Effectiveness of Hemp Oil and Orange Citric Oil as Fire Ant (*Solenopsis geminata)* Repellent

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Abstract: More and more people are looking for solutions to their pest problems outside of the mainstream pesticides and pest repellents found in stores, leading them to turn toward "home-remedies" such as essential oils. However, there is limited research about the effectiveness of certain essential oils on repelling and killing arthropods. This experiment covered the use of hemp oil and orange citric oil as possible repellants for fire ants (*Solenopsis geminate*) common to the Brazos Valley of Texas. Collection of ants was done from an ant mound next to the Texas A&M entomological building and the progression of the experiment was done inside the building. Petri dishes were used to contain the experiment, and the number of times the ants touched the substance was counted and compared against the others. The water control had the highest touch count and its average was statistically different (p value= 0.00028805) than the substances tested. Orange citric oil had consistent results among its three trials as well as exhibited a statistical difference between it and the control tests. Hemp oil was not as consistent in its results. Although two of the trials had counts very similar to each other, the third trial had more than double the counts than the other trials. These results show that orange citric oil was a very effective repellant to *Solenopsis geminata* compared to the water control. Hemp oil shows promise as a repellent but a larger experiment size would need to be used in order to determine the consistency of its effectiveness. Possible next steps in this line of research include expanding on hemp oil's effectiveness, determining if the ability of an oil to aerosolize changes its effectiveness, and if other essential oils are effective as repellents against *Solenopsis geminate*.

Key words: Solenopsis geminate, essential oils, hemp oil, citric oil

Traditionally, pesticides have been used to repel and kill common household pests, specifically ants. As further research is done linking severe, life-threatening diseases to chronic exposure to these pesticides, a need for a safer method of keeping unwanted pests out of the home is sought after (The Lancet, 2017). Recently, essential oils have been shown to repel and often even kill ants, specifically *S. geminata* (Hu 2017). Essential oils can be found at drugstores and are fairly inexpensive. These oils also have very low toxicity in vertebrates and are considered safe in the household if all known allergies are avoided (Francikowski 2019).

Orange citric oils contain a terpene, D-limonene, that is one of the most common found in nature. D-limonene has been categorized as "generally recognized as safe" and can even be used to flavor juices, ice creams, puddings, etc. and has a fairly low toxicity (Sun 2017). D-limonene has also been found to be an effective pesticide for many insects. This is due to the D-limonene molecule affecting the flux of sodium in and out of peripheral neurons. Maintaining consistent sodium concentrations is essential and any changes can cause many side effects in any species (Hu 2017).

Hemp oil is not as common to households as orange citric oil so there is less information, both from research-based and homeopathic-based sources, about this oil. Hemp oil is obtained from the plant *Eupatorium cannabinum L.*, also known as hemp agronomy, and is known to have antibacterial and cytotoxic properties (Judzentiene 2015). Hemp oil has been shown to be lethal (LC_{50}) to Brine shrimp larvae, in the phylum Arthropoda and subphylum Crustacea, at levels between 16.3 and 22.0ug/mL (Judzentiene 2015). This oil is of interest for the use of pest control as there are no known negative side effects in mammals, making it safe to use in larger amounts in households (Gulluni 2018).

S. geminata were chosen due to their ability to induce an anaphylactic reaction that requires immediate medical attention. Unlike most ants that bite and spray acid into the opening, *S. geminata* grab onto a victim's skin with the mandible and use a stinger located on the abdomen to inject a venomous compound, solenopsin, subcutaneously (Wijerathne 2018). Solenopsin is an alkaline toxin in the piperidine class and causes almost immediate local reactions resulting in a sterile pustule. However, in those in which are hypersensitive or allergic to molecules in the piperidine class, solenopsin can cause a systemic reaction and result in death if not treated quickly and properly. This systemic reaction is known to occur one to four hours after being stung. Typical treatment includes a dose of epinephrine and corticosteroids followed by supportive therapy (Potiwat 2015).

Material and Methods

Collection Procedure

Collection was completed on the campus of Texas A&M University directly outside the Heep Center (30°36'40.2"N 96°20'51.9"W). The date of collection was 16 April 2019 at approximately 7:00pm.

