The Use of Coffee Bean Oil to Repel *Amblyomma americanum* (Acari:Ixodidae)

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Abstract: There is growing interest in using natural alternatives to repel ticks, particularly essential oils as opposed to synthetic chemicals. Previous studies have found volatile monoterpenes in many essential oils to be repellent or acaricidal, leading us to test the repellency of coffee bean oil to ticks. *Amblyomma americanum* (Acari: Ixodidae) were the subjects tested in two separate studies. One determined the repellency by testing the number of ticks repelled from five different dilutions of coffee bean oil, with dichloromethane as the solvent and control. The other study investigated the farthest distance at which the oil could repel ticks. The study shows that concentrations as low as one percent were effective at repelling *A. americanum,* however, twenty percent repelled the greatest number of ticks. We conclude that the twenty percent solution was repellent up to sixty centimeters. Coffee bean oil does show the ability to repel *A. americanum*. This work shows that coffee bean oil is another example of essential oils that show ability to repel ticks.

Introduction

The incidence of tick-borne diseases is increasing at an alarming rate (Swei *et al*. 2020). In the United States, the number of tick-borne diseases in humans, companion animals, and wildlife surpasses all other vector-borne diseases (Little *et al*. 2014; Rosenberg *et al*. 2018). Furthermore, ticks transmit a greater diversity of pathogens than any other arthropod vector (Shaw *et al*. 2001; de la Fuente *et al*. 2008). The lone star tick, *Amblyomma americanum*, has a broad host range; readily feeding on companion animals, wildlife, and humans and transmits several pathogens that cause disease such as human monocytic ehrlichiosis, tick-borne relapsing fever, tularemia, red meat allergy known as alpha-gal, and southern tick-associated rash illness (Hopla 1953; Hopla 1955; Anziani *et al.* 1990; Ewing *et al.* 1995; Godsey *et al.* 2016). *A. americanum* is established in the eastern, southeastern, and mid-western United States with expanding distribution into the northeastern US and southern Canada (Sonenshine 2018). Established populations exist in canopied and brushy habitats located between prairies and forests and are thus, a major pest species in many national and state parks (Semtner *et al.* 1971; Koch and Burg 2006). This is an aggressive tick that will move

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quickly across several meters when attracted to host odors (Sonenshine 2018). The broad host range, expanding geographical distribution, preferred habitat, and vector competence makes *A. americanum* a serious health threat to both humans and animals. Preventing ticks from biting humans and domestic animals can stop these tick-borne diseases from spreading.

There are many recommendations from experts on how to protect people and their animals from tick bites. The United States Center for Disease Control (CDC) recommends the use of repellents, some of which can be used on the skin while others should only be used on clothing (Center for Disease Control 2019). Permethrin, a synthetic repellent, is applied to clothing and has been shown to be effective in repelling *A. americanum* (Schreck *et al.* 1982). For direct skin usage, DEET (N,N-diethyl-3-methyl benzamide) has been used since the 1950s and is an effective tick synthetic repellent (Carroll *et al.* 2008). In recent years, the use of synthetic chemicals to repel arthropods has raised concerns related to environmental and human health risk and more interest in the use of natural alternatives to prevent tick bites, particularly essential oils.

Essential oils are extracted from plants and contain volatile chemical compounds. The volatile terpenes, sesquiterpenes, sulfur, menthol, and phenylpropenes in these oils, specifically are thought to repel ticks (Nerio *et al.* 2010). For example, spiderwisp (*Gynandropsis gynandra*) (Malonza *et al.* 1992; Lwande *et al.* 1999), catnip (*Nepeta cataria*) (Birkett *et al.* 2011), bog myrtle (*Myrica gale*) (Jaenson *et al.* 2005), citronella (*Cymbopogon*) (Sukkanon *et al.* 2019), lemon eucalyptus (*Corymbia citriodora*) (Sukkanon *et al.* 2019) and carnation (*Dianthus caryophyllus*) (Tunón *et al.* 2006), contain monoterpenes and have shown to be effective repellents. Oil of Citronella, oil of lemon eucalyptus, and catnip oil have been approved for use on skin by the US Environmental Protection Agency (EPA). These have comparable efficacy to synthetic repellents, with low toxicity (Katz *et al.* 2008). Other essential oils such as garlic (*Allium sativum*), onion (*Allium cepa)*, citrus peel, tea tree (*Melaleuca alternifolia*), geranium, peppermint (*Mentha piperita*), spearmint (*Mentha viridis*), marjoram (*Marjorana hortensis*), sweet basil (*Ocimum basilicum*), and lavender (*Lavandula officinalis*) oils have shown promise in repelling ticks but have not been approved by the EPA as tick repellents (Don-Pedro 1996; Abdel-Shafy and Soliman 2004; Iori *et al.* 2005; Jaenson *et al.* 2006; Štefanidesová *et al.* 2017). Many of these successful plant-derived repellents derived from plant essential oils are highly volatile and have limited residual activity (Sukumar *et al.* 1991; Zhu *et al.* 2018).

