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Title: Developing a Natural Antifungal Foliar Spray with Synergistic UV-A Light Enhancement

Background:

Nursery plants are threatened by various phytopathogenic fungi, causing substantial economic losses. Fungicide protection is considered a major approach for management of these phytopathogens. However, this approach has encountered several challenges, including fungicide resistance, issues of phytotoxicity, concerns about chemical residues, environmental impact, and potential health risks. As a result, there an increasing need for alternative, more sustainable methods for disease management.

This research project recognizes the need for effective and eco-friendly approaches to disease control in the Oregon nursery industry. Specifically, it explores the potential of natural antimicrobials that have demonstrated enhanced efficacy with the synergistic effect of UV-A light for inactivating pathogenic bacteria and fungi. When exposed to light of appropriate wavelength, photoactive antimicrobials can absorb energy and initiate a series of reactions that generate reactive oxygen species (ROS). These ROS exhibit antimicrobial properties by causing oxidative damage to multiple targets of microbial cells, thus enhancing the efficacy of the antimicrobial agents while mitigating the development of resistance[1]. Successful applications of this novel approach for disease control have been observed in the food and agriculture sectors for addressing various phytopathogens and foodborne pathogens, including *Xanthomonas citri*, *Pseudomonas syringae*, *Botrytis cinereal*, and more [2,3].

This proposed research aims to develop and optimize a foliar spray based on these natural antimicrobials, combined with synergistic exposure to UV-A light for disease control. *Calonectria pseudonaviculata* (previously called *Cylindrocladium pseudonaviculatum*), the phytopathogen that responsible for boxwood blight, will be the target of the research. The successful development of this approach will yield an efficient, environmentally friendly, and cost-effective biofungicide for disease prevention in the Oregon nursery industry.

Project objectives and outcomes:

Objective 1: Screening the efficacy of five natural antimicrobials (gallic acid, curcumin, caffeic acid, riboflavin, clove oil) in inactivating *C. pseudonaviculata* in the presence and absence of UV-A.

Objective 2: Formulating and optimizing a foliar spray utilizing the promising compounds identified in Objective 1.

Objective 3: *In vitro* assessment of the optimized spray for controlling the spreading of *C. pseudonaviculata* on boxwood leaves.

Deliverables: 1) A promising foliar spray with natural ingredients for disease prevention, offering an eco-friendly and cost-effective solution for the Oregon nursery industry. 2) Knowledge as a foundation for future in-plant applications of the spray to combat diseases afflicting nursery crops.

Methods:

Evaluation of antifungal efficacy with the synergism of light: An *in vitro* germination assay will be performed for evaluation of antifungal efficacy. *C. pseudonaviculata* will be obtained from ATCC, activated, and grown on potato dextrose agar (PDA). To prepare the inoculum, 10 mL of peptone buffer solution will be added to a PDA with 7 days-old *C. pseudonaviculata*, and the colonies will be scraped using a sterile spreader to detach conidia from the colonies. Then the supernatant will be collected and filtered

using sterile gauze to separate the spores from the hyphae and conidiophores. The obtained conidia inoculum will be enumerated using a hemocytometer and adjusted to a concentration of $1x10^4$ conidia/mL. The conidia will then be spread-inoculated on PDA plates that are supplemented with different concentrations of antimicrobials (0 - 1000 ppm) and exposed to a UV-A light for 0, 30, 60, 90, and 120 min. After the light exposure, the agar plates will be incubated at 20 °C for 24 h, and the length of the germination tubes will be measured using an optical microscope. Percentage of germination of 50-100 conidia arbitrarily chosen from each plate will be assessed. A conidium will be considered geminated if the germ tube is twice the width of the conidium. For comparison, conidia that are treated only with antimicrobials or UV-A light will be separately included as control samples to evaluate the synergistic antifungal effects between the compounds and light irradiation. All measurements will be performed at triplicate. Statistical analysis will be performed using SAS or JMP.

Formulation and optimization: Box Behnken Design will be adopted to assist formulating and optimizing the foliar spray. The variables include the type of antimicrobial compounds, the concentrations, and UV-A dosage. The optimal condition will be determined based on *in vitro* efficacy for inactivating *C. pseudonaviculata* conidia as descried above. Ethylenediaminetetraacetic acid (EDTA), a commonly used chelating agent, will be included to improve antifungal efficacy if necessary.

Evaluation of disease inhibition on boxwood leaves: An *in vitro* assay will be performed to test the effect of the optimized foliar in controlling the spread of *C. pseudonaviculata* on boxwood leaves. Conidia *C. pseudonaviculata* of harvested as descried above will be spot inoculated on pre-sanitized leaves of boxwood. Afterwards, the leaves will be incubated at room temperature and observed for infection area (% of a leaf) over 10 days. Leaves inoculated with deionized water will be controls. All measurements will be performed at triplicate. Statistical analysis will be performed using SAS or JMP.

Timeline:

- *Jan 2024 - April 2024:* Screening of antifungal efficacy with and without synergistic UV-A
- *May 2024 – Aug 2024*: Formulation and optimization of the foliar spray; Interim report
- *Sep 2024 – Dec 2024: In vitro* disease inhibition assessment on boxwood leaves; Final report

Budget:

Proposed budget	**Dollar amount**	**Justification**
Salaries (PI)	4,176	PI's salary support for 0.5 calendar month (0.042 FTE)
Salaries (Graduate Research Assistant, GRA)	28,924	Salary support for a full time GRA (0.49 FTE) for 1 year
Benefits (PI)	1,097	PI's fringe benefits for 0.5 calendar month
Benefits (GRA)	7,466	A full time GRA's fringe benefits for 1 year
Supplies	5,000	Accommodate the cost of chemicals, microbiological culture and supplies, and other relevant lab consumables
Total	**46,663**	

Key references:

1. do Prado-Silva, et al. (2022). *Food Control*, *132*, 108527.
2. Ferreira, et al. (2021). *World Journal of Microbiology and Biotechnology*, *37*(12), 199.
3. Lima, et al. (2022). *Laser Physics Letters*, *19*(2), 025601.