JOURNAL OF
EMERGING INVESTIGATORS

Article

Repulsion of Ants Using Non-Toxic Household Products

Meenakshi Ambati[1] and Kameshwari Duvvuri[2]
[1]Joseph T. Henley Middle School, Crozet, VA
[2]University of Virginia School of Medicine, Charlottesville, VA

SUMMARY

Ant invasion causes damage exceeding $5 billion annually in North America. Commercial repellents often contain chemical compounds that are potential carcinogens and maybe harmful to human health. In this study, we aimed to identify natural products with ant-repelling properties. We tested chalk, cinnamon, ginger powder, lemon juice, rosemary powder, table salt, and turmeric powder using a custom ring apparatus designed to quantify ant-repellence. We discovered that cinnamon and lemon were the most effective ant repellents of the tested products. Specifically, we found that cinnamon oil and lemon juice increased the median time required for ants to exit the ring as compared to their vehicle controls; that the lipid-soluble component of cinnamon, i.e., oil, and the lipid-insoluble component of lemon, i.e., juice, were more efficacious at repelling ants than their counterpart fractions; that cinnamon oil and lemon juice repelled ants in a dose-dependent manner; that cinnamon oil was more efficacious than lemon juice; that lemon juice was more potent than cinnamon oil; and that cinnamon oil and lemon juice are synergistic in their ant-repellent properties. These data suggest that compounds found in non-toxic household products, such as cinnamon oil and lemon juice, could be used in low-dose combinations as potent, effective, eco-friendly, and safe ant repellents.

INTRODUCTION

Ant invasions of residential homes are seasonally commonplace. Use of commercial insecticides containing compounds such as bifenthrin, which is classified as a possible human carcinogen and is highly toxic to aquatic life and bees, are often the first response of homeowners and pest control agencies to ant invasions (1). In this study, we aimed to identify non-toxic household products that repel ants in a safe and effective manner as alternatives to commercial insecticides. Ants cause billions of dollars of damage to residential homes and to commercial crops every year, making this area of research broadly beneficial to homeowners, house residents, and the agricultural industry (2).

Previous research suggests that essential oils of plants such as basil, lemon grass, and peppermint repel a variety of arthropods (3). In addition, previous research suggests that various acids secreted defensively by animals act as ant deterrents (4, 5). As a result, we chose to test 7 non-toxic household items for ant-deterrent properties: chalk, fresh ground cinnamon, ginger powder, lemon juice, rosemary powder, table salt, and turmeric powder. We used water as a control.

Preliminary results suggested that cinnamon and lemon were the most effective ant repellents among the tested items. We then hypothesized that the lipid-soluble and lipid-insoluble fractions of cinnamon and lemon would differ in their effectiveness, because cinnamon and lemon have different parts, such as bark, oils, rind, and juice, that may vary in their chemical composition. Subsequently, we hypothesized that cinnamon oil and lemon juice would repel ants in a dose-dependent manner. Specifically, we expected that chemicals in cinnamon oil and lemon juice are responsible for the repellent properties of cinnamon and lemon, and that as the concentration of these chemicals increased, so too would their repellent activity. Next, we hypothesized that cinnamon oil and lemon juice would be synergistic, rather than merely additive, in their repellent activity. We formulated this hypothesis based on our expectation that cinnamon oil and lemon juice might trigger different senses of the ant due to the different physical and chemical properties of these substances.

RESULTS

The experiment was set up in a stainless steel, 12-inch pan. For each trial, we deposited one of the non-toxic household products in a 6-inch diameter ring within the 12-inch pan and then placed a single ant at the center of the pan (**Figure 1**). Ants explore new territories instinctively and attempt to exit the arena when placed at the center of the pan (6). However, if the ant is repelled by the substance in the 6-inch diameter ring, the ant would avoid the perimeter and spend a greater amount of time inside the ring. We used the time taken by the ant to exit the ring as a measure of the repellence of the substance. Water and grapeseed oil were chosen as negative controls for their inability to repel the ants. Specifically, water was used as a negative control for water-soluble substances and grapeseed oil was used as a negative control for lipid-soluble substances.

