The Effect of Highly Saturated Fats on *Drosophila* Behaviors and Viability

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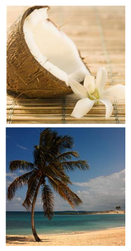
Abstract

Obesity is a global health epidemic that leads to the development of diseases including high blood pressure, heart disease, and Type 2 diabetes. We first utilized the fruit fly as a model system to determine the effects of obesity on specific behaviors by feeding third-instar larva media containing 30% coconut oil. Coconut oil is composed of approximately 99% fat. While allowing female flies to lay eggs in the media containing coconut oil, we serendipitously discovered that adult 4-day old female flies fed 30% coconut oil died within 3-5 minutes of exposure to the media. Subsequently, we determined that the effect of coconut oil on adult fly viability is dosage- and age-dependent. Conversely, feeding third-instar larva doses of coconut oil ranging from 10-30% did not affect viability, but high doses impaired locomotor activity. Further specific behavioral studies are currently being applied to larva and adult flies to dissect the effects of coconut and other heavily saturated oils on behaviors and viability. With an eye to the future, we are using this study as a foundation to screen for potential fruit fly and mosquito repellants that are safe to use for humans and biodegradable.

Introduction

Plant essential oils represent viable alternatives as repellants for the commonly used insect repellant DEET (N, N-diethyl-meta-toluamide) which is harmful to humans and the environment. *Drosophila* has hardly been used as a model system to examine the effectiveness of plant essential oils as natural repellants. To begin to develop an understanding of the properties of essential oils that either ward off or cause mortality to *Drosophila* during their adult lifetimes, we are screening the effect of coconut oil on adult fruit fly viability. Quite by chance, we discovered that four-day old adult fruit flies exposed to 30% coconut oil within their feeding media, died within 3-5 minutes. We are now examining the dose-, time-, and age-dependence of this effect and comparing it to the effects of other natural oil treatments.



CHEMICAL COMPOSITION VIRGIN COCONUT OIL

Schedule 2 (Source SII,1977)

Chemical contents	Total
Air(%)	0,5
Saturated Acid	
Kaprok Acid (%)	no
Kaprilik Acid (%)	8-9
Caprice Acid (%)	5-8
lauric Acid (%)	45-51
Miristik Acid (%)	17-18
Palmitik Acid (%)	8-10
Stearik Acid (%)	1-3
Arakhidik Acid (%)	0-1
Unsaturated Fat Acid	
Oleic Acid (%)	5-8
Linoleum Acid (%)	1-2
Chemical Property- Nature	
FFA (%)	5
Soap number	255-265
peroxide number	-
Sodium number	8-10
Bias Index	
Dangerous metal	negative

Average – 65% Oleic Acid

http://www.chemistryexplained.com/Di-Fa/Fats-and-Fatty-Acids.html

Methods

Culturing Flies
Oregon-R fruit flies were fed standard cornmeal-yeast-agar medium at 25°C (77°F) at approximately 40+/-2% humidity conditions within a biological incubator maintaining a diurnal 12 hr light-dark cycle.

Adult Viability Assay
Virgin female and male flies were collected under carbon dioxide anesthesia and aged appropriately before transferring to standard cornmeal-yeast-agar medium containing virgin coconut oil in doses ranging from 5-30%.

Adult Odor Preference Assay
A group of twenty 4-day old male or female virgin flies were tapped into a 2.5 x 20 cm glass tube and a connector was used to firmly attach a standard fruit fly medial vial containing the odorant dissolved in 1.5% agarose. A nylon mesh sheet was placed between the tubes and flies were then gently tapped onto the nylon mesh filter to begin the assay. Every minute for a ten- or 20-minute period, the numbers of flies located within the glass tube were counted within each of three equally divided spaces of the glass tube labeled as the closed end, middle, and open end located closest to filter. The values shown in Figures 2A and B represent the mean number of flies within each of these areas at the final 10-minute time point.