Fire ants (*Solenopsis geminate*) were collected from within their mound. All subjects used were collected at the same time. All subjects were placed into the same sealable plastic container. Petroleum Jelly was used to line the rim of the container so that the ants would not escape when the container was opened to retrieve subjects. The lid of the container was never sealed shut to prevent any possibility of asphyxiation prior to the start of the experiment. The ants were only in the collection for a time ranging from 30 minutes to 2 hours before being used in a trial.

Specimen Identification

The species of ant used to perform this experiment had a reddish-brown head and thorax with a black posterior. They also had a round head with large mandibles and were found in a small mound. Along with mandibles, these ants also had a posterior stinger with both being utilized to deliver a painful sting/bite combination. The aforementioned information combined with the geographic location, describe the *Solenopsis geminata*, also known by the common name Fire Ant.

Experiment Containment

Standard sized petri dishes (100mm) were used to contain the ants and substances during the duration of the experiment. Due to limited supplies, each of the three substances (water, hemp oil, and orange citric oil) had one of their three petri dishes made by a different company, resulting in the walls of the dish being slightly thicker and reducing the internal diameter of the dish by approximately 2mm. Petroleum jelly was used along the rims of the petri dishes to prevent ants from climbing out between the top and bottoms portions of the petri dishes. Ants were dropped into the petri dishes with the use of a small plastic spoon and the petri dishes were sealed shut once all ants were places inside. No material from the ants' mound was allowed into the petri dishes during the experiment so that they would not be attracted to portions of their home mound.

Subject and Trial Numbers

Each of the three substances would be tested in three separate trials each. Therefore, a total of nine trials were completed. Each trial was completed using five ants in each petri dish. Each ant was only used in a single trial so that no ants would be contaminated with a substance from another trial.

Substances Uses

Water was used as the control substance to compare the experimental substances against. Hemp oil and orange citric oil were used as the test substances as possible fire ant repellents. Two drops of each substance were dropped onto $1cm^2$ pieces of paper towel that were placed in the center of each petri dish. Paper towel squares were used to prevent the oils and water from spreading thin across the entire surface of the petri dish bottoms.

With the use of the towel squares, the oils and water were completely contained to the diameter of the squares. Paper squares were not intended to be needed; however, the orange citric oil was very thin so without the use of paper squares, it

would spread very easily across the entire bottom portion of the petri dishes. Each substance was therefore placed dropwise onto paper towel squares for consistency across substances.

Counting system:
Once all ants were placed in the petri dishes and the dishes were sealed, the number of times ants came into contact with the substance-soaked paper was counted during a duration of 30 minutes. Any ants who spent long times on the paper were only counted as a single touch. Any ant that was walking along the lid of the petri dish and fell onto the paper were not counted as touches due to this being an involuntary encounter.

Results

$H_0 : \mu_1 = \mu_2 = \mu_3$ $\quad$ $H_A : \mu_1 \neq \mu_2 \neq \mu_3$

Water, the positive control of this experiment, displayed the greatest number of touches by the ants which negates the prediction set prior. For trial 1, the ants touched the water 36 times including each time an ant drank the water as well as the time that it would remain on the water. In trial 2, there were even more touches committed by the ants, adding up to 43. These ants exhibited similar behavior as the first trial batch in that they would retain position on the area containing the water for long periods of time. Regarding the last trial performed with the water treatment, the ants touched the water 45 times, which was the maximum amount of touches observed through all the trials performed both for the water and the other treatments. In light of this data, it can be theorized that water actually exhibits attractive qualities in presence of fire ants.

