The Effects of Rice Bran Oil as a Repellent for *Aedes aegypti* Compared to Mineral Oil and Effective Natural Repellents

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Mosquitoes that spread viruses are called vector mosquitoes and can be extremely dangerous to humans, but bites from other mosquitoes can also lead to side effects such as itching, irritation, or swelling. Rice bran oil has not yet been tested as a repellent, but it possesses similar properties to mustard seed oil, which has been proven to have insect-repelling tendencies by previous studies (Mukesh et al., 2014). Discovering novel repellents is necessary because of recent studies that have been conducted regarding the resurgence of mosquito-borne diseases like arboviruses and malaria (Dahmana & Mediannikov, 2020). The purpose of this study was to determine the ability of rice bran oil to act as a mosquito repellent. The mosquito-repelling abilities of various essential oils that have been proven to be effective in preventing mosquito contact were compared with rice bran oil and mineral oil. It was hypothesized that rice bran oil would have significantly fewer *Aedes aegypti* landings than the odorless oil, as rice bran oil has a similar composition to mustard seed oil, an effective mosquito repellent. The number of *Aedes aegypti* landings in each oil was counted, as a significant number of mosquito landings would classify a compound as an ineffective repellent. A one-way ANOVA was conducted and found a significant difference between at least one pair of means, with $F(4,70)=86.78$, $p<.001$, and a Tukey test showed a significant difference between the rice bran and mineral oil. Rice bran oil was a more effective repellent than the control.

Introduction

Aedes aegypti, commonly known as the yellow fever mosquito, is responsible for spreading various diseases like dengue fever and the Zika virus. Mosquitoes that spread viruses are called vector mosquitoes and can be extremely dangerous to humans, but bites from other mosquitoes can also lead to side effects such as itching, irritation, or swelling (Dahmana & Mediannikov, 2020). In the past, a large variety of essential oils have been tested and proven to be effective mosquito repellents. Rice bran oil has not yet been tested as a repellent, but it possesses similar properties to mustard seed oil, which has been proven to have insect-repelling tendencies by previous studies. It has been discovered that mosquitoes are constantly developing resistance to different natural repellents over time. In one study, a Duke University neurotoxicologist noted that it is essential to continuously develop alternative repellents to eventually replace current ones (Nuwer, 2021). Discovering novel insect repellents is also necessary due to the many studies that have recently been conducted regarding a resurgence of mosquito-borne diseases like arboviruses and malaria evident in new regions due to the mismanagement of insecticides and the emergence of repellent resistance (Dahmana & Mediannikov, 2020).

There are various reasons as to why essential oils are able to repel mosquitoes and other insects. When examining the external structure of a mosquito, it is evident that mosquito legs have a highly hydrophobic surface structure. This often causes attraction forces between the surface of the mosquito legs and the oil (Wu & Kong, 2007). However, in one study it was discovered that mosquito contact time with oils is much lower when compared with hydrophilic liquids like water (Iikura et al., 2020). These findings suggest that the oil coating induces an escape response in the mosquito that causes it to be repelled from the oil. Another reason for the ability of oils to repel insects is due to the odors that they emit. In a mosquito's olfactory system, the odorant receptors are located on the antennae and maxillary palps, and this allows for the mosquito to detect odor chemicals on the dendrites of neurons on these areas (Wheelwright et al., 2021). Mosquitoes have odor receptors that are sensitive to smells, which is shown in their attraction to human blood.

Genes contributing to the behavioral evolution of mosquitoes have also prompted examinations into the odors of various essential oils and how they, like human blood, affect mosquito odor receptors. Most synthetic repellents are effective due to their ability to mask the smell of human blood, which consequently leads to less mosquito attraction (Afify et al., 2019). One study found that some synthetic repellents do not directly activate the olfactory neurons of mosquitoes but they instead function as “maskers,” a term used to describe odors that decrease odor-evoked responses of olfactory neurons (Afify et al., 2019). On the other hand, essential oils are able to act as effective repellents because of their ability to directly impact insects’ olfactory neurons.

