictus.* In order to test similar life stages as the laboratory raised mosquitoes, first through fourth instars were selected from the wild.

Essential Oils

The oils being tested were 100% tea tree oil (NOW, Bloomingdale, Illinois), 100% lemongrass oil (NOW, Bloomingdale, Illinois) and 100% nerolidol oil (TCI America, Portland, Oregon). Nerolidol and lemongrass were tested as separate oils, and tea tree oil was tested with nerolidol in a ratio of 1:2.

Testing the Oils

In order to ensure that the oil would be distributed in the water, filter paper (Melitta, Clearwater, Florida) was impregnated with 10 ml of each undiluted oil being tested, as well as a control with 10 ml of distilled water. The papers were left to dry over three days in a 23ºC room. Metal pans (30x20cm) (hBARSCI, Rochester, New York) were sanitized and filled with 400 ml of distilled water. The impregnated filter paper was placed at the bottom of the pan; no paper was left on the surface of the water. For the first trial, approximately 30 laboratory raised mosquitoes in the first, second, third and fourth instars were selected at random and placed in glass petri dishes (Living Systems Instrumentation, St. Albans, Vermont). The petri dishes were then placed in a randomly selected metal pan with the impregnated filter paper. In order to measure the number of deaths objectively, a strict definition of death was established beforehand (Campli et al. 2012). If the larva did not respond to stimulation by a pipette, it was observed under a dissecting microscope. If there was still no movement, it was declared dead. The number of deaths were recorded at 5, 10, 15, 20, 30, 60, 120, and 180 minutes. This process was repeated with wild caught mosquitoes for the last two trials. If there was any preference in the instar that was most effected by death in any trial, these differences were noted. Once the data was recorded, it was analyzed using ANOVA and a post-hoc Tukey calculator. Approximately 30 mosquitoes were selected from the wild larvae and reared in a 12x12x12 cage (Bioquip, Rancho Dominguez, California) and keyed out as *A. albopictus* adults.

Results

After analyzing the results of the total deaths in each trial, there was a significant difference when treating the mosquito larvae with nerolidol ($P<0.05$) and 1:2 ratio of tea tree and nerolidol ($P<0.05$) when compared to the control. Fig.1 shows that in all three trials, nerolidol and the 1:2 ratio of oils were the only substances to kill more than one larva in 180 minutes. In two out of the three trials, the two substances were able to kill all of the larvae in each pan. As shown in Fig. 2, the average deaths per time elapse for nerolidol was five, with a standard variation

Time elapsed	5 mins	10 mins	15 mins	20 mins	30 mins	60 mins	120 mins	180 mins
Trial 1								
Nerolidol	1	1	4	2	8	7	7	All Dead
Lemongrass	0	0	0	0	0	0	0	0
Ratio of 1:2	0	0	5	5	6	3	8	1
Control	0	0	0	0	0	0	0	0
Trial 2								
Nerolidol	25	5	All Dead					
Lemongrass	0	0	0	0	0	0	0	0
Ratio of 1:2	24	4	All Dead					
Control	0	0	0	0	0	0	0	0
Trial 3								
Nerolidol	29	0	0	0	1	2	All Dead	
Lemongrass	1	0	0	0	0	0	0	0
Ratio of 1:2	21	4	[illegible]	0	0	0	1	1 (All Dead)

Fig. 1. Total number of deaths per time elapse.

Treatment	Control	Nerolidol	Lemongrass	Ratio of 1:2
Observations N	24	24	24	24
Sum ∑Xi∑xi	0	120	0	86
Mean	0	5	0	3.5833
Sample Standard Deviation	0	8.5262	0	6.2964

of 8.5262. The ratio of 1:2 had an average of 3.5833 deaths per time elapse and a standard variation of 6.2964. In all three trials, lemongrass oil and the control did not cause any deaths in the mosquito larvae.