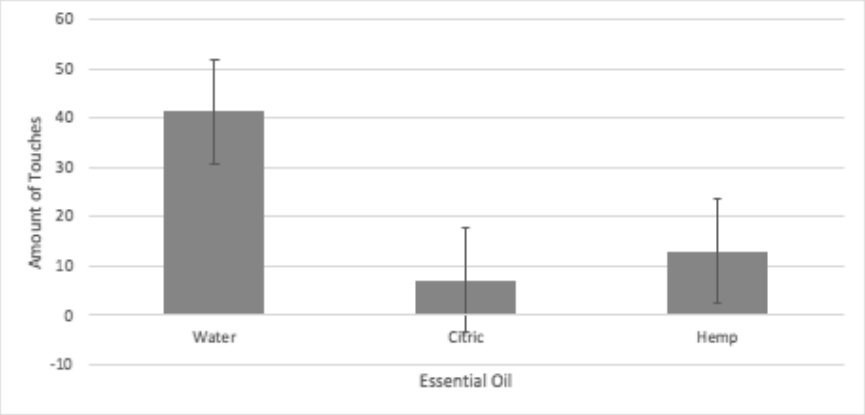
Pertaining to the hemp oil treatment, the second and third trial accumulated close values with the second resulting in 8 touches while the third had 10. However, the first trial displayed 21 touches which was double the amount for the other two trials making it an outlier in the data as well as offsetting the calculated means for this treatment. For trial one, the ants were seen walking over the treated area and also began to clean themselves after touching the oil. The self-cleaning response was also witnessed in trial 3. In trial 2, the ants touched the oil but would immediately turn away from the treated area.

In regards to the orange citric oil treatment, all three trials resulted in the same number of touches, 7, as seen in Table 1. However, more notably, the ants experienced very different behavior in the presence of this treatment in comparison to the other treatments.

Upon obtaining this data, it was theorized that the orange citric oil was the most efficient organic treatment in repelling the fire ants and possess qualities that can deal detrimental and, in some cases, deadly, repercussions upon contact with the ants. Water, which was introduced into the experiment as a control resulted in attracting the ants against premature predictions. Concerning the obtained statistics, an ANOVA test was performed. In reference to Table, the p-value for the data 0.00028805 which is indicative of strong evidence against the null hypothesis. With this knowledge it can be observed that each treatment yielded different values and were not all equal as suggested in the null hypothesis.

Table 1: Amount of Touches in Response to Treatment			
	Water	**Citric**	**Hemp**
Trial 1	36 touches	7 touches	21touches
Trial 2	43 touches	7 touches	8 touches
Trial 3	45 touches	7 touches	10 touches
Avg:	41.3333333	7	13
St. Dev:	4.72581563	0	7

Table 1. Experiment results for each trial and substance, average touches for each substance, and standard deviations for each substance



Graph 1. Averages of the 3 trials for each essential oil and consideration of positive and negative error bars

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Water	3	124	41.3333333	22.3333333
Citric	3	21	7	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1768.16667	1	1768.16667	158.343284	0.00022955	7.70864742
Within Groups	44.6666667	4	11.1666667			
Total	1812.83333	5				

Tab

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Water	3	124	41.3333333	22.3333333
Hemp	3	39	13	49

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1204.16667	1	1204.16667	33.7616822	0.00436584	7.70864742
Within Groups	142.666667	4	35.6666667			
Total	1346.83333	5				