Mustard seed oil is an oil extracted from the seeds of mustard plants. In India, this oil is extracted from black mustard seeds, or *Brassica nigra*, while in Russia and China it is extracted from brown mustard seeds, or *Brassica juncea* (Divakaran & Babu, 2016). Although the typical purpose of mustard seed oil is for cooking or hair health, there is also evidence to suggest that it can be utilized as an effective mosquito repellent. In one study, it was discovered that using a mustard oil base for a repellent offered 86.36% and 85% protection with a total protection time of 230 and 240 min, respectively (Mukesh et al., 2014). An oil with a similar composition as mustard seed oil is rice bran oil. Rice bran oil is the oil extracted from the outer shell of a rice grain, and it is most often used in cooking. The two oils have similar sodium and glycemic index levels, but more importantly, they have very similar odors, which is a source of justification for experimenting with rice bran oil as a mosquito repellent. Along with this, rice is a crop grown in many developing countries that do not possess adequate access to vaccinations against mosquito-borne diseases, including countries in Asia and coastal areas in Africa (Linares, 2002). In some situations, irrigated areas that grow rice in surpluses experience a higher mosquito population, and as a result, these areas can be at a higher risk for malaria cases if not treated properly (Chan et al., 2022).

The purpose of this research topic was to determine the ability of rice bran oil to act as a mosquito repellent. Rice bran oil has not been previously tested as a mosquito repellent even though it is often cheaper and more readily available in developing countries than most other essential oils, providing the study’s literature gap (Linares, 2002). In order to study this, the mosquito-repelling abilities of lemon oil, eucalyptus oil, and peppermint oil, which have been previously proven to be effective in preventing mosquito contact, were compared with rice bran oil and mineral oil, an odorless oil. The number of *Aedes aegypti* landings in each of the oils was measured, as a compound with significantly less mosquito landings than the control would be classified as more effective at repelling the mosquitoes. It was hypothesized that rice bran oil would have significantly less *Aedes aegypti* landings than the mineral oil, as the rice bran oil has a similar composition and odor as mustard seed oil, an oil that has previously been proven to be an effective *Aedes aegypti* repellent.

Literature Review

Mosquito Body Composition

A large part of the mosquito repelling abilities of hydrophobic liquids is due to the composition of mosquito legs. The fine geometrical structure of the mosquito leg surface generates a weight-supporting force on water surfaces, leading to one mosquito leg having 23 times the maximum repulsive force of a single mosquito leg (Wu & Kong, 2007). This trait evolved in mosquitoes in order to allow females to grip the surface in order to lay their eggs, meaning that casting a silicone oil-based liquid on a water surface prevents mosquitoes from using the superficial layer as a foothold to lay eggs. In one study done to test mosquito-repelling activities induced by tarsal contact with hydrophobic liquids, it was discovered that an oil-coated surface leads to an increased escape response from mosquitoes (Iikura et al., 2020). Another major characteristic in mosquitoes leading to attraction or repulsion to certain compounds is the odor receptors. One study discusses an evolutionary preference for human odor in domestic mosquitoes that is tightly linked to increases in the expression and ligand-sensitivity of the odorant receptor AaegOr3, which is present in high levels in human odor (McBride et al., 2014). This attribute provides an understanding of how female vector mosquitoes found a preference for human blood. Another reason for the ability of essential oils and other repellents to prevent insect contact is due to the odors that they emit. In one study, it was found that certain repellents possess the ability to inhibit a mosquito's odorant receptors, which as a result would cause less attraction to human blood (Tsitoura et al., 2015).

Mosquito-Testing Strategies

There are various methods of testing the efficacy of insect repellents. The arm-in-cage method is the most common test as described by the World Health Organization (WHO) and the Environmental Protection Agency (EPA). To evaluate a topical repellent according to WHO guidelines, the repellent must be applied to the participant's arm, which is then placed in the cage filled with host-seeking female mosquitoes. The repellent's efficacy is determined by the landing behavior of the mosquitoes (Moreno-Gomez et al., 2021). This method would not be possible while following the BSL-1 guidelines, so a more feasible method would be to test the repellents without using human participants. This method entails placing a Petri dish filled with the repellent being tested somewhere in the insect containment cage and then counting the number of times the mosquitoes make contact with the dish. This measurement would be compared to the number of landings in odorless oil as a control, as the odorless oil should not act as a repellent and would therefore yield more mosquito landings than the repellent compound being tested. This method of placing the treatments in a Petri dish and counting the number of times the mosquitoes landed in the substance has been used in many studies done to test the efficacy of essential oils as insect repellents. One example was in a study performed to test the efficacy of various volatile oils at repelling mosquitoes, where fewer mosquito landings implied higher repellent efficacy (Tawatsin et al., 2001).

