A Bright Solution: Acetic Acid but Not Coriander Oil Inhibits Microbial Growth in a Small Scale Continuous Flow Hydroponic System

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Introduction

Food justice and dignity are not accessible to everyone, for example, in the United States 12.7% of households [4] and 475 million people worldwide [5] are food insecure and cannot always intake recommended daily calories. Locally grown foods and hydroponic growing systems are one way to mitigate these issues. Bright Agrotech, a local producer of hydroponic and aquaponic systems, produces a Zip Tower recirculating hydroponic drip irrigation system that has had problems with clogging at the dripper, preventing the nutrient/water mixture from being supplied to the plants. The clog is suspected to be from the growth of a microbial biofilm. This has caused a loss in time, money and crops for agricultural producers and negative implications on the prospects of food security, sustainability, and local food production.

Coriander sativum essential oil has purported anti-biofilm adhesion properties and interferes with quorum sensing [2] .Acetic acid [3] has also been shown to decrease microbial growth through creating a more acidic environment and is reported to be non harmful to plants [1] at low concentrations.

We will attempt to isolate *Pseudomonas* spp. and *Enterobacter* spp. as these are common environmental bacteria. That are commonly found in biofilms[6].

Objectives/Hypotheses

Objectives

- To design in-laboratory small scale recirculating nutrient solution systems and test anti-clogging potential of added treatments of coriander essential oil, acetic acid, and a combination of the two.
- To identify microbial components in the nutrient water that could lead to biofilm formation.
- To test the toxicity of the added treatments on lettuce in the laboratory.
- To make suggestions to Bright Agrotech for minimizing dripper clogging and thus improving food production.
- To understand the effect of temperature on the microbial growth.

Hypotheses

1. We hypothesize that if essential oil extract from *C. sativum* is added to the nutrient solution, at concentrations of 5μL/mL, the growth and adhesion of potential biofilms and microbial growth in the drippers will be decreased.
2. We hypothesize that the addition of 0.25% final concentration of acetic acid to the nutrient solution will also decrease biofilm growth.
3. We hypothesize that the combination treatment will have a synergistic effect and will further limit biofilm formation.
4. We hypothesize that through the addition of these additives the plant growth will be unaffected when compared to a control set-up.
5. We hypothesize that species in the genera of *Pseudomonas and Enterobacter* are major components of the biofilm.

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Results

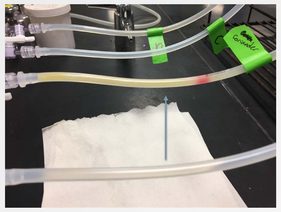


Figure 1: Discoloration and biofilm formation was highest in the coriander line (second from bottom) slight discoloration was observable in the control line (top).

Figure 2: Differences in turbidity in the nutrient water from flushing the lines after 6 weeks of flow. From left to right: control, coriander oil, acetic acid, combo.

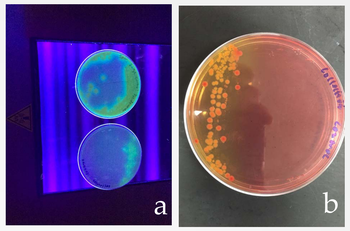
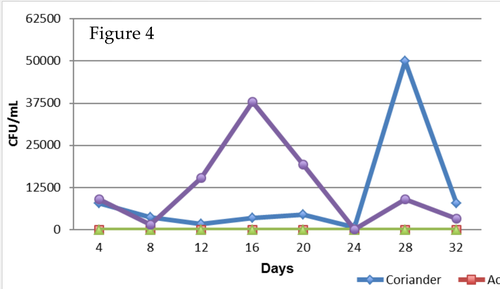


Figure 3: (a) Growth and fluorescence on *Pseudomonas* medium; a positive fluorescence test for the presence of *Pseudomonas* spp. (b) Growth with red colonies on DCLS#2 medium; a positive presence test for *Enterobacter* spp.



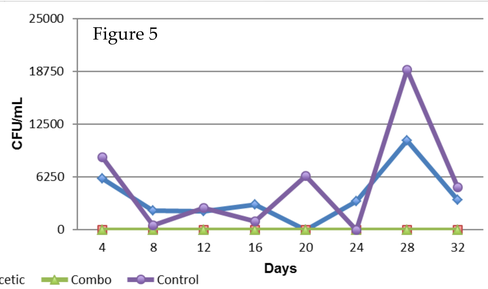


Figure 4: Room Temperature colony counts on BHI through time. Acetic acid and combination treatments inhibited microbial growth. Coriander treatment had the most growth at day 28 and the control saw growth over the course of 4 weeks. Figure 5: 30º C growth on BHI. Again acetic acid and combination inhibited growth while coriander and control treatments had significant colony counts. Overall we observed more growth at room temperature.