Table 3. ANOVA: single factor for hemp oil

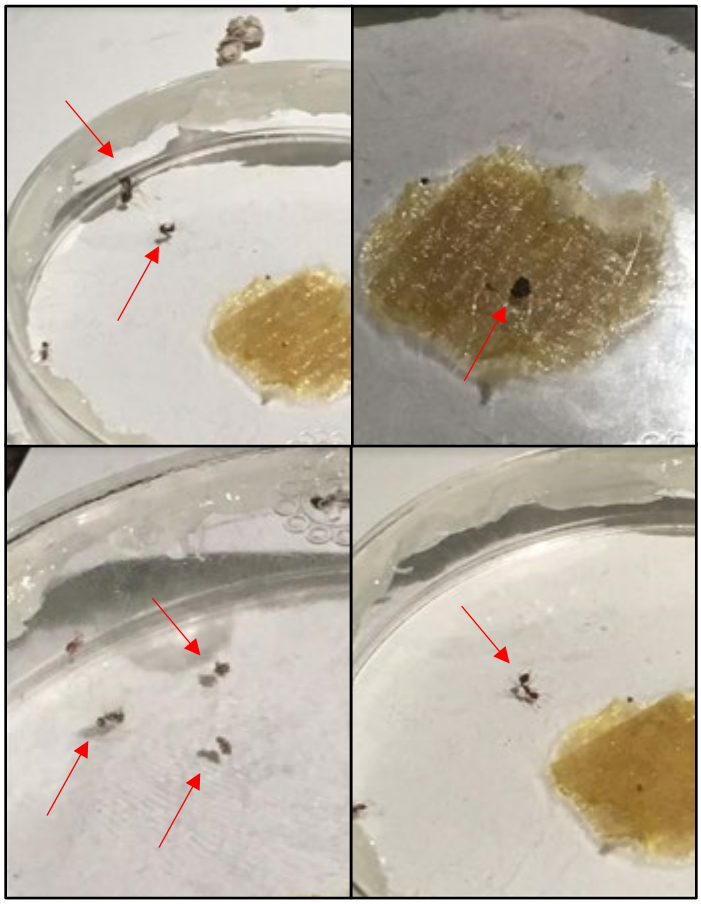


Figure 1. Ants seizing during the citric oil trials both close to the oil-soaked paper and further away (arrows point to individual ants)

Discussion

The objective of this experiment was to add to the growing knowledge about the effectiveness of different essential oils as pest repellent, specifically ant repellent. This was completed by comparing the number of times a sample of ants touched paper towel pieces soaked in orange citric oil and hemp oil versus a water-soaked control. This objective was chosen due to the popularity of essential oils, their availability in households, and the desire to stray away from the use of standard pest control products.

Orange citric oil was the most effective of the two oils used (see Table 1). The oil used had a very strong odor and dispersed easily, causing us to have to alter the methods and use a small piece of absorbent material to hold the oil droplets in the center of the petri dish. The three trials did not vary in the number of touches (see Tables 1 and 2). More trials would be needed to link the number of touches, seven, to the five ants that were placed in the petri dish.

Once placed in the petri dish, the ants participating the first trial frantically wandered around for the first few minutes before the first ant touched the orange citric oil-soaked towel. Being a species that typically follow one another, within the next few minutes, all ants had touched the towel once. The second and third trials proceeded similarly. In these trials, the ants began seizing almost immediately after touching the towel (see Figure 1). This result was possibly due to the D-limonene compound in orange citric oil that has an impact on the sodium flux within a cell (Hu 2017). In Drosophilia, researchers have linked sodium/potassium pump mutations to seizure susceptibility but there has not been any similar research done in the order Hymenoptera, providing an area for further exploration (Kaas 2015).

The high variance in the number of touches between the three trials of hemp oil (see Tables 1 and 3) leads to concerns about possible issues in the setup of this portion of the experiment. The average number of touches for hemp oil was lower than that of water while even the hemp oil trial with the highest touch count was still below any trails in the water control (see Table 1). There is a considerable difference between the hemp oil results and the control (see Table 3) so hemp oil does show promise as a possible ant repellent. However, no conclusion should be made about its effectiveness yet, due to the high variation between the hemp oil trials. A possible explanation of this variance could be the use of petroleum jelly as a containment method. Without the petroleum jelly, the ants would fit through the gap between the lid and the bottom of the petri dishes. The petroleum jelly was used to seal this gap but it may have added another point of interest to the ants that could have contributed to the unintended variations in the trials. It should be noted that the petroleum jelly was used in all trials for all three substances while only hemp oil experienced a large variation in the number of touches between trials.

Effects of hemp oil on arthropods is unknown but its effects on humans is better studied. *E. cannabinum* ethanolic extract has been shown to reduce the viability of certain colon cancer lines due to changes in gene regulation, mitotic disruption, and nuclear disruption within 24 hours (Ribeiro-Varandas 2014). The application of this finding to arthropods would be limited due to the high rate of proliferation that cancer cells show compared to the behavior of normal cells.