Current Established Repellents

The first milestone in topical repellent development was the discovery and implementation of *N,N*-diethyl-*3*-methylbenzamide, or DEET. Most marketed repellents have a high concentration of DEET in order to be effective and durable. However, some individuals prefer not to use DEET for a variety of reasons, including dermal irritation and other perceived health concerns, and may instead turn to alternative products (Carroll, 2019). Larvicides are an alternative that is often used to prevent mosquito contact, as they reduce the mosquito population in breeding places, where they are concentrated, immobilized, and accessible before they are able to become adults (Osanloo et al., 2019). The application of synthetic larvicides or the usage of an insect growth regulator makes up the process of larviciding.

Natural Repellents Versus Synthetic Repellents

Due to the skin irritation caused by repellents like DEET and the overall harmful effects on the environment caused by synthetic repellents, essential oils and other natural repellents fulfill the demand for eliminating insecticides and chemical repellents. Synthetic repellents like DEET work as acetylcholinesterase inhibitors, allowing them to block the insect's olfactory receptors for substances found in odor (Legeay et al., 2016). DEET, for instance, acts directly on the odorant to decrease its volatility and therefore reduces the amount of attractive odorants capable of activating mosquito olfactory receptors (Afify et al., 2019). However, alternatives to these synthetic repellents are being discovered due to the fact that the repellents only work when they are in extremely high concentrations. Natural repellents, on the other hand, are able to repel mosquitoes by doing the opposite and activating neurons in the insect. In one study, it was discovered that the natural repellents eugenol and lemongrass oil strongly activate a subset of olfactory receptor neurons, and this similarly occurs with almost every essential oil that has been proven as a practical insect repellent (Afify et al., 2019).

Rice Prevalence Around the World

The two species of cultivated rice in the world are *Oryza glaberrima*, or African rice, and *Oryza sativa*, or Asian rice (Linares, 2002). Currently, the African species of rice, which is native to sub-Saharan Africa, is being replaced in West Africa by the Asian species due to the introduction by the Portuguese in the middle of the 16th century. It has been found in one study that the malaria parasite prevalence is significantly higher in rice-growing versus non-rice-growing villages across various countries in Africa. Since 2003, malaria rampancy has become almost two times higher in rice-growing villages versus non-rice growing (Chan et al., 2022). This is because irrigated rice-growing communities in sub-Saharan Africa are exposed to greater malaria risk, meaning a higher mosquito population is present. The African mosquito vector *Anopheles gambiae* is long-lived and prefers to bite humans, causing them to be exceptionally efficient in transmitting malaria, which plays a significant role in Africa where 96% of the world's malaria mortality burden is accounted for (Chan et al., 2022). As a result, researchers are currently attempting to discover ways to inhibit mosquito population growth related to rice cultivation.

Methods

Once *Aedes Aegypti* larvae were acquired through Niles Biological in California, they arrived separated in two bags like the one shown in Figure A1. The two bags of water filled with around 60 larvae in each bag were poured into two plastic containers, which were then covered with saran wrap. These plastic containers were then placed in an insect containment cage provided by the local Department of Health and Environmental Control (DHEC) as displayed in Figure A2, and the saran wrap was torn in order to allow oxygen circulation. After around one week, the majority of the *Aedes aegypti* larvae had grown into adult mosquitoes. A Petri dish filled with nectar that was changed out daily was placed in the corner of the cage as the diet for the adult *Aedes aegypti*, and the containers with the remaining larvae were discarded. There were around one hundred mosquitoes remaining in the cage when the oils began to be tested.

