

United States Department of Agriculture
Project Initiation

Project Data for Accession Number 1034324

Project Title

Be proactive, not reactive: Enhancing microbial safety for small microgreens growers through integrated research and extension

Project Details

Sponsoring Institution National Institute of Food and Agriculture	Project Status ACTIVE
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Program Code [A1701] Critical Agricultural Research and Extension: CARE	

Project Director

Xiao, Z.

Recipient Organization

UNIVERSITY OF CONNECTICUT
438 WHITNEY RD EXTENSION UNIT 1133
Storrs-Mansfield, CONNECTICUT 062699018

Performing Department

Nutritional Sciences

Non Technical Summary

Over the past decade, microgreens have been recalled several times in the United States and Canada because they may have been contaminated with harmful bacteria. Because microgreens is a specialty crop that has not been well studied, these recalls highlight the need for better, science-based approaches to reduce food safety risks as the production and consumption of microgreens continue to grow. In this project, we aim to improve the safety of microgreens by combining research with hands-on education for growers. First, we will work with microgreens farmers in Connecticut and West Virginia to understand how they currently grow, harvest, and handle their crops, and we will test for the presence of common harmful bacteria. Based on this information, we will create easy-to-follow best-practice guidelines that align with federal food safety rules. Second, we will develop a natural, plant-based antimicrobial treatment and apply it before and after harvest using specialized spraying equipment to help reduce

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harmful bacteria on microgreens. Third, we will provide in-person and online training, workshops, and educational materials to help microgreens growers in Connecticut and West Virginia better understand food safety risks and adopt safer practices. By bringing together researchers, extension educators, and growers, this project will support the use of safe practices and new technologies. In the long term, it can help reduce foodborne illness, protect public health, increase consumer trust, sustain farm operations, and strengthen food security.

Animal Health Component

Animal Health Component: 0%

Research Effort Categories

Basic	30%
Applied	70%
Developmental	0%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
712	1430	2000	40%
712	1430	1100	60%

Knowledge Area

[712] Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins

Subject Of Investigation

[1430] Greens and leafy vegetables (includes endive, lettuce, spinach, turnip-greens, celery, rhubarb, parsley, asparagus)

Field Of Science

[2000] Chemistry

[1100] Bacteriology

Keywords

Essential Oils, Food Safety, Microgreens, Electrostatic Spray, Integrated Research and Extension

Goals / Objectives

The long-term goals of this proposed research and extension integrated project are to investigate the intersection between microbial safety and microgreen production related to the Produce Safety Rule, to provide scientific evidence for the regulations of microgreens growing, harvesting, packing, and distribution, to improve the overall safety and quality of microgreens, and ultimately to promote a sustainable food system for the local microgreens industry. The specific objectives of the project are to: 1) Survey local microgreen growers in Connecticut and West Virginia on their pre- and post-harvest practices and assess the prevalence of common pathogens on microgreens. Develop SOPs for

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small microgreens growers in accordance with FSMA Produce Safety Rule.2) Develop innovative Pickering nanoemulsions from food-derived proteins and natural phytochemicals as alternatives to chemical sanitizing agents. Use electrostatic sprayers to apply these formulas during pre- and post-harvest stages to improve the microbiological safety of microgreens.3) Mitigate food safety risk by developing in person and online training modules for microgreen growers in CT and WV and providing training through food safety extension services.

Project Methods

Objective 1. Conduct a survey on the current pre- and post-harvest practices and develop standard operation procedures

Task 1.1. Survey the pre- and post-harvest practices for local microgreen growers

Conducting a survey: We will conduct an in-depth survey of 10 commercial microgreens growers in CT and WV. Questionnaires will collect their knowledge and current pre-harvest and post-harvest practices of microgreens and related microbial safety concerns. Data analysis: Survey data will be analyzed using Qualtrics and results will be used to identify areas for improvement and development of educational material. Evaluation: We expect the survey results could provide information on current pre- and post-harvest practices in local microgreen growers in CT and WV and their knowledge and attitude towards microbial safety.

Task 1.2. Assess the prevalence of common pathogens

Conducting a prevalence study: Microgreens samples will be collected from local farms in CT and WV and immediately processed for the isolation and identification of Salmonella. Xylose Lysine Deoxycholate medium will be used, and typical black centered colonies will be isolated for biochemical characterization and real-time PCR. Confirmed Salmonella strains will be subjected to genomic characterization using whole genome sequencing on MiSeq platform. Evaluation: We expect that Salmonella spp. will be isolated from microgreen samples. Genomic characterization will reveal antimicrobial resistance and virulence gene determinants.

Task 1.3. Develop SOPs

Developing SOPs: The project will review current SOPs of various microgreens growers in CT and WV and develop SOPs based on FSMA Produce Safety Rule. Evaluation: We expected that detailed SOPs will be developed based on our presumptive SOP procedures.