Treatment Comparisons	Tukey HSD Q Statistic	P-Value	P>0.05
Control Vs. Nerolidol	4.5939	0.0085127	Yes
Control Vs. Lemongrass	0	0.8999947	No
Control Vs. 1:2	3.9429	0.0076847	Yes
Nerolidol Vs. Lemongrass	4.6221	0.0081524	Yes
Nerolidol Vs. 1:2	1.3157	0.7639695	No
Lemongrass Vs. 1:2	3.895	0.008399	Yes
[illegible]	[illegible]	[illegible]	[illegible]
Laboratory reared Vs. Wild reared (Treated with 1:2)	0.0848	0.8999947	No

Fig. 3. Post Hock HSD test Results.

The ANOVA with post-hoc Tukey HSD test results are shown in Fig. 3. There was a significant difference between the control group when compared with nerolidol (*P*=0.0085) in killing *A. albopictus* larvae. When compared to the control, the 1:2 mixture had a significant difference as well (*P*=0.0077). The tea tree and nerolidol ratio of 1:2 had no significant difference when compared with nerolidol (*P*=0.7670). When comparing the wild and laboratory reared larvae, treating with nerolidol and the ratio of 1:2 had no significant difference (*P*=0.9000) in the first trial compared with the last two.

Discussion

This experiment found that nerolidol and a tea tree and nerolidol ratio of 1:2 were significantly effective at killing the larvae of *A. albopictus* in various larval instars. In all but one trial, all of the mosquito larvae were dead within 2 hours. There was no significant difference between the two substances (*P*=0.9000). With testing both oils, there was no preference in the instars that were affected the most.

Plant based insecticides can be an inexpensive and effective way of controlling mosquitoes that can vector dangerous diseases. In areas that have a high contraction rate of mosquito borne diseases, there is a resistance to traditional pesticides that needs to be addressed. Organic oils have been shown to be more culturally acceptable and can help control mosquito populations (Moore et al. 2007).

Tea tree oil has been shown to have antibiotic, antifungal, and antimicrobial properties in several independent studies. Tea tree is an extract from the Australian plant *Melaleuca alternifolia*. The oil has also shown to cause the loss of viability and the inhibition of respiration that is glucose-dependent. This effect is greater in organisms that are in the exponential growth phase (Carson et al. 2006). These properties would affect the ability for the growing larvae of *A. albopictus* to survive after being exposed to the oil.

Nerolidol occurs naturally in several plants and is known for its' floral odor. It is used in cosmetics and as a flavoring agent in food. Nerolidol is extracted from plants during the production of (3E)-4,8-dimethy-1,3,7-nonatriene, a volatile that safe guards plants from damage caused by herbivores. Nerolidol is reported to be antimicrobial, anti-parasitic, anti-inflammatory, and anti-Leishmaniasis to name a few properties. When the toxic and hemolytic effects of nerolidol were analyzed, it was found that nerolidol was more potent when compared to the terpenes that cause a spike in membrane fluidity. These results show that while nerolidol increased membrane fluidity, it had a higher ability to cause a disruption in the cells membrane. This could also increase the cytotoxic potential in cells. In an in vitro experiment, nerolidol had shown the ability to decrease ATP. This would mean a decrease in energy transfer within cells. With this decrease in energy, the oil would cause the cells to go into hepatic cell cytotoxicity. The ending result would mean death (Chan et al. 2016). These properties would explain the significant difference in larval death shown in nerolidol when compared to the control group.

While some essential oils are relatively safe to use around mammals if properly applied, further studies should be done in order to understand the full impact they could have on the environment (Moore et al. 2007). This study tested 100%, undiluted oils against mosquito larva. It is important to note that in high concentrations, oils do have a negative effect if it comes in contact with mammal skin, and should never be ingested. With most essential oils, a safe concentration for mammal skin is typically less than 4% (Chan et al. 2016).

