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Title: Measuring Volatile and Non-volatile Antifungal Activity of Biocontrol Products

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interview Statements are read by JoVE's voiceover talent.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **28**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **JoVE's Voiceover Talent**: This protocol provides a method for making accurate side-by-side comparisons of the relative efficacy of volatile and non-volatile antifungal compounds at different fungal growth stages **[1]**.

1.1.1. Use Figure 2B *Video Editor: please sequentially emphasize graphs OR no animation*

REQUIRED:

1.2. **JoVE's Voiceover Talent**: This method is well-suited for evaluating the antifungal activity of plant-derived products used in dried or liquid forms that contain a wide diversity of molecules **[1]**.

1.2.1. Use 4.2. Talent adding PDA to tube of garlic powder

OPTIONAL:

1.3. **JoVE's Voiceover Talent**: This method can provide valuable information about the mode of application of plant-derived products and is particularly well-suited in the field of biocontrol **[1]**.

1.3.1. Use 6.4.2. Talent transferring plugs to lids

Introduction of Demonstrator on Camera

1.4. **JoVE's Voiceover Talent**: Demonstrating the procedure will be Valentina Gligorijevic, an Engineer-Assistant from the Sup'Biotech laboratory **[1]**.

1.4.1. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera *Videographer: please film*

Protocol

2. Conidia Recovery

2.1. For conidia collection, layer 3 milliliters of 0.05% Tween-20 on a *Trichoderma* mycelium culture **[1]** and use a rake to release the conidia from the conidiophores, taking care not to press down on the mycelium to prevent the hyphae from being torn away **[2]**.

2.1.1. WIDE: Talent adding Tween-20 to plate, with Tween-20 container visible in frame NOTE: CU at the end

2.1.2. Conidia being raked NOTE: CU + MED

2.2. When all of the conidia have been released, use a micropipette to quickly recover **[1]** and add the conidia solution to a 15-milliliter tube for counting **[2]**.

2.2.1. Solution being aspirated

2.2.2. Talent adding solution to tube, with hemocytometer visible in frame

3. Fungal Plate Preparation

3.1. To prepare fungal plates, deposit 100 microliters of spores at a 3 x 10^6 spores/milliliters concentration onto a 9-centimeter Petri dish containing PDA (P-D-A) medium **[1-TXT]** and use a sterile spatula to add 10 grams of 2-millimeter-diameter glass beads to the plate **[2-TXT]**.

3.1.1. WIDE: Talent adding spore to plate **TEXT: PDA: potato dextrose agar medium**

3.1.2. Talent adding beads to plate **TEXT: 4800 spores/cm^2/plate = 925 spores/5 mm/diameter-agar plug** NOTE: take 2

3.2. Then gently shake the plate forward and backward **[1]**, spin and rotate the plate in 90-degree segments until it has been fully rotated to evenly distribute the spores across the surface of the agar **[2]**.

3.2.1. Plate being tilted *Videographer: Important/difficult step*

3.2.2. Plate being spinned and rotated *Videographer: Important/difficult step*

3.3. Then incubate the plate at 30 degrees Celsius until growth inhibition analysis **[1]**.

3.3.1. Talent placing plate at 30 °C

4. Garlic Contact-Inhibition Plate Preparation

4.1. To perform a contact-inhibition assay with garlic powder, use a sterile spatula to weigh the desired garlic powder quantity into individual 50-milliliter tubes **[1]** to obtain concentrations generally ranging from 0.25 to 16 milligrams/milliliter **[2]**.

4.1.1. WIDE: Talent adding garlic to tube on balance

4.1.2. Shot of tubes with varying amounts of garlic powder

4.2. Add 10 milliliters of approximately 45-degree Celsius PDA to each tube **[1-TXT]** and carefully invert each tube several times to evenly distribute the powder throughout the agar **[2]**.

4.2.1. Talent adding agar to tube(s) **TEXT: Optional: Check agar temperature against inside of wrist**

4.2.2. Talent inverting tube

4.3. Quickly pour the homogenized suspensions into 5-centimeter-diameter Petri dishes **[1]** and allow the agar to solidify at room temperature **[2]**.

4.3.1. Talent adding agar to plate

4.3.2. Shot of solidified agar

5. Contact-Inhibition Assay

5.1. To perform a contact-inhibition assay, use a 5-millimeter-diameter sterile stainless-steel tube to plot a circle in the center of the agar in the control and antifungal compound-treated dishes **[1]** and use a sterile toothpick to dispose of the agar cylinders **[2]**.

5.1.1. WIDE: Talent making circle in dish

5.1.2. Plug being removed

5.2. Next, use a new 5-millimeter diameter sterile stainless-steel tube to plot 15-20 circles randomly into the fungal plates **[1]** and use sterile toothpicks to carefully transfer the spore-, hyphae-, or mycelium-covered cylinders into the empty regions of the PDA plates **[2]**.

5.2.1. Circles being plated *Videographer: Important step*

5.2.2. Cylinder being transferred *Videographer: Important step*

5.3. Then return the plates to the 30-degree Celsius incubator **[1]**.

5.3.1. Talent placing plate(s) in incubator NOTE: take 2

6. Vapor-Phase Antifungal Inhibition Assay

6.1. To prepare plates for a vapor-phase antifungal inhibition assay, pour 10 milliliters of PDA medium into the lid of a 5-centimeter-diameter Petri dish containing 10 milliliters of PDA medium containing antifungal compounds or PDA medium alone **[1]** and allow the agar to solidify at room temperature **[2]**.