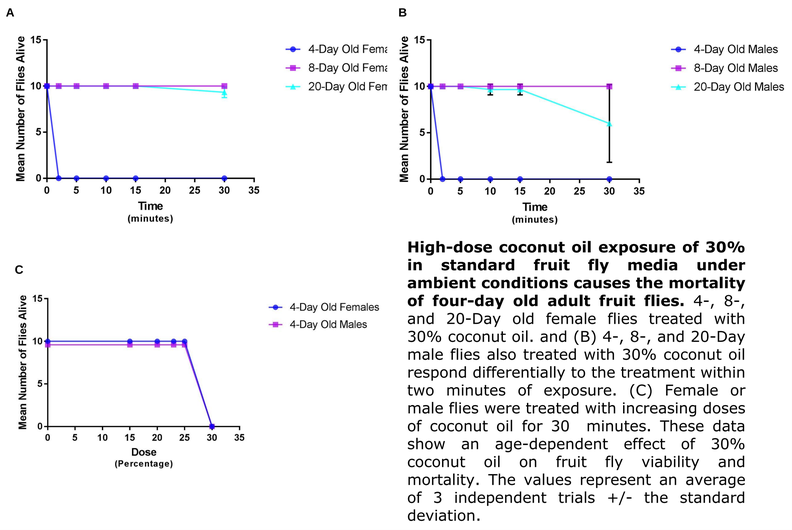
Systemic Treatment of Larva with Oils
Hundreds of flies were maintained in large population cages. Each cage contained an apple-juice agar plate with a dab of yeast paste food (Baker's yeast and distilled H_20). During peak egg-laying time, females laid eggs for ~15-20 minutes. Under the same environmental conditions used for basic husbandry, the eggs were allowed to transition into first-, second-, and third-instar larva for 96 hours on either regular yeast paste (control) or yeast-paste containing specific doses of either coconut oil or walnut oil. All experimental media were dyed blue using McCormick's food color at a final concentration of 0.1% to determine whether larvae ingested the food. Third-instar larvae were subjected to rhythmic behavioral assays.

Larval Locomotor Behavioral Assay
Refer to Figures 3 (immediate right)

Results

Fig. 1

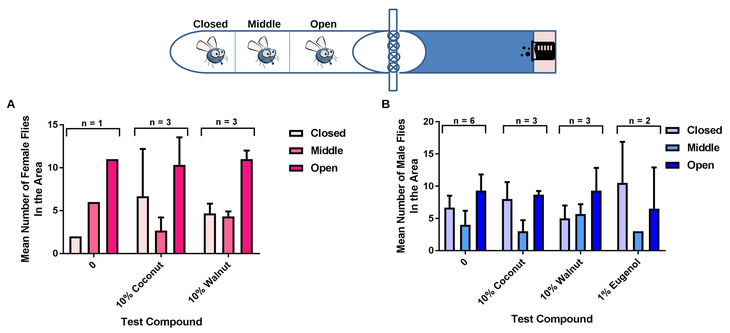
Coconut Oil Treatment Affects the Viability of Four-Day Old Adult Fruit Flies



High-dose coconut oil exposure of 30% in standard fruit fly media under ambient conditions causes the mortality of four-day old adult fruit flies. 4-, 8-, and 20-Day old female flies treated with 30% coconut oil. and (B) 4-, 8-, and 20-Day male flies also treated with 30% coconut oil respond differentially to the treatment within two minutes of exposure. (C) Female or male flies were treated with increasing doses of coconut oil for 30 minutes. These data show an age-dependent effect of 30% coconut oil on fruit fly viability and mortality. The values represent an average of 3 independent trials +/- the standard deviation.

Fig. 2

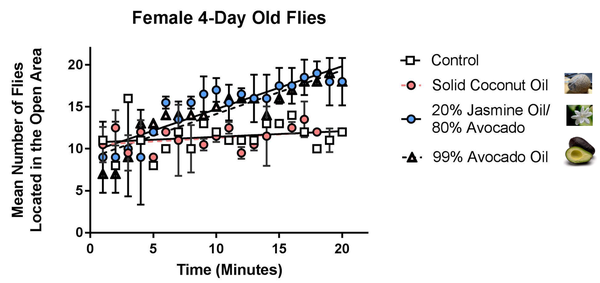
Preliminary Data: A 10% Dosage of Coconut Oil Does Not Affect Female or Male Odor Preference



Low-dose coconut oil and walnut oil odorant exposure of 10% in 1.5% agarose does not appear to affect odor preference after 10 minutes of exposure. Groups of 20 four-Day old female and male flies exposed to either 10% coconut oil or 10% walnut oil. (A) These preliminary data suggest that low odorant doses of coconut oil and walnut oil dissolved in a base of 1.5% agar do not affect preference behavior of female flies after 10 minutes of exposure. (B) Similarly, 4-day old male flies do not exhibit a preference for these compounds as prepared. Two groups of 20 male flies were also exposed to 1% Eugenol, a known attractant. The *n* stands for the number of groups assayed. The values represent the mean number of flies located within the closed, middle, or open part of the glass tube (left side). The error bars denote standard deviations of the mean.

