



Module 4: Plant Growth, Management & Pest Control

Hands-On Activity (HOA) B: DIY Spore Print & Culture

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Module 4 HOA-B Instructions Worksheet

Grouping: Pairs or small groups (2–4 students)

Time: 25-40 min initial setup, plus 1-2 short follow-up sessions for observations over the next several days.

Precautions & Safety:

- **Handling Mold:** Powdery mildew (a common plant fungus) is generally harmless to people, but handle infected leaves carefully. Avoid shaking or waving the leaf to prevent releasing spores into the air. **Do not inhale** any spores or allow them to contact your eyes or nose. Wash your hands with soap after handling the infected plant material.
- **Agar Plates:** Once you have set up your agar plates with spores, **keep them sealed**. Do not open the Petri dishes after sealing, to avoid unintended mold spores escaping or other contaminants getting in. You will be able to observe growth through the closed lid. The teacher will instruct on proper disposal of plates—**do not** open them on your own.
- **Clove Oil:** Clove oil is a strong natural substance. **Wear gloves** when handling clove oil or treated plates, and avoid contact with skin or eyes. Use only the small amount directed. If any clove oil gets on your skin, wash immediately with soap and water. Note that clove oil has a strong smell; that's normal, but if you feel uncomfortable, inform your teacher.
- **General Lab Safety:** No eating or drinking during this activity. Keep your workspace clean and organized. Wipe up any spills (e.g., water or agar) immediately to prevent slips. As always, follow your teacher's instructions for safe lab behavior.

Materials (per group):

- **Powdery mildew-infected leaf** (from a plant with obvious white “powdery” fungal spots – e.g., tomato, a squash, melon, or rose leaf). *You need a piece of a plant that shows active powdery mildew.*
- **2 sterile Petri dishes with nutrient agar** (pre-poured agar medium for fungus growth).
- **Clove oil** (a small dropper bottle provided by your teacher; you'll only use 1–2 drops).
- **Water** (clean tap or distilled water in a dropper bottle – for the control treatment).
- **Disposable pipettes or droppers** – 2 (one for water, one for clove oil).
- **Cotton swabs** – optional, to help spread treatments if instructed.
- **Parafilm or tape** – to seal Petri dish edges.
- **Permanent marker** – to label Petri dishes (e.g., “Water Control” and “Clove Oil”).
- **Nitrile or latex gloves** – for handling the leaf and chemicals safely (each student handling materials should wear a pair).
- **Ruler** – to measure fungal growth (colony size) on the plate through the lid.
- **Paper towels** – for cleaning up and hand-drying after washing.
- **Aprons/Lab Coats**



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Objective:

Work as a team to **capture and culture fungal spores** from an infected leaf and test a natural antifungal treatment. You will create a “spore print” by pressing a powdery mildew-infected leaf onto agar plates. Then, apply **water (control)** to one plate and **clove oil** to another. Over the next several days, you’ll observe and measure the mold growth on each plate. By comparing the growth of the fungus (e.g., fungal mycelium) with and without clove oil, you will learn how an everyday natural product can inhibit plant pathogens. This hands-on activity demonstrates a key idea in sustainable agriculture: using **integrated pest management (IPM)** strategies – in this case, monitoring a disease and using a targeted, eco-friendly treatment – instead of relying solely on synthetic chemicals. You’ll practice microbiology lab techniques (such as sterile plate handling), observation and data-collection skills, and systems thinking by connecting this mini-lab to real-world pest control in food systems. *In short, you’re exploring how we can manage plant pests more safely and sustainably!*