The 7 household substances exhibited varying degrees of ant repellence (**Figure 2**). Of these substances, cinnamon

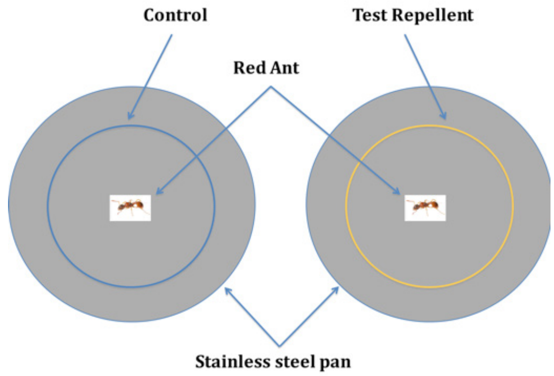

Figure 1. Schematic of apparatus. A stainless steel, 12-inch pan was used as the arena. For each trial, one of the non-toxic household products was deposited in a 6-inch diameter ring within the 12-inch pan. A single ant was placed at the center of the pan to commence each trial.

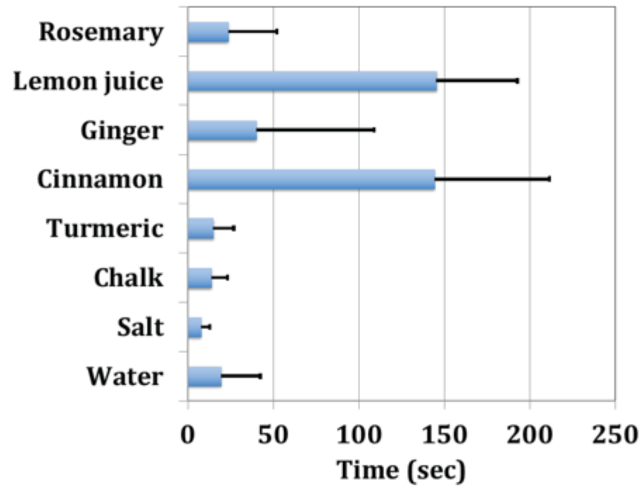

Figure 2. This bar graph shows the times taken by the ants to leave the rings of the various substances. The blue bar represents the mean time taken to leave the ring and the black bar represents the standard error of the mean.

powder (P = 0.005 compared to negative control) and lemon juice (P = 0.002 compared to negative control) were the most effective; thus, we further investigated these 2 substances in subsequent experiments.

We tested whether the lipid-soluble or lipid-insoluble components of cinnamon were responsible for its ant repellence. To determine this, we extracted cinnamon oil from cinnamon by heating it in grapeseed oil. We ran the mixture through a sieve and separated the oil from the insoluble cake. Then we exposed the ants to rings containing grapeseed oil, cinnamon oil, or the insoluble cinnamon cake. Cinnamon oil extract displayed significantly better ant repellence compared with cinnamon cake or grapeseed oil ($P < 0.05$) (**Figure 3A**).

We then tested whether the lipid-soluble or the lipid-insoluble components of lemon were responsible for its ant repellence. To determine this, we extracted the lemon oil from the peel of the lemon and we heated it in grapeseed oil. We also squeezed out the juice of the lemon. We exposed the ants to rings containing grapeseed oil, lemon oil, water, or lemon juice. Lemon juice displayed significantly better ant repellence than lemon oil (P = 0.013) (**Figure 3B**). Overall, these data indicate that the lipid-soluble fraction of cinnamon and the aqueous (lipid-insoluble) fraction of lemon are superior repellents compared to their aqueous (lipid-insoluble) and lipid-soluble fractions, respectively.

Kaplan-Meier survival curve analyses showed that the median time to exit the ring was significantly greater with lemon juice compared with water (P = 0.0001), and significantly greater with cinnamon oil compared with grapeseed oil (P = 0.003) (**Figure 4A-B**).

Next, we performed dose-ranging studies of cinnamon oil and lemon juice by testing concentrations from 0.5% to 100% in grapeseed oil and water, respectively. The dose-response curves were fit using an exponential curve model. The mean time to exit the ring increased with increasing concentrations of cinnamon oil and lemon juice (**Figure 5A-**

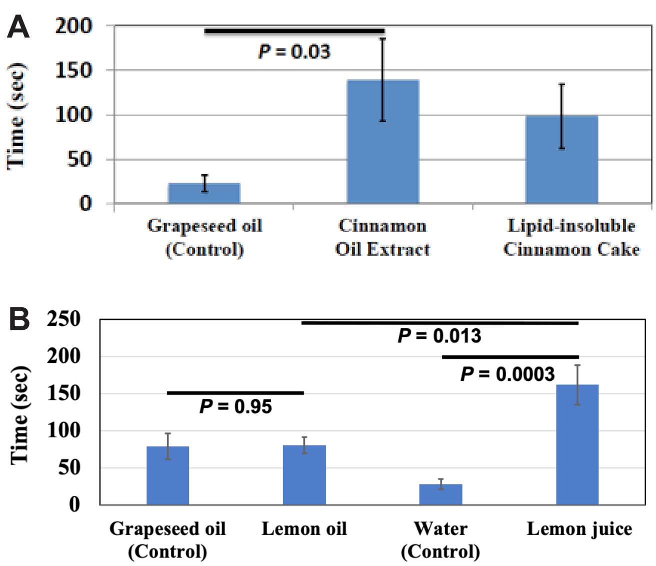

Figure 3. Cinnamon oil extract was more effective than its control, grapeseed oil (A). Lemon juice was more effective than its control, water, and more effective than lemon oil (B). P values by Student's t test.