Fig. 3

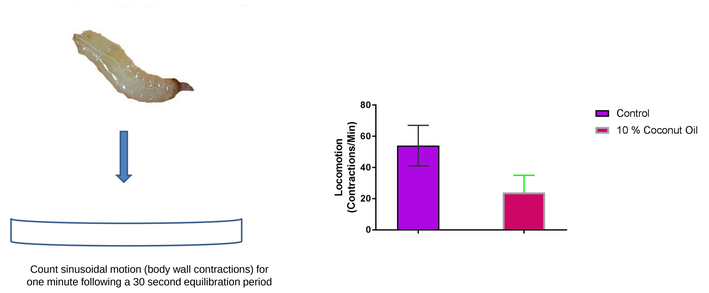
Preliminary Data: Pure Coconut Oil Does Not Affect Female Odor Preference



Pure coconut oil odorant exposure does not appear to affect odor preference after 20 minutes of exposure. Groups of 20 four-Day old female flies exposed to either pure coconut oil, pure avocado oil, 20% jasmine oil dissolved in a base of olive oil, or 1.5% odorless agar. These preliminary data suggest that coconut oil fragrance does not affect preference behavior of female flies after 20 minutes of exposure. Avocado oil was first use as a base to dissolve 20% jasmine oil. Surpisingly, flies were attracted to avocado oil alone. Jasmine is known to be a natural fruit fly attractant and served as an alternate, positive control for eugenol (see Fig. 2). Currently, 4-day old male flies are also being assayed for these oils as well as for the attractant jasmine. The *n* stands for the number of groups assayed thus far. The values represent the mean number of flies located within the open part of the glass tube (right side closest to the mesh partition). The error bars denote standard deviations of the mean. The lines are extrapolated linear fits of the data.

Fig. 4

Body Wall Contractions
Oscillatory Rhythmic Behaviors



Count sinusoidal motion (body wall contractions) for one minute following a 30 second equilibration period

Conclusions

1) 100% oil-treated larvae consumed the oils dissolved into yeast paste dyed with blue food color since all intestinal tracts of these larvae were blue in color (data not shown).

2) Towards publishing, the data shown in Figure 1 require at least two more trials per all conditions assayed. However, a high percentage of coconut oil has a severe viability impact on young, four-day old flies while having less of an impact on the health of older flies of either sex.

3) Thus far, preliminary data suggest that male and female flies are not attracted to 10% coconut oil, 10% walnut oil, or even 1% eugenol, a known attractant. We then modified the assay to carefully replicate published methods in which the assay is timed for a longer period of time (20 minutes) and we assayed pure coconut oil.

4) Adult 4-day old flies appear to be attracted to avocado oil suggesting a chemoreceptor exists for detecting oils containing long chain, monounsaturated fatty acids (Oleic acid).

4) We are assessing mouth hook contractions as well as non-rhythmic behaviors; this work is in progress.

Future Directions

1) Design a more strategic screening assay dependent on the exact ratio of saturated versus non-saturated oils (i.e. coconut oil versus corn oil).

1) To complete the studies shown here in their entirety using mineral oil as an odorless base solvent.

1) To increase productivity, we are setting up another study area with an incandescent light to double-up data gathering as a team. Flies need to be exposed to a constant light source for 30 minutes prior to the assay and then assayed for 20 minutes.

1) Ideally, we were attempting to develop an enhanced insect repellant using natural oils rather than essential oils. These data suggest that the oils tested thus far are not good candidates.

1) To expose flies to 30% coconut oil and examine them using scanning electron microscopy to determine if the oil is blocking the breathing apparatus.

1) Since larval and young adult stages of flies are negatively affected by coconut oil, we are seeking an understanding of this effect to uncover a new and possibly improved target for pesticide development and use that is safe for humans and the environment. A new insecticide strategy envisions a natural and safe "plugging reagent" as the insect pierces the skin to feed (i.e. mosquito) based on a new working hypothesis of how coconut oil may be asphyxiating flies.

Acknowledgements

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