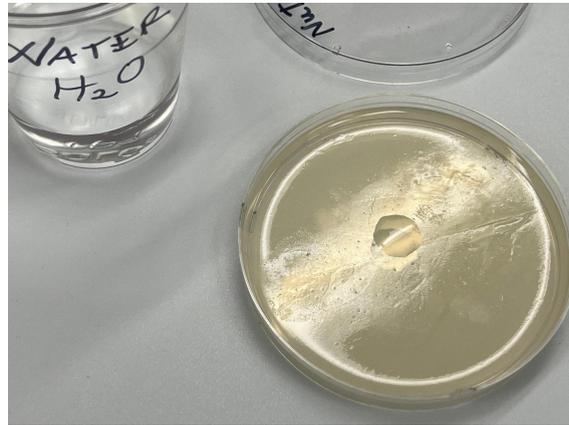
Instructions

Before You Begin: Make sure your **agar plates and droppers are prepared** by your teacher. Put on your **gloves**, and have all materials ready at your station. Label one Petri dish as “**Water – Control**” and the other as “**Clove Oil**” so you don’t mix them up. Read all steps first, so your group can work efficiently (one person can handle the leaf, another the droppers, etc.). Remember to minimize the time Petri dish lids are open – this keeps other microbes out!



Step 1: Apply Treatments (Water vs. Clove Oil). Now, treat each plate differently:

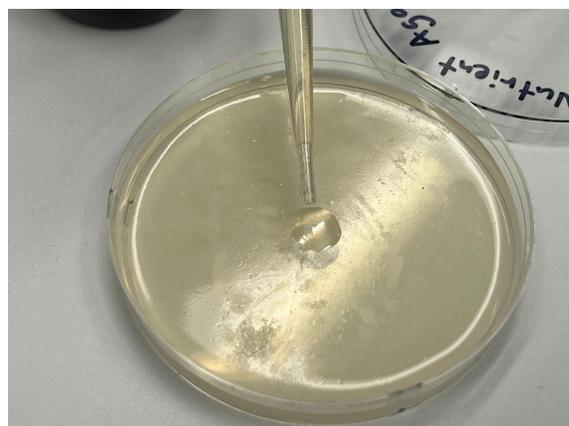
Step 1.1 Control Plate (Water): Using a clean dropper, place 1–2 drops of **water** onto the center of the agar. This simulates normal conditions (no antifungal treatment) but provides a bit of moisture for the spores.



Step 1.2 If needed, use a sterile cotton swab to very lightly spread the water across the agar surface.

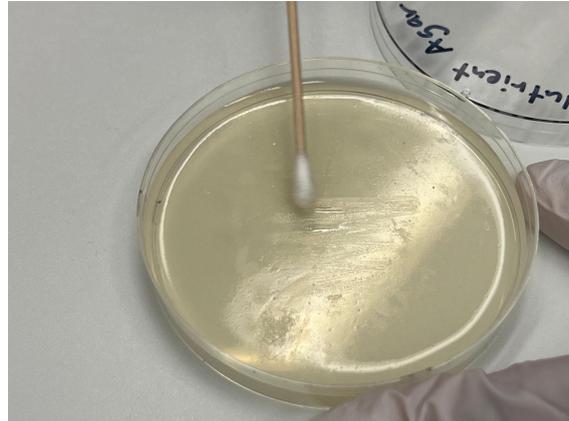


Step 1.3 Clove Oil Plate: Using a separate dropper (or the one provided with the clove oil bottle), carefully place **1 drop of clove oil** onto the center of the agar of the second plate. If the drop is very small, you may add a second drop, but do not over-saturate the plate. *Clove oil doesn't mix with water; it's okay if it beads up on the agar.*





Step 1.4 If instructed by your teacher, you can use a sterile swab to gently spread the clove oil drop over the central area of the agar. You will likely smell the strong clove aroma – that's a sign of the oil's presence.



Step 2: Press the Leaf (Spore Printing).

Step 2.1 Carefully place the powdery mildew-infected leaf on the agar surface of each Petri dish, pressing the infected side (the side with white powdery spots) down.





Step 2.2 Gently press for a few seconds to deposit spores onto the agar. You can use a different part of the same leaf (or a second leaf) for the second plate to ensure both plates get plenty of spores. *Be careful that the leaf or your fingers don't scrape the agar – just a light pressure.*



Step 2.3 After pressing, remove the leaf. You likely won't see much on the agar yet (maybe a faint powdery pattern), but you have now "planted" invisible fungal spores on each plate.



