

Isolation of Naturally Occurring Pesticides

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Discipline: Biology, Environmental Science, Ecology

Grade Levels: Grades 7 through 12

Goal: To show that insecticides occur in nature.

Materials: Mortar and pestle (for plants)

Acetone

Corn Oil (Mazola®)

Weighboats

Drosophila melanogaster (wingless)

Instant Drosophila food

Cotton balls

Squirt bottle containing water

1 package of cheesecloth

Plastic vials

Small funnels

Gloves

Electric coffee or spice grinder (for spices)

Spices: 5 grams each - fennel, cumin, cayenne pepper, black pepper, nutmeg, tobacco, dried mustard

Plants: 5 grams each - Chrysanthemum flowers, Marigold leaves, Marigold flowers

Safety Considerations: Teacher should advise students to avoid breathing acetone vapors or getting acetone on their skin.

Teacher Note: If you use wingless flies you don't have to anesthetize them. Transfer of live flies is best achieved by placing a funnel on the empty vial. The fly vial is then tapped to cause flies to drop to the bottom, and the cotton is removed. The vial is quickly inverted onto the funnel. Gently tap the empty vial to cause ~ 20 flies to drop into it.

Procedure/Preparation (teacher version):

1. Make up stock solution of corn oil/acetone: add 0.5 ml corn oil to 100 ml acetone
2. Weigh out 5 grams of each sample: fennel, cumin, cayenne pepper, black pepper, nutmeg, tobacco, dried mustard, Chrysanthemum flowers, Marigold leaves, and Marigold flowers.

3. Processing of Samples (Students can process during lab too)

- a. *Plants*: Put plant sample in mortar, sprinkle some sand over the sample and grind until thoroughly crushed, about 2 minutes. Add 10 ml of acetone and grind thoroughly, being careful not to splash liquid. If the mixture is too thick, add 5 more ml of acetone. Strain the acetone/extract mixture by pouring through cheesecloth in a funnel on top of a 125-ml Erlenmeyer flask. After most of the liquid has drained into the flask, extract the remaining liquid by forming a bag around the plant material and squeezing (make sure that you wear gloves). The liquid in the flask is your extract.

- c. *Spices*: Grind spice sample in grinder until pulverized (if its not already pre-ground). Transfer ground sample into cheese cloths and tie into packets. Have students place the packets in a weighboat and add 10 ml of acetone. Stir the packet in the acetone for ~ 30 seconds. Squeeze out the liquid into the weighboat (make sure that they wear gloves). The liquid in the weighboat is their extract.

Student instructions after extract preparation:

4. Mix 1 ml of the extract with 0.5 ml of corn/acetone stock solution. Pour into a plastic vial.
5. Quickly twirl jar in a hood to evaporate the acetone. The oil/extract mixture should coat the sides of the jar.
6. Add dry instant food so that it just covers the bottom of the jar. Carefully add water until the food has been hydrated.
- Note: The food is needed to prevent the flies from sticking to the oil in the bottom of the jar – which will kill them.*
7. Add ~ 20 *Drosophila melanogaster* adults to an empty vial and stopper with cotton. Transfer the 20 (or so) flies to the vial coated with extract.
8. Record results after 5, 10, 15, 30 minutes (and 24 hours if you'd like).
9. Assign one of the students a control: 0.5 ml corn oil and 1 ml acetone. Coat sides of vial and add food and flies.
10. Compile the class data on the "Data Compilation Sheet."
11. Rank the toxicity or pesticide effectiveness (i.e. the spice/plant with the most flies dead in the shortest time would receive the highest ranking).

Extensions:

1. Try different plants and different plant parts.
4. Try different spices.
5. Capture different species of wild *Drosophila* and test them.
6. Use different types of insects.
7. Try chromatography of extracts.
8. Instead of corn oil use Kimwipes~. A Kimwipe® is saturated with 1 ml of extract. The Kimwipe~ is then spread out in the hood and dried for 10 minutes. The Kimwipe® is then placed in the bottom of the jar and saturated with 1.5% sugar (sucrose) solution. Be careful not to get any water on the sides of the jar. Add 20 adult *Drosophila melanogaster* to the jar and put in cotton stopper. Record results as above.