The first oil to be tested was mineral oil, which served as the control due to its lack of an odor and therefore the unlikelihood of having any insect-repelling potential. Water was not used as a control, as mosquito legs behave differently between hydrophobic and hydrophilic liquids, so a lipid would serve as a more accurate point of comparison with the essential oils. 10 drops of the mineral oil were placed on a Petri dish that was then placed in the corner of the cage opposite to the Petri dish of nectar. This was done by the researcher first putting on a thick beekeeper glove in order to avoid being bitten by the mosquitoes. Then, the mesh net on the side of the containment cage as shown in Figure A2 was untied enough to allow the gloved hand to enter the cage without allowing the mosquitoes to escape. Once the Petri dish was placed in the cage, the number of times that the *Aedes aegypti* landed in the Petri dish of mineral oil in the span of one minute was counted using a stopwatch and clicker. After this quantity was recorded, the Petri dish was then taken out through the mesh net and properly disposed of. These methods were repeated for 15 trials using the same oil. After the mineral oil was tested for 15 trials, rice bran oil, lemon oil, eucalyptus oil, and peppermint oil were tested in the same manner. The latter three oils have been proven to be effective *Aedes aegypti* repellents in previous research as mentioned beforehand, so these oils served as a point of comparison to the rice bran oil besides the control in order to determine a more accurate degree of efficacy of the rice bran oil. A diagram displaying the five different oils used is located in Figure A3. After the *Aedes aegypti* landings were counted and recorded for 15 trials of each of the five oils, a one-way ANOVA was conducted to determine whether there was a significant difference between at least one pair of means, as this would imply the possibility of rice bran oil having a significant difference compared to the control, which was mineral oil.

Results

In order to determine the mosquito-repelling abilities of each oil, quantitative data was collected as the number of times *Aedes aegypti* landed in each oil within one minute. The raw data from the fifteen trials for each of the five oils is located in Table A4. The central tendency values are displayed in Table A1. From these statistics, it was found that the mineral oil, which served as the control in this experiment, had the most *Aedes aegypti* landings counted on average (M=47.93). The rice bran oil was found to have a lower number of average mosquito landings than the mineral oil (M=38.53). The lemon oil also yielded a lower number of mosquito landings (M=22.07), and the eucalyptus oil showed a slight decrease in mosquito landings compared to the lemon oil (M=21.80). The peppermint oil was found to have the lowest average number of mosquito landings (M=10.60).

The bar graph displayed below in Figure A4 shows the average number of *Aedes aegypti* landings in each of the tested oils. It is evident that the mineral oil yielded the highest number of mosquito landings, while the peppermint oil yielded the lowest.

In order to determine whether there was a significant difference between at least one pair of means, a one-way ANOVA test was conducted. The results of this test including the sum of squares, the degrees of freedom, and the mean of squares for the factor and the error are located in Table A2. The F-test value was calculated to be 86.78, and the p-value was <.001. Because the p-value was calculated to be less than the alpha value of 0.05, the test was statistically significant and it was determined that there was a statistical difference between at least two of the tested oils.

After it was determined through the one-way ANOVA test that there was a significant difference between at least one pair of means, a post-hoc test was conducted to evaluate which oils the difference was significant between. A Tukey test was chosen for the post-hoc test due to each of the variable groups having the same sample size. The results for the Tukey test are located in Table A3. With a critical value of 3.92 and a mean squared error of 38.22, it was found that the T value was 6.26. From the Tukey test, it was found that there was a significant difference between the mineral and rice bran oil, mineral and lemon oil, mineral and eucalyptus oil, mineral and peppermint oil, rice bran and lemon oil, rice bran and eucalyptus oil, rice bran and peppermint oil, lemon and peppermint oil, and eucalyptus and peppermint oil. This was due to these groups having a mean difference higher than the T value of 6.26. Because the mean difference between the lemon and eucalyptus oil was 0.27, which is lower than the critical value of 6.26, it was determined that there was no significant difference between the mean values of these groups.