Step 3: Seal and Store the Plates.

Step 3.1 Immediately close each Petri dish and **seal it** with a strip of Parafilm or tape around the edge of the lid so it's airtight. Double-check that each dish is labeled correctly (especially if you removed the lid during setup – lids should go back on the correct base).





Step 3.2 Write your group name or initials and the date on each plate as well. Place the sealed plates in the designated **incubation area** as instructed by your teacher. Generally, plates should be kept at room temperature, out of direct sunlight. *Do not disturb or reopen the plates now that they are sealed.* The spores need time to germinate and grow.

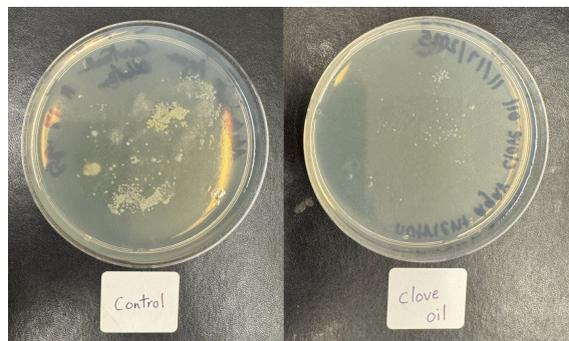


Step 4: First Observation (Follow-Up Day 1-2)

Step 4.1 After a couple of days (your teacher will specify when – typically 1–2 days after setup), you'll make your first observations. **Do not open the plates** – keep them sealed. Observe each plate **through the lid**

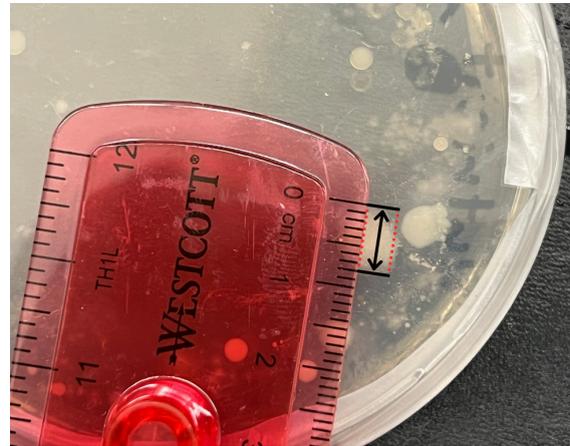


Step 4.2 What to look for: Do you see any signs of fungal growth on the agar? Powdery mildew fungus might appear as white, fuzzy or cottony patches growing out from where the leaf was pressed. The control plate (water only) will likely show more growth at this point than the clove oil plate. The clove oil plate may show little to no growth in the area where the oil was applied, though there might be some tiny colonies at the edges or faint growth if some spores germinated.





Step 4.3 Measuring: If fungal colonies are visible, use a ruler to measure the **diameter** of the largest colony or the overall fuzzy area on each plate (in millimeters). You can hold the ruler against the bottom of the plate for an approximate measurement. If there are multiple small colonies, note how many and their sizes roughly, or estimate the percent of the plate surface that has mildew.



Step 4.4 Recording data: On your **Data Collection Worksheet**, record today's date and observations for each plate. Write down descriptions (e.g., "**Plate 1 (Water):** white fungal colony ~15 mm across in center; **Plate 2 (Clove):** only a few tiny white specks ~2–3 mm). If no growth is yet visible on one or both plates, note "no visible growth." It might just need more time – powdery mildew can be a bit slow to start on agar.