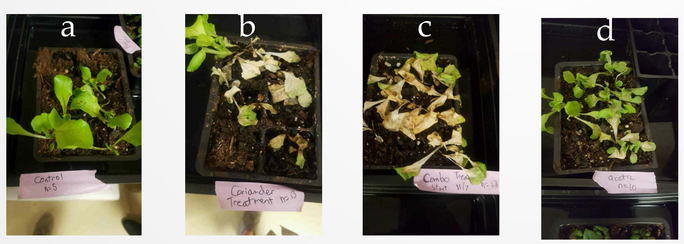


Figure 6: Lettuce plants 24 hours post treatment: (a) control, (b) coriander, (c) combination, (d) acetic.

We observed severe necrosis of the leaves and eventual death in all plants except the control.

Discussion

We did not develop a clog in any of the drippers after 6 weeks of flow. We believe this is due to low flow rate and given enough time we believe a clog would have developed.

Acetic acid showed a high inhibition of bacterial growth, both alone and in combination with coriander oil, supporting our second hypothesis. Coriander oil alone did very little to limit growth, in fact it seemed to increase growth at times and could be acting as an additional metabolite for these microbes. Therefore we reject hypothesis 1. Literature seemed to suggest the anti-biofilm properties of coriander [2] but the studies were done in a hospital setting not on environmental microbial biofilms.

Introducing these treatments killed two week old lettuce plants, leading us to reject our fourth hypothesis, in spite of literature research that suggested plants can tolerate these chemicals. This discrepancy could be due to the manner in which we treated the plants by watering in the treatments which could have "shocked" the plants instead of gradually dripping in the treatment/water mixture. Perhaps the lettuce wasn't established enough to tolerate the treatments and could be a sensitive species.

Notice in Figures 4 and 5 there are some discrepancies in the total culturable bacteria as the flow systems continued. Day 24 was the first flow after refilling the syringes in the pump from our stock nutrient water that was stored in the refrigerator. We suggest the drop in growth could be from some bacterial death in storage and needed more time at higher temperature to grow enough to spike again at day 28.

Because the Acetic Acid Treatment showed complete inhibition of all microbial growth, this agent shows promise for use in hydroponics at lower concentrations that would not be deleterious to plant growth.

Methods

We set up 4 small scale 140 mL syringe pump flow systems (both at room temperature and at 30º C to mimic temperature ranges seen in greenhouse settings) to pump nutrient water obtained from Bright Agrotech through their hydroponic drippers. This is to mimic a large greenhouse hydroponic system. Each flow system had different treatments: one with coriander oil at 5 μL/mL, a second with a 0.25% final concentration of acetic acid, a third had a combination of coriander oil and acetic acid, and the final was the control untreated nutrient water. These were pumped through the dripper at 0.5 mL/Hr over the course of 5 weeks and collected into falcon tubes every 4 days.

Each flow through was enumerated on Brain Heart Infusion (BHI) and *Pseudomonas* media and was tested with aquarium strips.

At the end of the 6 week period a biofilm had formed in the control and coriander treatment tubing. We manually agitated the tubes and T-streaked the collected sediment on the following:

- BHI agar
- *Pseudomonas* Isolation agar
- Desoxycholate Citrate Lactose Sucrose Agar (DCLS#2), which selects for *Enterobacter* spp.

We also tested out treatments on lettuce plants to ensure that these treatments could be useful options for hydroponic farming.

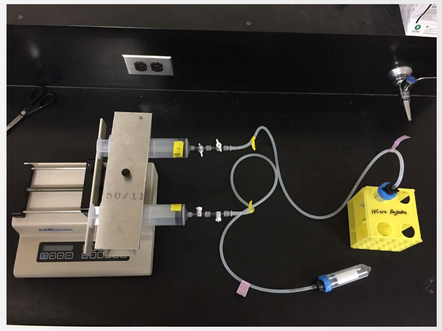


Figure 7: Representation of our small scale flow system; Syringe pump, syringes, flow lines, Bright Agrotech Drippers, and falcon collection tubes.

Conclusion

- Acetic acid is an effective inhibitor of microbial growth in the dripper systems.
- Though we were not able to grow a clog in the dripper we were able to grow a biofilm in the tubing.
- *Enterobacter* spp. and *Pseudomonas* spp. are present in the biofilm.
- Coriander oil did not decrease microbial growth.

Future Directions

- In the future, we would like to test lower concentrations of acetic acid on lettuce survival and antimicrobial effectiveness. This would allow it to be useful in hydroponic farming as a potential solution to biofilm formation.
- We would like to test treatments on a wider variety of plants.
- We would also like to identify more species that are present in hydroponic biofilms.

Resources

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