Objective 2. Develop antimicrobial Pickering nanoemulsions and apply it using electrostatic sprayers

Task 2.1. Prepare EO-loaded Pickering nanoemulsions

Preparation and characterization of ZCPs and ZCTPs: ZCPs will be prepared by a pH-cycling method. For ZCTPs, tannic acid will be added with continuous stirring for 18 h, exposed to air at room temperature. Finally, the pH will be slowly adjusted to 7.0 to form nanoparticles. Freshly prepared nanoemulsions will be characterized by DLS for particle size, PDI, zeta potential, and morphology.

Preparation and characterization of OEOs

Pickering nanoemulsion and OEOs nanoemulsion: The OEOs will be added into ZCTPs dispersions and mixed by a high-speed homogenizer in an ice bath for preparing the coarse emulsions and then subjected to a sonication to obtain OEOs Pickering nanoemulsions. Tween-80 will be used to obtain OEOs nanoemulsions control. Freshly prepared nanoemulsions will be characterized by DLS for particle size, PDI, zeta potential, and morphology.

Assessment of antimicrobial efficacy of OEOs Pickering nanoemulsions and OEOs nanoemulsions: The antimicrobial efficacies will be determined by Diameter of Inhibition Zone, Minimum Inhibitory Concentration, and Minimum Bactericidal Concentration. Salmonella Enteritidis and Listeria monocytogenes are employed as examples of G⁻ and G⁺ pathogenic bacteria, respectively.

Data analysis: All experiments results conducted in triplicate will be presented as mean values $\pm$ standard deviation. $P < 0.05$ indicates significant difference.

Evaluation: We expect to develop nano-sized Pickering emulsions that can effectively kill pathogenic bacterial. The Pickering nanoemulsion will show a better effect than the conventional nanoemulsion.

Task 2.2. Apply Pickering nanoemulsions through electrostatic spraying system

Bacterial strains and growth conditions: Bacteria (Salmonella and Listeria monocytogenes) will be cultured in tryptic soy broth with 0.6% yeast extract for 18 h at 37 °C, and then sub-cultured for expansion and diluted to approximately 10⁸ CFU/mL for inoculation.

Seed sterilization and plant growth: Radish and broccoli seeds are treated with commercial sanitizer (Tsunami 100, Ecolab) diluted in sterile deionized water for 5 min followed by 3 rinses before transferring to the growth system. Seeds will germinate in the dark condition and continually grow with a 12-hour light cycle at 25/18 °C with the relative humidity of 50-70%.

Inoculation of microgreens: For pre-harvest inoculation, spot inoculation method will be used. Bacterium inoculum will be prepared around the concentration of 10⁸ CFU/mL. For post-harvest inoculation, microgreens are immersed into of bacterium inoculum (10⁸ CFU/mL) at 1:10 ratio and agitated gently at room temperature and air-dried in a laminar flow safety cabinet.

Application of Pickering nanoemulsions through electrostatic spraying system: Essential oil-loaded Pickering nanoemulsions will be applied

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through an electrostatic spraying system. All treated microgreen samples will be packaged and sealed in sterile bags and stored at 4 °C and microbial populations will be counted at predetermined time intervals. Data analysis: All experiment results conducted in triplicate will be presented as mean values $\pm$ standard deviation. $P < 0.05$ indicates there is a significant difference between groups. Evaluation: We expect to find significant microbial reductions detected on microgreen samples after applying electrostatic spraying systems than conventional garden sprayer, and the usage of antimicrobial agents is decreased. Objective 3. Develop in-person and online training modules of best practices and provide food safety training via extension Task 3.1. Develop in-person workshops and online training modules on FSMA best practices In-person training workshop: We will develop and host an in-person training workshop. In the morning, it will include an introduction to cleaning and sanitation practices in a microgreen processing equipment, common microbial safety concerns on microgreens, consequences of pathogen contamination, the effects of packaging conditioning on the inactivation process, and managing possible contamination in the microgreen facility. In the afternoon, the team will demonstrate the postharvest processing with various sanitizing and storage conditions of various microgreens. A follow-up Q&A section will be used to collect additional comments and thoughts. A one-day food safety workshop: During the second year of the project, the team will develop a flyer for the workshop, create the event program, and contact researchers, extension specialists, and local microgreen growers. In the morning, we will discuss the microgreen post-harvest process, quality control, microbial safety, and antimicrobial application. In the afternoon, we will conduct a "writing your own food safety plan" section to assist growers to customize their own food safety plans. Online learning modules: A series of stand-alone online learning modules will be created to accommodate some growers' need, which can be accessed remotely at any time. The recorded videos will be distributed through the websites of UConn and WVU Extension Services. Evaluation: We expect that workshop will supply the essential information and confidence for local microgreens growers in CT and WV to establish a safe pre- and post-harvest procedures. Task 3.2. Provide food safety training to local microgreen growers through UConn and WVU food safety extension services Food safety training: The extension team will provide food safety training for local microgreen growers to disseminate the research findings. The training materials will include pre- and post-harvest food safety; modules addressing specific concerns such as: Salmonella and Listeria monocytogenes, food defense, sanitation, etc. Materials will be offered as training modules on various topics on food safety. Evaluation: We expect that local microgreen producers could increase their knowledge on food safety regulations on microgreens and best practices for their production.