Other plant-derived compounds that are not as highly volatile have been shown to repel ticks. High levels of fatty acids in coconut oil have been shown to repel three different species of ticks (Zhu *et al.* 2018; Sukkanon *et al.* 2019; Barrozo *et al.* 2021) and have a longer repellent activity than DEET. Hence there is interest in studying other plant-based essential oils comprised of different active ingredients that could provide greater efficacy and extended repellent activity.

Coffee bean (*Coffea arabica)* oil is an inexpensive, natural oil that is primarily (80-85%) composed of triglycerides with diterpenes making up 13 - 15 % of the oil (Ratnayake *et al.* 1992; Al Kanhal 1997; Speer and Kölling-Speer 2006). The fatty acid composition of coffee bean oil is dominated by linoleic acid and palmitic acid with some minor contribution of stearic acid, oleic acid, arachidic acid, and alpha-linolenic acid (Speer and Kölling-Speer 2006).

To determine if coffee bean oil could be used as an efficient tick repellent, we tested five different concentrations of coffee bean (*Coffea arabica*, Plant Therapy®) oil to determine the most effective concentration at repelling *A. americanum*. This assay also investigated the efficacy of the coffee bean oil extract at several distances. To the best of our knowledge, there are no published studies on the repellent properties of coffee bean oil on ticks, or on the maximum distance at which any essential oil is repellent to ticks.

Methods

Ticks

Five hundred adult *A. americanum* ticks of both sexes were purchased from Texas A&M Tick Research Laboratory (College Station, Texas). Adults were housed temporarily in the Department of Biology at the University of Oklahoma (Norman, Oklahoma) in glass incubators (38.1 cm x 43.2 cm x 30.5 cm) and maintained at 90 % relative humidity using a saturated potassium chloride solution.

Repellency Assay

Coffee bean, *Coffea arabica*, oil was purchased from Plant Therapy® (Twin Falls, Idaho) and diluted to 1, 2, 5, and 20 percent using dichloromethane (DCM) purchased from Sigma-Aldrich (St. Louis, Missouri). Four sheets of Whatman™110 mm filter paper were cut in half; one half was treated with a coffee bean oil dilution (1 ml) (treatments) while the other half was treated with DCM (1 ml) (control). Treatment and control solutions were pipetted onto the center of each filter paper and allowed to diffuse across the filter paper. Each treatment was placed in a separate petri dish alongside the control leaving a centimeter of space between the treatment and control. The space between the two halves was deemed the "neutral zone." To eliminate a DCM affect, the same set up was used, but one side remained untreated while the other side was treated with one milliliter of DCM.

A slightly modified area preference method was used to test repellency (Tapondjou *et al.* 2005; Olivero-Verbel *et al.* 2010; Caballero-Gallardo *et al.* 2012; Lü and Shi 2012). Five ticks were simultaneously placed in the petri dish at random orientations. Ticks were monitored for a total of 15 minutes. At 5-minute intervals, each tick was scored with either 0 for staying in the neutral zone, 1 for moving toward the control, or -1 for moving toward the treatment. At the completion of the 15 minutes, the ticks were removed, killed and stored in 70 % ethanol for long-term preservation. The procedure was repeated five times using fresh assays and ticks.

Distance Efficacy

The distance efficacy of coffee bean oil extract was determined by constructing a testing arena. The testing arena was created by drawing four concentric circles (radii = 15 cm, 30 cm, 45 cm, and 60 cm) on a poster board (117.6 cm x 142.4 cm) (Figure 1). Five mL of the 20 % coffee bean oil dilution was placed in a watch glass on the center of the arena. The 20 % dilution was chosen because it was the most repellent treatment in the repellency assay. For each trial, five ticks were randomly oriented and placed at one of the four distance intervals and each tick's position was recorded every five minutes for 15 minutes. At the end of each 5-minute interval, each tick was scored with a 0 for staying in place, 1 for moving away from the oil, or -1 for moving toward the oil. Trials were repeated five times at each distance interval (15, 30, 45, and 60 cm) with different ticks for every replication.