A limitation of this experiment is the small number of materials tested. Although the hemp oil and the orange citric oil have shown promising results, there are still other types of essential oils that could be tested which could yield better, or at least different, results. Other essential oils such as vanilla, mint, and citronella have been shown to have different levels of toxicity towards ants, with citronella having similar repellent effects as citrus oil (Francikowski 2019). The repellency of hemp and orange citric oil combined needs to be researched next before drawing a definite conclusion of their effectiveness. A study conducted tested the effectiveness of individual essential oils as well as their effectiveness when different combinations of essential oils were mixed and the results from the study suggested "[essential oil] mixtures were generally more repellent than individual [essential oils], with the lemon and vanilla 1:1 mixture acting as the strongest repellent (Francikowski 2019)." However, this experiment tested the repellency of individual oils and came to the conclusion that orange citric oil by itself had the strongest ability to repel in a small, closed container.

The ability of the orange citric oil to aerosolize compared to the ability of hemp oil to aerosolize must also be considered and studied before any firm conclusions can be made. With their touch averages being very similar (Graph 2) and the experiment being performed in an enclosed space, the concentration of oil components in the air could greatly affect their abilities to influence arthropod behavior. This could be completed by conducting the experiment in a larger container or a container without a sealed top.

Acknowledgements
We would like to thank Jose Juarez for making this research project possible.

References Cited

Cilek, J. E., C. F. Hallmon, and R. Johnson. **2011**. Efficacy of Several Commercially Formulated Essential Oils Against Caged Female Aedes albopictus and Culex quinquefasciatus when Operationally Applied via an Automatic-Timed Insecticide Application System. Journal of the American Mosquito Control Association. 27: 252–255.

Francikowski, J., B. Baran, M. Cup, J. Janiec, and M. Krzyżowski. **2019**. Commercially Available Essential Oil Formulas as Repellents Against the Stored-Product Pest Alphitobius diaperinus. Insects. 10: 96.

Gulluni, N., T. Re, I. Loiacono, G. Lanzo, L. Gori, C. Macchi, F. Epifani, N. Bragazzi, and F. Firenzuoli. **2018**. Cannabis Essential Oil: A Preliminary Study for the Evaluation of the Brain Effects. Evidence-Based Complementary and Alternative Medicine. 2018: 1–11.

Hu, W., N. Zhang, H. Chen, B. Zhong, A. Yang, F. Kuang, Z. Ouyang, and J. Chun. 2017. Fumigant Activity of Sweet Orange Essential Oil Fractions Against Red Imported Fire Ants (Hymenoptera: Formicidae). Journal of Economic Entomology. 110: 1556–1562.

Judzentiene, A., R. Garjonyte, and J. Budiene. **2015**. Variability, toxicity, and antioxidant activity of *Eupatorium cannabinum* (hemp agrimony) essential oils. Pharmaceutical Biology. 54: 945–953.

Kaas, G. A., J. Kasuya, P. Lansdon, A. Ueda, A. Iyengar, C.-F. Wu, and T. Kitamoto. 2016. Lithium-Responsive Seizure-Like Hyperexcitability Is Caused by a Mutation in the Drosophila Voltage-Gated Sodium Channel Gene paralytic. eNeuro. 3.

(The Lancet) The Lancet. **2017**. The Lancet. 389: 1076.

Potiwat, R., and R. Sitcharungsi. 2015. Ant allergens and hypersensitivity reactions in response to ant stings. Asian Pacific Journal of Allergy and Immunology. 33: 267–275.

Ribeiro-Varandas, E., F. Ressurreição, W. Viegas, and M. Delgado. 2014. Cytotoxicity of Eupatorium cannabinum L. ethanolic extract against colon cancer cells and interactions with Bisphenol A and Doxorubicin. BMC Complementary and Alternative Medicine. 14.

Sun, J. 2007. D-Limonene: safety and clinical applications. Alternative Medicine Review. 12: 259–264.

Wijerathne, B. T., G. K. Rathnayake, R. S. Dias, N. D. Wickramasinghe, S. P. Thalgaspitiya, A. H. Karunaratne, and S. B. Agampodi. 2018. Uncommon Skin Response to the Stings of Tropical Fire Ant Solenopsis geminata. Wilderness & Environmental Medicine. 29: 551–554.