Discussion

The purpose of this experiment was to determine the efficacy of rice bran oil as a mosquito repellent. Mosquitoes tend to develop resistance to repellents that are used over a long period of time, placing importance on constantly discovering new effective repellents. Discovering novel insect repellents is also necessary due to the many studies that have recently been conducted regarding the resurgence of mosquito-borne diseases like arboviruses and malaria in new regions (Dahmana & Mediannikov, 2020). Rice bran oil was justified as a potential candidate for a mosquito repellent due to its similarity in odor to previously proven natural repellents, and its significance was evident in major part because of higher mosquito populations found in irrigated rice-growing communities in developing countries without proper access to vaccinations against diseases caused by mosquito vectors (Chan et al., 2022). It was hypothesized that rice bran oil would have significantly fewer *Aedes aegypti* landings than the mineral oil, as the rice bran oil has a similar composition and odor as mustard seed oil, which is an established effective natural insect repellent. This hypothesis was supported, as the Tukey test showed a significant mean difference between the mineral oil and rice bran oil. Because the mineral oil served as the control due to its lack of an odor, the significantly lower number of *Aedes aegypti* landings in the rice bran oil compared to the mineral oil supported the hypothesis. The p-value that was calculated in the one-way ANOVA test in Table A2 was also lower than the alpha value of 0.05, further proving that the difference between the mosquito landings in the rice bran oil and the control did not occur by chance.

Along with being compared to mineral oil, which served as the control, rice bran oil was also compared to three oils that have been proven in the past to act as effective natural repellents: lemon oil, eucalyptus oil, and peppermint oil. Through doing so, it was found that, similar to the rice bran oil, there was a significant mean difference between each of these three oils and the control. From the descriptive statistics in Table A1, it can also be seen that although the rice bran oil yielded a lower number of *Aedes aegypti* landings than the mineral oil, its average number of *Aedes aegypti* landings was still higher than those of the lemon oil, eucalyptus oil, and peppermint oil, implying that the rice bran oil was effective as a repellent compared to the control, but it was not as effective as the lemon oil, eucalyptus oil, and peppermint oil.

From the mean values, there are also patterns that can be discerned regarding the efficacy of lemon oil, eucalyptus oil, and peppermint oil. The lemon oil and eucalyptus oil had a mean difference much lower than the critical value, implying that their abilities to repel the *Aedes aegypti* are similar. The peppermint oil had the lowest average number of mosquito landings and a significant mean difference from each of the other oils, meaning that it was the most effective in preventing the mosquitoes from landing on the Petri dish. The rice bran oil had a significant mean difference from the mineral oil and had a lower average number of mosquito landings, meaning that it was more effective at repelling the mosquitoes than the mineral oil. However, the rice bran oil had a higher average number of mosquito landings than the other oils that were tested, meaning that it did not repel the mosquitoes to the same extent that the lemon, eucalyptus, and peppermint oils were able to. The latter three oils had odors that were more potent than the rice bran oil, which may have contributed to the disparity between mosquito landings.

This study was the first to examine rice bran oil's efficacy as a repellent for *Aedes aegypti* or any other vectors, but the results found in this experiment correspond with those found in similar studies that examined the mosquito-repelling abilities of the other three oils tested, or lemon, eucalyptus, and peppermint oil. In one study, peppermint essential oil was proven to be an efficient larvicide and repellent against dengue vectors (Kumar, Wahab, & Warikoo, 2011). In another study examining the bioefficacy of three Citrus essential oils against *Aedes albopictus*, it was found that lemon peels were also effective in repelling mosquitoes (Giatropoulos et al., 2012). In a study done to determine the larvicidal efficacy of neem oil and eucalyptus oil, it was found that eucalyptus oil was more effective against mosquito larvae (Kaura et al., 2019).

The sources of uncertainty in this study could have been prevented significantly if the experiment were performed in a different environment. The experiment was conducted in a garage during the winter, meaning that the low outside temperatures could have impacted the patterns of *Aedes aegypti* activity over time. Another way in which the environment could have impacted the results is through the possible addition of another odor that may have come from the outside environment. Because the efficacy of a repellent often relies on the potency of its odor, external odors coming from the outdoor environment may have impacted the results. If the experiment were to be performed in a proper lab environment, it would also be likely that more *Aedes aegypti* larvae would survive due to the monitored temperature.

A possible extension to this study could be to examine the efficacy of a different oil or natural substance, and this would be done by comparing the possible repellent to mineral oil along with other proven effective natural repellents. It may be valuable to test the efficacy of mustard seed oil compared to rice bran oil, as they have similar odors and compositions and it would be interesting to examine if this translates into similar repelling abilities. Another possible future study could be to determine whether certain oils possess a larvicidal ability rather than simply a repelling one. This could be conducted through the examination of the long-term effects of exposing mosquitoes to a certain natural repellent.