Example:

Plate	Observation 1 Date: 11/18/25 (Day 1 after setup) <i>Describe growth</i>
Control (Water)	Growth: ~ 15 mm colony diameter Description: ~ 15 mm fuzzy white patch and several ~ 3 mm specks clustered together
Clove Oil	Growth: ~ 2 mm colony diameter Description: only ~ 2 mm tiny spots and faint



Step 5: Second Observation (Follow-Up Day 3-5)

Step 5.1 A few days later (for example, by Day 3-5 after setup), check the plates. The **Control (Water) plate** will likely have a substantial patch of white, fluffy mold where the spores grew uninhibited. It may even produce the powdery look (new spores) on the colony surface.



Step 5.2 After a few days have passed (for example, by Day 1 after setup), check the plates again. By now, differences should be pronounced. The **Clove Oil plate** may have only a much smaller patch of mold, or possibly none if the treatment was very effective. Some growth might be visible at the very edges of where the oil was applied (or where oil didn't reach), or the colonies might be thin and stunted.



Step 5.3 Again, **measure** the approximate size of the fungal growth on each plate. Record your observations and measurements on the data sheet for this final observation day. This will be your endpoint for comparing treated vs. untreated.

Example:

Plate	Observation 2 (Final) Date: 11/20/25 (Day 3 after setup) Describe growth
Control (Water)	Growth: ~ 50 mm colony diameter Description: covers about ~ 75% of plate, thick white mildew
Clove Oil	Growth: ~ 10 mm colony diameter Description: several small colonies ~ 10 mm where leaf was printed and edge of the plate is clear



Step 6: Analyze Your Results.

Step 6.1 Use your final measurements to calculate the **percent inhibition** of fungal growth by the clove oil. Here's how: take the size of the mold colony on the control plate and compare it to the size on the clove plate. For example, if the control colony diameter is 50 mm and the clove oil colony is 10 mm, the clove treatment reduced growth by 40 mm. Divide that difference by the control ($40/50 = 0.8$) and multiply by 100 – that would be **80% inhibition**. A 0% inhibition would mean the clove had no effect (both plates grew the same), whereas 100% inhibition means the clove oil completely stopped the fungus (no growth on the treated plate). Calculate this value for your experiment and record it.

Percent Inhibition = $[(\text{Control growth} - \text{Clove oil growth}) / \text{Control growth}] \times 100\%$

Example:

- Control plate final growth size = 50 mm of colony diameter
- Clove plate final growth size = 10 mm of colony diameter
- Difference = $50 \text{ mm} - 10 \text{ mm} = 40 \text{ mm}$

Percent Inhibition = $[(50 \text{ mm} - 10 \text{ mm}) / 50 \text{ mm}] \times 100\% = 80\% \text{ inhibition}$

Step 6.2 Now, with your group, **discuss the outcome** using the questions on your data collection worksheet (e.g., Which plate had more growth? Why? What does the percentage inhibition tell us? How might clove oil be working to inhibit the mold?). Make sure everyone in the group understands the results so you can share out or write up answers. This is where you connect what happened in the plate to the bigger picture of pest management.

Which plate had more growth? Why?

- State observations and compare the growth outcomes of each plate

What does the percentage inhibition tell us?

- State the calculated percentage inhibition and interpret its connection to clove oil inhibiting fungal growth.

How might clove oil be working to inhibit the mold?

- Consider what clove oil contains that can harm microbes.



Step 7: Clean Up

Step 7.1 When your teacher gives the go-ahead (usually after the final observation), collect your sealed plates and any used droppers or materials for proper disposal. **Do not open the plates.** The teacher will sterilize or dispose of them safely (they may contain live fungal cultures). Throw away used gloves and wash your hands thoroughly. Clean the work area with disinfectant if instructed.



Reflection & Connection:

After completing the observations, reflect on these questions with your group and be prepared to discuss or write about them (see your Data Collection Worksheet for space to answer):

- **What did you observe about fungal growth on the control vs. the treated plate?** Which had more growth, and how would you describe the difference?
- **How effective was the clove oil in inhibiting the mold?** Use your measurements (and the percentage inhibition you calculated) to support your answer.
- **Why do you think the clove oil affected the powdery mildew?** What might be happening to the fungus when it encounters clove oil?
- **How does this activity demonstrate an aspect of Integrated Pest Management (IPM)?** Think about how we identified a problem and used a targeted solution. Why is using a natural treatment like clove oil considered a sustainable or eco-friendly approach to pest control?