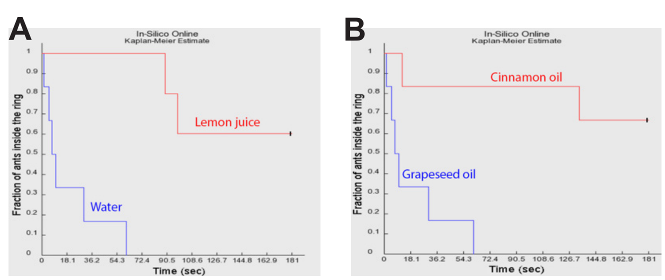

Figure 4. Kaplan Meier survival curves showing the fraction of ants inside the lemon juice (A) or cinnamon oil (B) rings over time. In the water control group, all the ants left the ring before 60 sec. However, in the lemon juice group, half the ants remained inside the ring by the time the experiment ceased (log-rank P = 0.0001). In the grapeseed oil control group, all the ants left the ring before 60 sec. In the cinnamon oil group, nearly 70% of the ants remained inside the ring by the time the experiment ceased (log-rank P = 0.003).

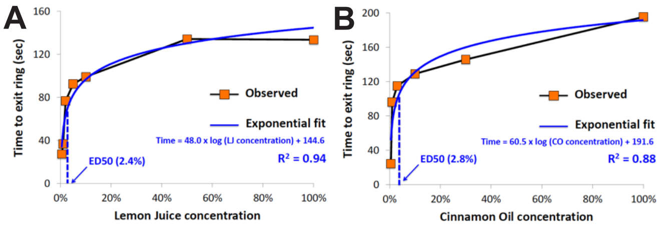

Figure 5. Scatter plots of the observed mean times to exit the ring (orange squares) for various concentrations of lemon juice (A) and cinnamon oil (B). The response was dose dependent: as the concentrations increased the time increased as well. These data followed an exponential trend (blue lines are the exponential curves of best fit). The coefficients of determination were: R^2 = 0.94 for lemon juice and 0.88 for cinnamon oil.

B). The coefficients of determination (R^2) were 0.88 and 0.94 for cinnamon oil and lemon juice, respectively. This excellent fit indicated a dose-dependent effect of both substances. In addition, most of the variation (88% and 94%) in repellence could be attributed to variation in cinnamon oil or lemon juice concentration.

The efficacy values, defined as the ratio of observed inhibition to theoretical maximal inhibition were 95.4% for cinnamon oil versus 60.9% for lemon juice (**Table 1**). Therefore, cinnamon oil was more effective than lemon juice at repelling ants. Efficacy refers to the maximal effect of a substance regardless of the dose used. Potency is a measure of the activity of a substance in terms of the concentration required to produce a defined effect, quantified in this study as the concentration required to repel at 50% of the maximal effect. The median effective dose, or ED50, values were calculated by plotting a log-linear curve (7). The half-maximal value of inhibition was identified on the y-axis, and the ED50 for this point on the exponential fit line was identified on the x-axis. The ED50 was 2.41% for lemon juice, whereas the ED50 was 2.79% for cinnamon oil (**Table 1**). Therefore, lemon juice was more potent than cinnamon oil at repelling ants.

Next, we sought to determine whether the combination of cinnamon oil and lemon juice would be additive, synergistic, or subtractive in their ant-repellence. We analyzed the interaction between cinnamon oil and lemon juice using the Bliss Independence Model (8). The observed data for the combination of these two substances showed inhibition greater than additivity and the combination indexes at all dose combinations were < 1.0 (**Table 2**).

These data, when plotted on a log-linear graph, demonstrated that the combination of cinnamon oil and lemon juice outperformed both substances individually, and exceeded the expected ant-repellence if their combination was merely additive (**Figure 6**). Therefore, the combination of cinnamon oil and lemon juice was synergistic in repelling ants.