Due to the unknown consequences of nerolidol in the environment, the results of this experiment could help those living in an area that has places that accumulate puddles of water, like old tires and containers. Small amounts of water could end up harboring mosquito larvae if left untreated (Chan et al. 2016). The oils should be where dogs or children would not be exposed.

Lemongrass oil is currently used as an insecticide and has been tested in low concentrations. One drawback is the longer amount of time it takes to kill or harm the larvae compared to tea tree and nerolidol oil (Soonwera and Siriporn 2016). For further studies, nerolidol and tea tree should be tested at several concentrations to determine the lowest amount needed in order to still be significantly effective in killing larvae. Another limitation of this study was only having one trial in using laboratory reared mosquitoes and two trials of wild caught. In the end, they were not significantly different from each other; however more trials would yield a more accurate *P* value.

References

Boothe, E. C. 2015. QUANTIFYING DISPERSAL BEHAVIOR AND ABATEMENT EFFICACY FOR Culex quinquefasciatus AND Aedes albopictus IN COLLEGE STATION, TEXAS.

http://oaktrust.library.tamu.edu/bitstream/handle/1969.1/155276/BOOTHE-THESIS-2015.pdf?sequence=1&isAllowed=y

Campli, E. D., S. D. Bartolomeo, P. D. Pizzi, M. D. Giulio, R. Grande, A. Nostro, and L. Cellini. 2012. Activity of tea tree oil and nerolidol alone or in combination against Pediculus capitis (head lice) and its eggs. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3480584/

Carson, C. F., K. A. Hammer, and T. V. Riley. 2006. Melaleuca alternifolia (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1360273/

Chan, W. K., L. T. H. Tan, K. G. Chan, L. H. Lee, and B. H. Goh. 2016. Nerolidol: A Sesquiterpene Alcohol with Multi-Faceted Pharmacological and Biological Activities. http://www.mdpi.com/1420-3049/21/5/529

Kimbaris, A. C., E. Kioulos, G. Koliopoulos, M. G. Polissiouc, and A. Michaelakis. 2007. Coactivity of sulfide ingredients: a new perspective of the larvicidal activity of garlic essential oil against mosquitoes. http://onlinelibrary.wiley.com.ezproxy.library.tamu.edu/doi/10.1002/ps.1678/pdf;jsessionid=3D7DD102F086B0DA9A11B38976995168.f03t04

Kraemer, M. U. G., M. E. Sinka, K. A. Duda, A. Mylne, F. M. Shearer, O. J. Brady, J. P. Messina, C. M. Barker, C. G. Moore, R. G. Carvalho, G. E. Coelho, W. V. Bortel, G. Hendrickx, F. Schaffner, G. R. W. Wint, I. R. F. Elyazar, H. J. Teng, and S. I. Hay. 2015. The global compendium of Aedes aegypti and Ae. albopictus occurrence. https://www.nature.com/articles/sdata201535

Maia, M. F., and S. J. Moore. 2011. Plant-based insect repellents: a review of their efficacy, development and testing. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3059459/

Moore, S. J., N. Hill, C. Ruiz, and M. M. Cameron. 2007. Field Evaluation of Traditionally Used Plant-Based Insect Repellents and Fumigants Against the Malaria Vector Anopheles darlingi in Riberalta, Bolivian Amazon.

Soonwera, M., and P. Siriporn. 2016. Effect of Cymbopogon citratus (lemongrass) and Syzygium aromaticum (clove) oils on the morphology and mortality of Aedes aegypti and Anopheles dirus larvae. https://link.springer.com/article/10.1007/s00436-016-4910-z

Vontas, J., E. Kioulos, N. Pavlidi, E. Morou, A. D. Torre, and H. Ranson. 2012. Insecticide resistance in the major dengue vectors Aedes albopictus and Aedes aegypti. http://ac.els-cdn.com/S0048357512000818/1-s2.0-S0048357512000818-main.pdf?_tid=4ef7b514-31d0-11e7-8b85-00000aacb35f&acdnat=1494015820_dfed84276eed90e50f55ad687be4b90e