



Introduction

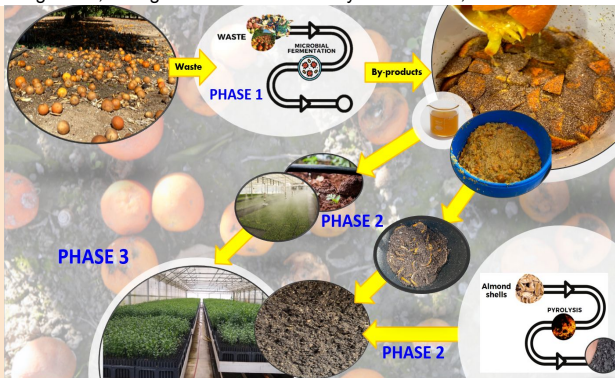
Agricultural waste by-products as soil mix amendments have been shown to be effective in increasing soil organic carbon and nitrogen levels, as well as introducing beneficial microbes into the soil. This in turn reduces the need for chemical fertilizers and increases crop tolerance or resistance against various pathogens (1, 2).

Previous studies have demonstrated the effectiveness of using Biochar (BC), which is pyrolyzed plant matter, in increasing plant growth and nutrient content while reducing symptoms of Tomato mosaic virus in infected tomato plants (3). Similarly, the use of Bokashi (BOK), which involves fermenting food waste anaerobically with a mix of microbes, has been shown to increase the amount of beneficial bacteria in the rhizosphere and enhance resistance against *Phytophthora parasitica* in tomato plants (4).

By amending these agricultural waste by-products into the soil, farmers can promote sustainable agriculture practices and reduce their reliance on chemical pesticide & fertilizers, while also promoting soil health and crop productivity. Further research in this area could yield even more promising results and could help to establish these amendments as standard practice in sustainable agriculture.

Aim

The aim of this project is to investigate the potential impact of using BC and BOK amendments on disease resistance in citrus plants infected with Citrus tristeza virus (CTV) and Citrus exocortis viroid (CEVd). Additionally, this project seeks to explore how the application of BC and BOK affects the citrus microbial community, and to determine whether the microbes present in the amended substrate possess plant growth-promoting or antimicrobial properties against common root rot pathogens such as *Phytophthora* and *Pythium*.



Methods

- Control soil mix: equal parts perlite, peat moss, and coconut coir.
- Treated soil mix: Soil mix blended with 12.5% BOK and 10% BC.
- Biochar is provided by Corigin Solutions and BOK is obtained from PHASE 2 using Bokashi Cycle™ bran and citrus waste from UCR AgOps (5).
- One year old C35 and Carrizo rootstocks were transplanted into treated and untreated soil mixes, grafted with Duncan and Citron, and inoculated with CTV and CEVd.



- Bacteria is isolated from roots treated in control and ag waste amended soil (using Isolation Bio Prospector).
- Zone of inhibition tests are performed against *Phytophthora spp.* and *Pythium spp.* according to images below (6). Scan 500 tool is used to make measurements.

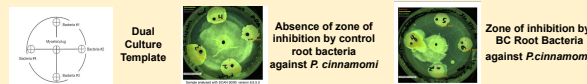


Figure 1. Schematic representation of PHASE 1-3 procedure for agri-food waste processing for the production of carbon-based soil amendments and bio-fertilizers.

PHASE 1. A two-week-long Bokashi anaerobic fermentation, during which the inoculated beneficial microorganisms break down the organic waste, resulting in the production of Bokashi liquid (Bokashi-Liquid) and solid (Bok) by-products, containing various metabolites and nutrients that can be used to improve soil fertility and plant growth (Fig.1);

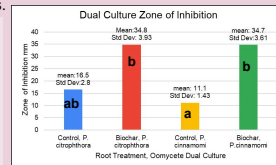
PHASE 2. A two-week-long aerobic composting of Bok (PHASE 1), to create carbon-based soil amendments with improved agronomic performance;

PHASE 3. A standardized greenhouse plant application of blended bokashi by-product (from PHASE 2) with biochar to the soil media and the diluted Bokashi-Liquid (from PHASE 1) via fertigation as carbon-based soil amendments and bio-fertilizers.

Results

- In a pilot trial, bacterial isolates were collected from citrus roots grown in standard soil mix and from roots grown in a soil mix containing 10% biochar (BC). These isolates were then plated on selective media with a *P. cinnamomi* or *P. citrophthora* plug in the center. The bacterial isolates from the BC soil mix showed an average of 210% greater zone of inhibition against *P. citrophthora* and 312% greater zone against *P. cinnamomi* compared to the control isolates (Figure 2).
- The bacterial isolates are currently undergoing sequencing and analysis to determine their specific properties and potential applications.
- Meanwhile, the results of the impact of agricultural waste on virus/viroid disease expression are still pending. These results will provide valuable insights into the effectiveness of agricultural waste as soil amendments to promote plant health and reduce the incidence of viral and viroid diseases.

Figure 2: Bacterial isolates from roots grown in a control soil mix (blue/yellow) and a biochar BC10% soil mix (red/green) were each dual-cultured with *P. citrophthora* (blue/red) and *P. cinnamomi* (yellow/green). Different letters above bars represent significant differences between treatments at a significance level of $P < 0.05$.



Conclusion

The findings from this project will have positive implications for the citrus industry and sustainable agriculture practices. By identifying the potential of biochar and bokashi by-product waste amendments to enhance disease resistance and promote plant growth, farmers could reduce their reliance on synthetic chemicals and improve the health and productivity of their crops. Additionally, the identification of beneficial microbes in the amended substrate could lead to the development of novel biological control methods for root rot pathogens, which could further reduce the need for chemical pesticides. Overall, this project has the potential to advance our understanding of the role of soil amendments in promoting plant health and disease resistance, and to pave the way for more sustainable and eco-friendly circular-based agricultural practices.