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Determination of Inhibitory Effects of Essential Oils on Fungal Pathogen Botrytis cinerea

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Determination of Inhibitory Effects of Essential Oils on a Fungal Pathogen *Botrytis cinerea*
A Proposal for the Research and Creative Experience for Undergraduates (RCEU) Program, Summer 2015

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Project Summary

Sequencing methods suggest that there are about 5.1 million fungal species on Earth with most of them still undiscovered. Fungi remain the most understudied organisms, with 50 researcher studying bacteria for every 1 studying fungi. We will target *Botrytis cinerea*, known as gray mold, which has been one of the major pathogens affecting harvested fruit and vegetables resulting in billions of dollars of world-wide agricultural losses annually. *B. cinerea* infection is usually treated with harsh synthetic antifungals that are dangerous for the environment, negatively affect bee communities, and lead to development of human diseases, e.g. cancer. Thus it is desirable to find new, harmless antifungals. Plants are continuously exposed to fungal spores and some of them have developed means of protection against them, either in the form of phytochemicals. One set of phytochemicals – volatiles from plant essential oils have proven very effective in fungal inhibition. Plants producing essential oils thus represent an excellent alternative to synthetic antifungals – grown in vicinity or with a crop of interest, they may prevent widespread fungal infections. We have previously identified several essential oils that inhibit *B. cinerea* growth. The goal of this research proposal is to find more essential oils with anti-*Botrytis* activities and determine the microscopic effects of mechanism of action of these inhibitors.

Student Duties

B. cinerea fungal growth

The student will take care of B. cinerea culture growth. Being a fast growing fungus, cultures of B. cinerea need to be maintained continuously. The student will have to isolate fungal spores, filter them to remove other fungal parts, determine their number and plate them on an agar plate supplied with potato dextrose media.

Inhibitor Screening

The student will perform *B. cinerea* inhibitor screening in three different ways. First broth microdilution will be used to determine whether an essential oil has any inhibitory activity against the fungus and if so, what concentrations efficiently inhibit fungal growth. In the next step, a series of disc diffusion studies with sub-inhibitory concentrations of the essential oil will be performed, fungal samples collected and subjected to confocal microscopy. Because of the volatility of essential oils, we will also use non-contact inhibition assay (i.e. fungus will be exposed to vapor phase only), collect samples and observe the microscopic effects of volatiles using confocal microscopy. Proper controls of fungal growth will be used at every step.

Determination of Microscopic Effects of Essential Oils on B. cinerea Growth
Samples of *B. cinerea* grown in presence of inhibitory essential oils will be analyzed using confocal microscopy. Fluorescent staining will be employed to visualize any damages to fungal cell wall and to determine viability of the fungus. The fluorescent images will be combined with DIC images to produce 3D representation of the essential oil effects.

Manuscript Preparation
The obtained data are expected to be a part of a research paper and the student will be expected to contribute to manuscript preparation by preparing high quality images and assisting with writing.

Expected Results and Deliverables
The microscopic effects of essential oils that were previously identified to inhibit *B. cinerea* growth will be determined. New essential oils with anti-*Botrytis* activity will be identified and further studied. Depending on time, the newly identified inhibitory essential oils might be analyzed by GC-MS and the volatile composition determined.

Benefits to the Student
The RCEU participant will learn the foundation of fungal culture growth and propagation, different types of inhibitory assay set-up, fluorescent labeling of different fungal parts, and data collection using the confocal microscope. Being a part of a larger research laboratory, the student will also be exposed to routine biochemistry work on a daily basis. Most importantly, the student will learn how to work with primary literature, design follow-up experiments based on observations, and use critical thinking which goes hand in hand with any scientific experiment.

Mentor Supervision and Interaction
Mentor will interact with the student on a daily basis, introducing the student to necessary lab techniques, checking on the progress of the project, assisting with data collection and experimental design. In addition, the RCEU participant will take an active part in the weekly lab meeting where he/she will be required to present the outcomes of the project. The project will culminate with poster presentation, which will be closely supervised by the mentor.