6.1.1. WIDE: Talent adding medium to dish *Videographer: Important step*

6.1.2. Shot of solidified agar with bottom of the Petri dish containing antifungal compounds in frame *Videographer: Important step*

6.2. Using a 50-milliliter centrifugal tube as a calibration tool, make a circle of PDA in the center of the agar **[1]** and use a sterile spatula to remove the agar around the circle **[2]**.

6.2.1. Talent making circle *Videographer: Important step*

6.2.2. Talent removing circle *Videographer: Important step* NOTE: CU of the result at the end

6.3. Use a 5-millimeter-diameter sterile stainless-steel tube to plot a circle in the center of the medium in the lid **[1]** and use a sterile toothpick to discard the agar-cylinder **[2]**.

6.3.1. Circle being plotted *Videographer: Important step*

6.3.2. Cylinder being discarded *Videographer: Important step*

6.4. Then use a 5-millimeter diameter sterile stainless-steel tube to randomly make plugs in the prepared fungal plates **[1]** and use a sterile toothpick to carefully transfer spore-, hyphae-, or mycelium-coated plugs into the lids of the assay plates **[2]**.

6.4.1. Talent making plug(s) *Videographer: Important step +picking*

6.4.2. Talent transferring plug(s) into lid(s) *Videographer: Important step*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
3.2., 5.2., 6.1.-6.4.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
3.2. This step needs attention to properly distribute the spores on the surface of the plate and obtain reproducible results. To ensure success, we specifically use 2 mm beads and avoid excessive rotation.

Results

7. Results: Representative Antifungal Compound Inhibition of Spore, Early Hyphae, and Mycelium Growth

7.1. Different diameters of fungal-radial growth **[1]** can be observed 24 hours after the transfer of agar areas from under the cellulose disks **[2]**, highlighting the presence of residual fungal hyphae on the agar after disk transfer **[3]**.

7.1.1. LAB MEDIA: Figure 2C
7.1.2. LAB MEDIA: Figure 2C *Video Editor: please emphasize different circles of growth in disk*
7.1.3. LAB MEDIA: Figure 2C

7.2. The quantification of residual hyphae can be confirmed by the measurement of growth, with an up to 22% diameter variability **[1]**.

7.2.1. LAB MEDIA: Figure 2D *Video Editor: please sequentially emphasize data bars OR no animation*

7.3. In this analysis, *Trichoderma spp.* SBT10-2018 (S-B-T-ten-two-thousand-eighteen)-growth inhibition triggered by three antifungal compounds was evaluated using contact- and vapor-phase inhibition assays as demonstrated for each fungal stage **[1]**.

7.3.1. LAB MEDIA: Figure 3

7.4. A higher spore sensitivity to carbendazim was observed **[1]** compared to early hyphae **[2]** and mycelium networks when *Trichoderma* and antifungal compounds were in contact **[3]**.

7.4.1. LAB MEDIA: Figure 3B *Video Editor: please emphasize Spores graph*
7.4.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize Early hyphae graph*
7.4.3. LAB MEDIA: Figure 3B *Video Editor: please emphasize Mycelium graph*

7.5. In contrast, carbendazim had no antifungal effect on *Trichoderma* when the fungus was placed at distance from the fungicide **[1]**, in accordance with the low volatility of this substance **[2]**.

7.5.1. LAB MEDIA: Figure 3B *Video Editor: please emphasize white data bars in all graphs*
7.5.2. LAB MEDIA: Figure 3B

7.6. When *Thymus vulgaris* essential oil was used as an antifungal compound **[1]**, a higher spore sensitivity was again observed **[2]** as compared to early hyphae and mycelium growth inhibition at a 0.01% essential oil concentration **[3]**.

7.6.1. LAB MEDIA: Figure 3C
7.6.2. LAB MEDIA: Figure 3C *Video Editor: please emphasize black 0.01 Spores data bar*
7.6.3. LAB MEDIA: Figure 3C *Video Editor: please emphasize black 0.01 Early hyphae and Mycelium data bars*

7.7. As expected, *Thymus vulgaris* essential oil presented identical antifungal activity irrespective of the distance between the fungus and the oil **[1]**.

7.7.1. LAB MEDIA: Figure 3C *Video Editor: please emphasize white data bars in all graphs*

7.8. When garlic powder was used **[1]**, a higher efficacy was observed against spore germination **[2]** and early hyphae elongation **[3]** than for mycelium growth **[4]**.

7.8.1. LAB MEDIA: Figure 3D
7.8.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize Spores graph*
7.8.3. LAB MEDIA: Figure 3B *Video Editor: please emphasize Early hyphae graph*
7.8.4. LAB MEDIA: Figure 3B *Video Editor: please emphasize Mycelium graph*

Conclusion

8. Conclusion Interview Statements

8.1. **JoVE's Voiceover Talent**: Particular attention must be paid during the fungal plate preparation to make sure that the spores are evenly distributed on the surface of the agar plate to ensure comparable results **[1]**.

8.1.1. Use 3.2. plate being tilted and/or rotated