Statistical Analysis

Statistical analyses were carried out in SigmaPlot, version 14.0 (Systat Software, Inc., Palo Alto, CA, USA). Since the data was categorical, we used a chi-squared analysis to determine statistical significance for both the repellency assay and distance efficacy tests

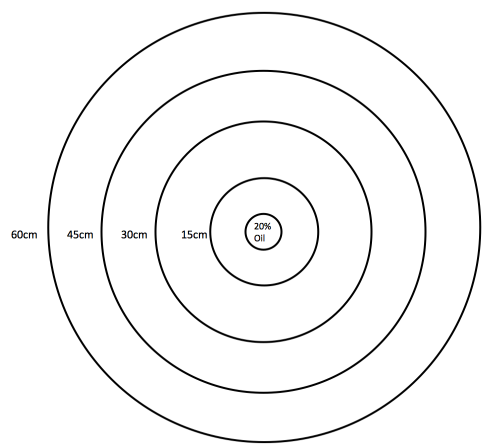

Figure 1. Distance assay arena. For each trial, 5 ml of 20 % concentration of oil were placed in a watch glass in the center of a 38.1x 43.2x 30.5 cm poster board. Five *A. americanum* ticks were placed at one distance interval (15, 30, 45, or 60 cm) for each test and positions were scored after 5, 10, and 15 minutes.

(Dowdy *et al.* 2011). For the repellency assay, we analyzed each time interval (t = 5, 10, and 15 minutes) independently and when significance was found, we ran pairwise comparison among all the different dilutions of each time period.

Results

Repellency Assay

More than half of ticks moved away from the coffee bean oil and after 15-minute exposure there was nearly a two-fold increase in the percentage of ticks that moved away from the coffee bean oil (Table 1). All four dilutions repelled a significantly greater number of ticks than the control treatment ($P < 0.001$; Table 2) hence DCM had no effect on tick repellency. While the first three dilutions (1 %, 2 %, and 5 %) repelled a similar number of ticks, the 20 % concentration repelled the greatest number of ticks (Table 1).

The repellency of coffee bean oil was not consistent over the entire testing interval (Table 2). At the five-minute interval, more ticks moved away from all the coffee bean oil dilutions than from the control. However, there was no difference in the number of ticks moving away from the different coffee bean oil dilutions. After ten minutes, there was no difference between the control and all coffee bean oil treatments. At the fifteen-minute time interval, all coffee bean oil dilutions repelled more ticks than the control treatment again. Among the coffee bean dilutions, the 20 % dilution repelled more ticks than the 2 % and 5 % dilutions while the 1% dilution repelled the same number of the other three dilutions.

Distance Efficacy

The purpose of the distance test was to determine the farthest distance at which the 20 % dilution was repellent to *A. americanum*. The 20 % dilution of coffee bean oil repelled ticks at all distances tested (Table 3); ticks were least repelled when the repellent was farthest away and more repelled when the repellent was close by. However, this was not a consistent trend as seen in the percent of ticks repelled when placed at 30 cm (44 %) compared to 45 cm (68 %) away from the oil. The final tick positions at the end of each time interval did not significantly differ with distance from the oil (t = 5, $\chi^2_6 = 5.91$, $P = 0.43$; t = 10, $\chi^2_6 = 8.77$, $P = 0.19$; t = 15, $\chi^2_6 = 5.91$ $P = 0.43$).

Table 1. The effect of coffee bean oil on *A. americanum* tick repellency as tested with dilutions of one, two, five, and twenty percent against the control (only DCM). Repellency was measured as the percent of ticks (out of 25 ticks at each concentration of oil) whose position was further away from the coffee bean oil than when the trial started at each of the time intervals.

Treatment (% dilution)	Ticks (%) that moved away from the coffee bean oil		
	5-minute	10-minute	15-minute
Control	20	44	52
1	56	72	76
2	60	64	68
5	64	64	64
20	64	64	96

Table 2. Statistical analysis values from chi-squared tests on the repellency of the different dilutions of coffee bean oil on *A. americanum* at the three different time intervals. Bolded data represent significant values. N.C. indicates the test was not conducted.

		5-min. interval		10-min. interval		15-min. interval	
Test	D.F.	χ^2 value	*P* value	χ^2 value	*P* value	χ^2 value	*P* value
Overall Dilution (%)	8	**16.81**	**0.032**	12.52	0.130	**36.24**	**< 0.001**
Control - 1	2	**7.05**	**0.029**	N.C.	N.C.	**15.43**	**< 0.001**
Control - 2	2	**10.00**	**0.007**	N.C.	N.C.	**10.93**	**0.004**
Control - 5	2	**10.12**	**0.006**	N.C.	N.C.	**11.71**	**0.003**
Control - 20	2	**9.96**	**0.007**	N.C.	N.C.	**16.27**	**< 0.001**
2 - 20	2	1.65	0.44	N.C.	N.C.	**6.86**	**0.032**
5 - 20	2	1.65	0.438	N.C.	N.C.	**8.17**	**0.017**
1 - 2	2	2.46	0.292	N.C.	N.C.	1.11	0.574
1 - 5	2	0.93	0.627	N.C.	N.C.	1.35	0.510
1 - 20	2	0.38	0.826	N.C.	N.C.	4.25	0.120
2 - 5	2	0.62	0.732	N.C.	N.C.	0.12	0.941