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Appendix

Figure A1: Bag of Aedes aegypti Larvae as Provided by Manufacturer

Figure A2: Insect-Containment Cage Used for Mosquitoes

Figure A3: Picture of the Different Oils Used in Experimentation

Figure A4: Experimental Design Diagram

Title of the Experiment The Effects of Rice Bran Oil as a Repellent for Mosquitoes Compared to Mineral Oil and Effective Natural Repellents					
Hypothesis Rice bran oil would have significantly less mosquito landings than the mineral oil, as the rice bran oil has a similar composition and odor as mustard seed oil, an established effective natural insect repellent.					
Independent Variable Type of oil					
Levels of Independent Variable	Mineral oil (control)	Rice bran oil	Lemon oil	Eucalyptus oil	Peppermint oil
Number of Repeated Trials	15	15	15	15	15
Dependent Variable Number of mosquito landings in each oil					
Constants - Environment of experimentation - Nectar given to mosquitoes - Source of each individual oil tested - Type of containment cage used					

Table A1: *Descriptive Statistics for Aedes aegypti Landings in Each Oil*

Type of Oil	*Mean Score*	*Standard Deviation*	*Variance*	*Sample Size*
Mineral oil	47.93	6.70	44.92	15
Rice bran oil	38.53	9.16	83.98	15
Lemon oil	22.07	4.93	24.35	15
Eucalyptus oil	21.80	4.80	23.03	15
Peppermint oil	10.60	3.85	14.83	15

Figure A5: Average Number of Aedes aegypti Landings in Each Oil

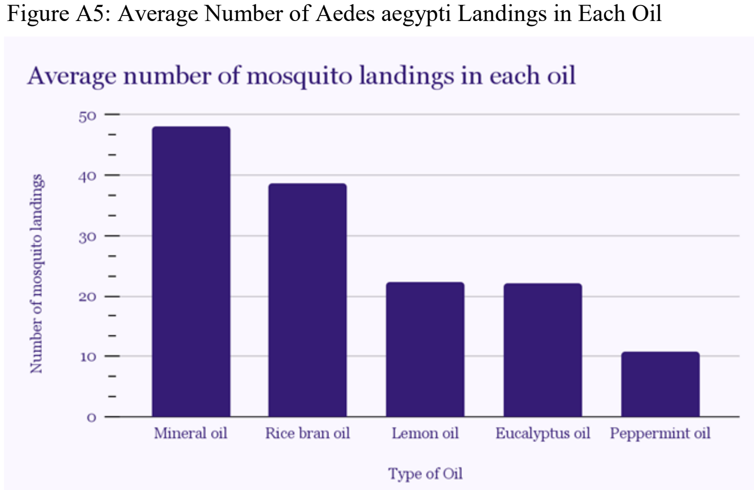

Table A2: *One-way ANOVA Summary Table of Results*

Source of Variation	*SS*	*df*	*MS*	*F*	*p-value*	*F crit*
Between groups	13267.79	4	3316.95	86.78	<.001	2.50
Within groups	2675.6	70	38.22			
Total	15943.39	74				

Table A3: *Tukey Test Table of Results*

Groups	*Mean Difference*
Mineral and rice bran	9.4
Mineral and lemon	25.87
Mineral and eucalyptus	26.13
Mineral and peppermint	37.33
Rice bran and lemon	16.47
Rice bran and eucalyptus	16.73
Rice bran and peppermint	27.93
Lemon and eucalyptus	0.27
Lemon and peppermint	11.47
Eucalyptus and peppermint	11.2

Table A4: *Raw Data of Mosquito Landings for Each Oil*

	Mineral Oil	*Rice bran oil*	*Lemon pure essential oil*	*Eucalyptus pure essential oil*	*Peppermint pure essential oil*
Trial 1	43	39	22	23	13
Trial 2	56	36	25	17	9
Trial 3	49	41	18	19	14
Trial 4	38	43	31	17	11
Trial 5	41	33	19	30	8
Trial 6	47	37	27	23	6
Trial 7	59	46	22	19	11
Trial 8	56	36	13	22	20
Trial 9	43	51	18	30	14
Trial 10	47	44	21	27	7
Trial 11	52	41	27	26	9
Trial 12	56	49	20	20	5
Trial 13	39	32	22	18	8
Trial 14	44	12	17	22	13
Trial 15	49	38	29	14	11