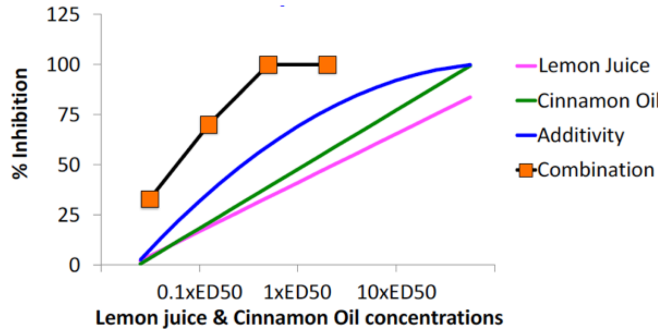

Figure 6. The combination of cinnamon oil and lemon juice is represented by the orange squares and the black line. The line of best fit for lemon juice is represented by the pink line while the line of best fit for cinnamon oil is represented by the green line. This graph shows the % inhibition for each concentration of the combined lemon juice and cinnamon oil. The inhibition was synergistic.

Table 1. Potency and efficacy of lemon juice and cinnamon oil in repelling ants.

Repellant	Potency (ED50)	Efficacy (Maximal inhibition)
Lemon Juice	2.41%	60.9%
Cinnamon Oil	2.79%	95.4%

Potency values, measured as median effective dose (ED50), reflect percentages of lemon juice diluted in water or of cinnamon oil diluted in grapeseed oil. Efficacy values, which reflect ratio of observed inhibition to theoretical maximal inhibition, are provided as percentages.

DISCUSSION

In the first phase of this project, we observed that, of 7 household substances, cinnamon oil and lemon juice repelled ants more effectively than their negative controls. In the second phase, we hypothesized that the lipid-soluble and lipid-insoluble components of cinnamon and lemon would differ in their effectiveness. We hypothesized that cinnamon oil and lemon juice would repel ants in a dose-dependent manner, i.e., as the concentration of their respective repellent chemicals increased, their effectiveness would as well. We determined that the principal components of cinnamon that repelled ants were lipid-soluble and that the principal components of lemon that repelled ants were aqueous. In the third phase, we hypothesized that cinnamon oil and lemon juice might be synergistic in their repellent activity, based on our expectation that each of these compounds might trigger different senses of the ant because cinnamon oil and lemon juice differ in their physical and chemical properties. We concluded that both cinnamon oil and lemon juice repelled ants in a dose-dependent manner, suggesting that specific,

Table 2. Efficacy of lemon juice and cinnamon oil, individually and in combination, in repelling ants

	Lemon Juice + Cinnamon Oil (% Inhibition)			
	1/32 x ED50	1/8 x ED50	1/2 x ED50	2 x ED50
Lemon Juice (Best Fit)	4.3	19.0	33.7	48.3
Cinnamon Oil (Best Fit)	3.2	21.0	38.8	56.6
Lemon Juice + Cinnamon Oil *Expected Additivity*	7.4	36.0	59.4	77.6
Lemon Juice + Cinnamon Oil *Observed*	32.8	70.0	100.0	100.0
Combination Index	**0.226**	**0.514**	**0.594**	**0.776**

Efficacy values, which reflect ratio of observed inhibition to theoretical maximal inhibition, are provided as percentages. Expected additivity values were calculated per the Bliss Independence Model (8) using the formula described in the Methods.

defined molecules in these substances are responsible for repelling ants. The exponential fit for concentration versus repellence supports that these molecules could be acting on specific ant receptors with first-order kinetics. A first-order reaction is also referred to as a unimolecular reaction as it depends only on the concentration of one reactant; it is quantitatively described by an exponential relationship. Thus, our finding that repellence is an exponential function of concentration suggests that there may be sensors in ants, such as receptors, that recognize specific molecules in the test substances. Such a mechanism could be responsible for the observed repellence effect. Lastly, we determined that, when combined, cinnamon oil and lemon juice performed synergistically.

Interestingly, cinnamon oil was more effective than lemon juice, whereas lemon juice was more potent. These contrasting observations suggest that the molecules in lemon juice responsible for its ant-repelling power operate at very low concentrations, but that, overall, the compounds in cinnamon oil have a greater ability to repel ants. By applying the Bliss Independence Model, a tool employed in pharmacology to quantify the interaction of two toxins, we determined that the combination of cinnamon oil and lemon juice was synergetic in repelling ants. Based on our observations, we conclude that all of our hypotheses should be accepted.

The apparatus we constructed was robust enough to quantify ant-repellence of a diverse array of substances with varying physical and chemical properties and over a range of concentrations. This two-dimensional arena could also be used to test the ability of substances to repel other non-aerial organisms. Indeed, by inverting the paradigm, i.e., using the time to reach the ring from the center, this apparatus could also be used to determine attraction of test substances.