Discussion

For decades, CDC has recommended that the repellents permethrin and DEET be used to prevent tick bites. Concerns raised regarding the health and environmental safety of these synthetic chemicals have created an interest in using natural repelling products, such as essential oils to repel ticks. While many essential oils have been tested both on- and off-host and some approved by the US EPA, coffee bean oil has never been tested. The results of these preliminary off-host assays indicate that coffee bean oil, an essential oil, may repel adult *A. americanum*. Across the range of dilutions tested, the most effective was the 20 % dilution, which repelled ticks up to 60 cm away from the oil. Twenty-four of twenty-five ticks moved away from the 20% dilution, and more than half of the ticks still moved away from the oil when they were up to 60 cm away from it. The difference in the percent of ticks repelled from the 30 cm and 45 cm treatment was likely a random occurrence. There were no significant differences in the distance assay.

Using a variety of methodologies, other studies have shown essential oils to have repellency activity against varying life stages of *A. americanum* (Ellse and Wall 2014). Meng *et al.* (2016) in a vertical filter bioassay determined that of the eight essential oils tested (oregano, clove, thyme, sandalwood, cinnamon, cedarwood, and peppermint), oregano oil was the most effective and peppermint oil the least effective in repelling *A. americanum* nymphs. The LC_{50} values for all essential oils tested, ranged from 0.113 to 0.297 mg/cm^2, were significantly higher than that of DEET ($P < 0.05$). Luker *et al.* (2021) using a novel tick carousel assay determined that six hours post-treatment, oil of lemon eucalyptus repelled more adult *A. americanum* than the synthetic repellents tested (DEET, Picaridin, and IR3535). Elemol, a principal constituent of the essential oil of Osage orange, *Maclura pomifera* (Moraceae) in a vertical filter bioassay and a fingertip bioassay did not differ significantly in repelling *A. americanum* nymphs than the popular synthetic repellent DEET (Carroll *et al.* 2010).

Table 3. Distance efficacy of a twenty percent dilution of coffee bean oil on *A. americanum* movement measured the percent of ticks (out of 25) that moved away from the coffee bean oil. The tick positions did not significantly differ with distance from the oil across the different time intervals.

Distance (cm) from coffee bean oil	Ticks (%) that moved away from 20 % coffee bean oil		
	5-minute	10-minute	15-minute
15	92	96	92
30	44	76	84
45	68	84	84
60	80	76	76

Repellent activity of at least ten essential oils have been tested for their ability to repel varying life stages of *A. americanum* using a variety of in vitro and in vivo bioassay methodologies (Phillis III and Cromroy 1977; Carroll *et al.* 2010; Meng *et al.* 2016; Luker *et al.* 2021). Our preliminary work was the first to test coffee bean oil in a horizontal in vitro repellency and distance assay. The 20 % dilution resulted in greatest repellency while the distance of repellency was not significant. The distance study was unique, as no known studies have measured the farthest distance at which an oil is effective.

The results of this study suggest that coffee bean oil may be effective in repelling ticks and hence warrants further study. *A. americanum* is an aggressive tick, quickly moving toward its host. It is strongly attracted to carbon dioxide emitted from humans and animals and has simple eyes that may detect movements of host silhouettes against contrasting background (Phillis III and Cromroy 1977; Carroll *et al.* 2010). With the preliminary evidence that coffee bean oil may have repellent activity, its ability to repel should be tested against these host cues and hence, an in vivo vertical fingertip bioassay is warranted for both *A. americanum* nymphs and adults. Furthermore, in the current study the time interval for testing repellency was maximized at 15-minutes. In the fingertip bioassay this interval should be extended to closer reflect conditions in the field. The distance assay should be repeated however modified to a fingertip bioassay. We were unable to determine the maximum distance at which the oil was effective because none of the results from the distance assay were significant. Farther distances should be tested with the 20 % dilution to find the true limit of the oil's repellency. Lastly, to be considered an effective repellent, coffee bean oil should be tested against DEET and must show higher repellency activity than DEET for people to consider its use.

It is important to note that while natural products can be effective in repelling ticks, they may not be safer than synthetic chemicals. Natural products just like synthetic must be tested for their effectiveness and safety passing regulations set forth by the US EPA before they are used on humans or animals. Studies like this are only the first step in determining if an essential oil, coffee bean oil is worthy of future consideration for tick or other arthropod repellency.

Our preliminary findings suggest that coffee bean oil may be an effective tick repellent; however, further repellency assays are warranted. Future research should include a vertical in vivo fingertip bioassay with additional *A. americanum* life stages, longer repellency duration, distance of repellency, and a comparison to DEET. If future research confirms coffee bean oil to have high tick repellency, to be safe and effective, coffee bean oil could be tested by the US EPA.

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