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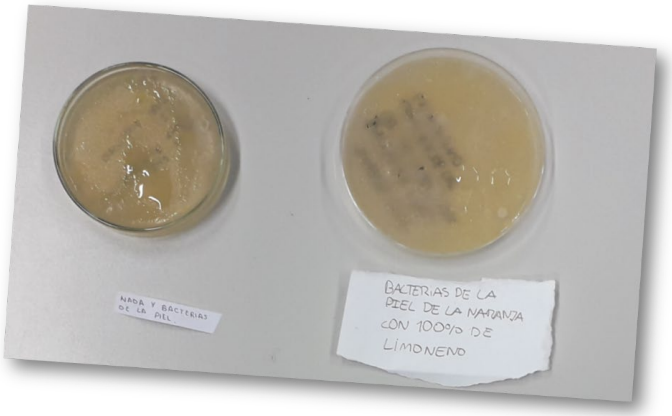
Extension activities

Citrus science: learn with limonene

Extension activity 1: Antibacterial activity

Many essential oils show antimicrobial activity.[1] In this experiment, students can investigate the antibacterial properties of limonene. It should take around 45 min to set up and then the results are recorded after 7–14 days.



Bacterial growth in petri dishes

Image courtesy of the authors

Materials

- 24 white cotton pads
- small paper discs
- 7 petri dishes with lids
- 1 unwaxed/organic orange
- bleach
- 96% ethanol
- growing medium, e.g.: 300 ml water; 10 g beef broth extract, 10 g of white sugar, 4 g agar
- *Escherichia coli* cultures or swabs from classroom surfaces

Procedure

1. The teacher or technician should make up agar plates before the lesson. Subsequent steps are performed by students.
2. Using gloves, rub a damp (not dripping) cotton ball or swab over the skin of an orange.

3. Soak the cotton ball or swab in a test tube with a small amount (1–2 ml) of distilled water.
4. Dilute the sample (10×); put 1 ml of water extracted from the cotton and add 9 ml of water.
5. Withdraw 1 ml of the diluted sample, add it to a new cotton ball or swab, and spread over the entire surface of six of the seven Petri dishes.
6. Seed the seventh petri dish with *E. coli* or bacteria swabbed from the classroom. To this dish, add a circle of paper with 0.1 ml of undiluted limonene on it.
7. Put 0.1 ml of limonene on a circle of paper and add it to one of the six remaining petri dishes (seeded with bacteria from the orange skin).
8. Make up a dilution of 25 % limonene in alcohol.
9. Put 0.1 ml of limonene sample on a circle of paper. Also try orange peel: squeeze the skin on the paper first and then put it in the petri dish.
10. Prepare controls with alcohol (solvent control), nothing on the paper (negative control), and bleach (positive control).
11. Place lids on the petri dishes, seal with tape, and write your name and the chemicals added. Store the plates upside down at room temperature.
12. After 7–14 days, take photos of the petri dishes and write down the results.
13. When the experiment has finished, add bleach to sterilize the petri dishes.

Results/Discussion

Bacterial sowing	**Added chemical**	**Bacterial growth**
Bacteria from the surface of the orange skin	Bleach (Control –)	–
	Nothing (Control +)	+
	Alcohol (Control for solutions)	+
	Limonene 100%	+
	Limonene 25% diluted in alcohol	+
	Orange peel extract	+
E. coli or bacteria isolated from the classroom	Limonene 100%	–

The positive and negative controls of bleach, nothing, and ethanol produce the expected results, so the experiment is well done. Neither limonene nor orange peel oil inhibit the growth of microorganisms on the surface of the orange skin.

Studies have shown that limonene can inhibit the growth of various bacteria,[2–4] but it does not inhibit the growth of orange skin bacteria. This can be explained by an evolutionary adaptation process of some types of bacteria common in the Mediterranean area where plants that produce limonene grow.

In the same way, where citrus fruits grow, it is natural for ripe fruit and leaves to fall to the ground, dispersing limonene: therefore, even in this way, some families of bacteria are adapted to degrade this substance.

Extension activity 2: Anti-germination activity

Essential oils can inhibit the germination of seeds. In this activity, students can investigate the anti-germination capability of limonene. It should take around 30 min.

Materials

- 30 dry lentil seeds or other fast-germinating seeds
- water
- 96% ethanol
- (*S*)-limonene
- orange peel
- cotton wool
- 6 petri dishes 7–9 cm in diameter with lids
- 10 plastic pipettes

Procedure

1. Moisten a piece of cotton; it should not drip water.
2. Place the cotton in a petri dish.
3. Place 5 seeds on top of the wet cotton.
4. Cover and close one of the three petri dishes.
5. Put a piece of orange peel in the second petri dish and close it.
6. Put a small ball of cotton in each of the other three petri dish.
7. Put a drop of pure (*S*)-limonene on the small ball of cotton in the third petri dish (100% limonene concentration).
8. Dilute 1 ml of limonene with 1 ml of alcohol (50% limonene concentration).
9. Put a drop of limonene (50% concentration) on the small ball of cotton in the fourth petri dish.
10. Dilute 1 ml of limonene with 3 ml of alcohol (25% limonene concentration).
11. Put a drop of limonene (25% concentration) on the small ball of cotton in the fifth petri dish.
12. Dilute 1 ml of limonene with 9 ml of alcohol (10% limonene concentration).
13. Put a drop of limonene (10% concentration) on the small ball of cotton in the sixth petri dish.
14. Put lids on all the petri dishes, seal with tape to prevent opening, and store the petri dishes at room temperature. They can be stored under light or dark conditions; it should just be the same for as all samples.
15. The results should be observed after 3–5 days.

Results/Discussion

Inhibition of seed germination by limonene

Image courtesy of the authors

	Number of sprouted lentils	**% of sprouted lentils**
Nothing (Control +)	5/5	100
Limonene 100%	0/5	0
Limonene 50%	0/5	0
Limonene 25%	0/5	0
Limonene 10%	0/5	0
Orange peel	5/5	100

- Limonene inhibits the germination of lentils; it works with all the concentrations used: 100%, 50%, 25% and 10%.
- Orange peel does not inhibit germination. This could be because the concentration of limonene is lower than that in the other experiments.

Anti-germination activity has been reported for limonene and other essential oils and could be a mechanism for the producer plants to limit competition from nearby plant species.[5,6]

References

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