Discussing these questions will help you see how a simple lab test connects to real farming practices. In agriculture, farmers often monitor their crops for signs of disease (just like we looked for spores) and then intervene in targeted ways (like using a bio-based fungicide or other IPM methods) to protect the plants while minimizing harm to the environment.

Tips for Success:

- **Label Everything:** You've already labeled your plates – great! Always label your samples clearly with treatment and group names. This avoids confusion later, especially when colonies start growing and plates all look similar.



- **Minimize Exposure:** When working with the agar plates, keep lids off for as short a time as possible. Open one plate at a time and close it as soon as you've pressed the leaf and added the treatment. This keeps other airborne mold or bacteria from contaminating your experiment (and keeps the powdery mildew spores in place).
- **Use Separate Tools:** Don't accidentally mix up your droppers or swabs between the water and clove oil. Using separate tools for each ensures your control plate really stays a true control (unaffected by clove oil).
- **Even Distribution:** If using a swab to spread liquids, be gentle and try to cover the area where the leaf was pressed. An even, light spread of clove oil gives the fungus less of a chance to find untreated spots to grow.
- **Be Patient:** Fungal growth can take a little time. If you don't see anything after the first day or two, don't panic. Give it a couple more days. Conversely, if one plate becomes fully overgrown, that's useful data too – just make your observation and note it.
- **Observe Carefully:** When looking at your plates, hold them up to good light. Sometimes a very thin fungal growth is hard to see – look for any white or grayish film on the agar. You might also notice a **difference in texture**: the agar where mold grows could look fuzzy or less shiny. Take notes even on subtle observations.
- **Calculate Accurately:** When computing percentage inhibition, double-check your measurements and math. This number will help quantify clove oil's effect, so you want it to be as accurate as possible. If you're unsure how to calculate it, ask for help – it's a good practice in applying math to science.
- **Think Big Picture:** Remember that this isn't just about one little leaf or plate. As you work, consider how this experiment is a small-scale model of a real-world scenario: detecting a crop disease and managing it with a sustainable method. This mindset will help you answer the reflection questions thoughtfully.

Skills You'll Use:

- **Microbiology Technique:** practicing basic sterile technique (keeping plates closed and uncontaminated, handling a biological sample safely) – similar to what researchers and lab technicians do when culturing microbes.
- **Observation & Measurement:** honing your ability to observe biological growth over time and measure it quantitatively (using rulers, calculating differences, and percentages).
- **Data Recording:** systematically recording what you see in tables and notes, which is key to any scientific investigation.
- **Data Analysis:** comparing two treatments (with vs. without clove oil) and interpreting what the differences mean. You'll use calculations (percent inhibition) to support your conclusions with evidence.
- **Critical Thinking:** thinking through why you got the results you did – what does it imply about clove oil's properties? How do fungi grow? – and considering the reliability of your experiment (Were there any errors or variations? What could be improved?).
- **Systems Thinking:** connecting this experiment to the broader concept of integrated pest management and sustainable agriculture. You're seeing firsthand how **monitoring** (spore detection) and **targeted action** (natural antifungal) can be parts of a larger system to keep plants healthy while reducing chemical use.

By the end of this activity, you will have done more than just grow mold – you will have explored a mini-version of how farmers and scientists tackle crop diseases in eco-friendly ways. **Powdery mildew** on a plate today can represent pests in a field tomorrow, and the clove oil trick mimics real alternatives farmers might use instead of synthetic pesticides. This



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ties into our big goal of creating **sustainable food systems**: using knowledge and natural resources wisely to protect crops with minimal harm to our environment. 🌱🔬 **Have fun**, be safe, and keep your curiosity alive – you're learning skills and concepts that extend far beyond this single experiment!