Several intriguing questions emerge from this study. For example, which molecules in cinnamon oil and lemon juice are responsible for repelling ants? To what extent is the acidic nature of lemon juice responsible for its repellence and could other acids serve a similar function? What mechanisms do these compounds trigger in ants to elicit repellence? Given that several molecules have been reported to repel multiple arthropods (3), could cinnamon oil and lemon juice repel other pests?

In conclusion, we have demonstrated that lemon juice and cinnamon oil, which are non-toxic household products, are effective in repelling ants. The results of this study could be used to create eco-friendly, non-toxic, and relatively inexpensive ant repellents. Future investigations could also unravel ant odor receptor biology. Our findings add to the growing body of evidence that the invasion of various pests can be controlled by household products that do not carry the attendant harmful effects of commercial pest control chemicals.

METHODS

Ant repellence by household substances

Red ants (Carolina Biological Supply Company) were placed, one at a time, in the center of a 12-inch stainless steel pan, inside a 6-inch ring containing one of the following test items: chalk (Crayola), fresh ground cinnamon (Deep Foods), ginger powder (Deep Foods), lemon juice (Kroger), rosemary powder (Kroger), table salt (Morton Non-Iodized), and turmeric powder (Deep Foods); or one of the negative controls: water and grapeseed oil (Trader Joe's). Each substance was deposited "free-hand" in the shape of a ring 5 mm wide and 1-2 mm in height. The surface was cleaned with isopropyl alcohol between trials and with soap and water or acetone between groups. The time taken by each ant to exit the ring was measured with an iPhone StopWatch. The experiment was stopped at 240 seconds and repeated 6–8 times.

Lipid-soluble versus lipid-insoluble components of cinnamon and lemon

Cinnamon bark (Deep Foods) was ground to powder (Jura-Capresso spice grinder), and its fat-soluble components were extracted by heating it in grape seed oil (Trader Joe's) on an electric stove and filtering it through a sieve when cool. Dilutions (0.5–100%) of cinnamon oil and freshly squeezed juice of lemons (Kroger) were prepared in grape seed oil or water, respectively. Red ants were placed, one at a time, inside a 6-inch ring of various concentrations of cinnamon oil, lemon juice, or controls in the center of a 12-inch stainless steel pan. The time taken by each ant to exit the ring was measured. The experiments were stopped at 240 seconds and repeated 6–12 times.

Combination of cinnamon oil and lemon juice

The potencies (ED50: Median Effective Dose 50%) of cinnamon oil and lemon juice were calculated using Microsoft Excel. Combinations of cinnamon oil and lemon juice at equal potency concentrations (2xED50; 1/2xED50; 1/8xED50; 1/32xED50) were prepared. Red ants were placed inside a ring of these cinnamon oil and lemon juice combinations, or a ring of combined controls (grape seed oil and water). The time taken by each ant to exit the ring was measured. The experiments were stopped at 240 seconds and repeated 6–12 times.

Statistical analysis

Means and standard errors of the mean (SEM) were calculated for each group and normalized by subtracting the average control group time. Groups were compared using Student's t-test, and P values were obtained using MS Excel. Mean differences were considered statistically significant if $P < 0.05$.

Median times to leave the ring were assessed using Kaplan-Meier analysis (http://in-silico.net/tools/statistics/survivor) to generate survival curves, and log-rank tests were performed to determine *P* values. Scatter plots were fit by exponential trend-lines and coefficients of determination (R^2) were determined (MS Excel regression analysis).

The efficacy of a substance was calculated as the ratio of observed inhibition (the longest mean time taken to exit the ring in the presence of a substance at its best concentration – mean time taken to exit the ring in the presence of control) to theoretical maximal inhibition (240 seconds – mean time taken to exit the ring in the presence of control). The ED50 values were calculated by plotting a log-linear curve. The half-maximal value of inhibition was identified on the y-axis, and the ED50 for this point on the exponential fit line was identified on the x-axis.

The interaction between two substances was analyzed using the Bliss Independence Model (8). Expected Additivity and Combination Index metrics were calculated according to these formulas:

Expected Additivity = Inhibition (Substance 1) + Inhibition (Substance 2) – [Inhibition (Substance 1) x Inhibition (Substance 2)]

and

Combination Index = (Expected Additivity / Observed Inhibition)

with the following interpretations:
Combination Index < 1 : Synergistic
Combination Index = 1 : Additive
Combination Index > 1 : Antagonistic

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Received: May 13, 2019
Accepted: September 2, 2019
Published: September 10, 2019