A New Generation of Fungicides: Testing the Efficacy of Essential Oils on the Inhibition of *Fusarium virguliforme* Growth

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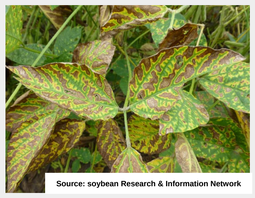
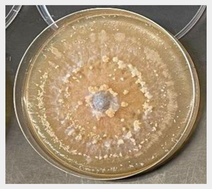
Background



Foliar symptoms of soybean sudden death syndrome (SDS)



Fusarium virguliforme (Fv), the fungal agent of SDS

- Soybean sudden death syndrome (SDS), caused by the fungus *Fusarium virguliforme* (*Fv*) is a top cause of crop damage in the US
- Seed treatment options for managing SDS are limited and there is growing interest in alternatives to synthetic fungicides

Lemongrass plant, clove flower buds, thyme plant

- Essential oils (EOs) have been demonstrated to have antimicrobial and insecticidal properties
- EOs are non-toxic, biodegradable, and generally regarded as safe (GRAS)

Materials and Methods

Inhibition of Mycelial Growth

Fv culture on PDA plate; quadrants marked for mycelial growth measurements

- Petri dish assay; potato dextrose agar (PDA) amended with lemongrass, thyme, or clove leaf oil at concentrations ranging from 0.0-1.0% (v/v), n=2
- Radial mycelial growth measured every two days for twelve days

Inhibition of Spore Germination

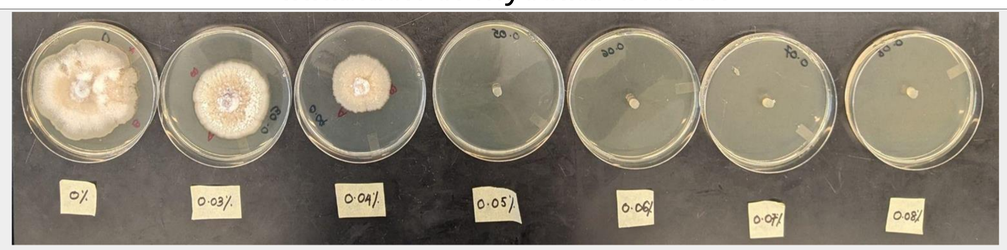
- Spore suspension added to cavity slides filled with EO-amended PDA of different concentrations
- Slides incubated for 17 hours in humidity chamber and then observed under the microscope, n=2
- Counted germinated and ungerminated spores to calculate % germination

Fv spore germination after 17 h (control)

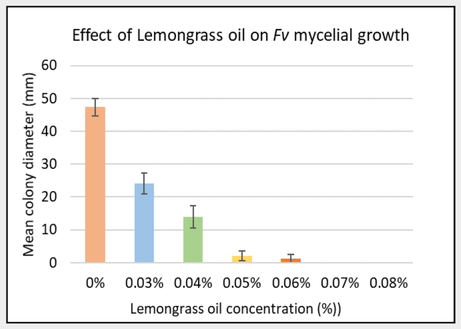
Fv spore in 1.0% lemongrass oil PDA after 17 h

Results

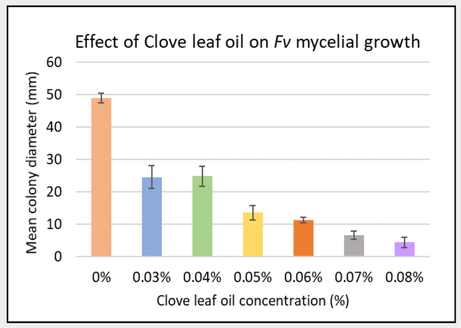
Inhibition of Mycelial Growth



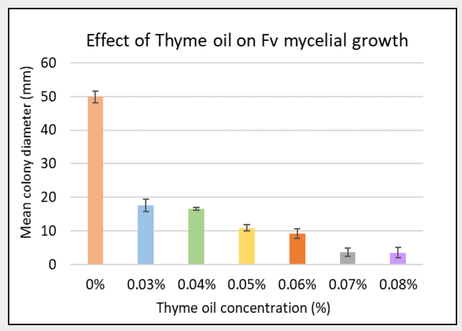
Suppression of *Fv* mycelial growth shown on PDA plates amended with concentrations of 0.00-0.08% lemongrass (LG) oil



Mycelial growth of *Fv* on PDA amended with different concentrations of lemongrass oil (n=10, 5 reps x 2 runs); *P*=0.002; SEM=5.6



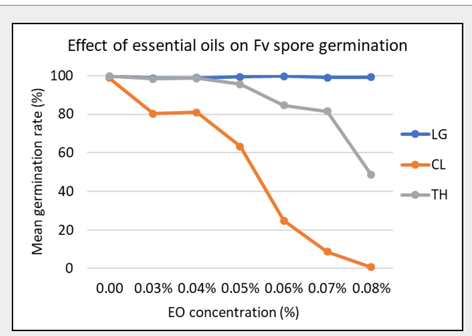
Mycelial growth of *Fv* ion PDA amended with different concentrations of clove leaf oil (n=10, 5 reps x 2 runs); *P*=0.013; SEM=5.4



Mycelial growth of *Fv* on PDA amended with different concentrations of thyme oil (n=10, 5 reps x 2 reps); *P*=0.0007; SEM=3.5

- Lemongrass oil was effective at suppressing *Fv* mycelial growth at concentrations as low as 0.05%
- Clove oil and thyme oil were not as effective, yet still suppressed mycelial growth at concentrations near 0.10%

Inhibition of Spore Germination

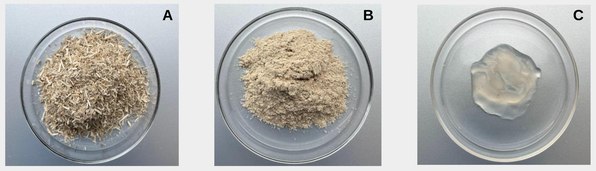


Spore germination (%) of *Fv* on PDA amended with different concentrations of lemongrass (LG, *P*=0.37, SEM=0.3), thyme (TH, *P*=0.03, SEM=8.5) and clove (CL; *P*=0.002, SEM=13.0) leaf oil (n=12, 6 reps x 2 runs).

- Clove leaf oil was the most potent suppressor of *Fv* spore germination out of the three oils tested
  - <2% germination at concentration of 0.08%;
  - <20% germination at concentration of 0.07%
- Lemongrass oil did not suppress Fv spore germination at concentrations of 0.03-0.08%; however, preliminary studies suggest that lemongrass may suppress germination at higher concentrations (≥0.15%)
- Thyme oil was more effective than lemongrass oil at the concentrations tested, with >50% spore germination at the highest concentration tested

Ongoing work

- Screen additional essential oils to determine the most effective against the SDS pathogen
- Identify delivery methods that maximize the effectiveness of the essential oils
- Nano-encapsulation of essential oils using soybean residue nanocellulose (in collaboration with Dr. Liu, Ag. & Biosystems Eng., ISU)



Preparation of nanocellulose from soybean residue: A) Raw soybean stove; B) washed and ground soybean stover; C) stover-derived nanocellulose.

- Test effectiveness of essential oils in suppressing SDS symptom development in plant assays

Control (no LG) | Organic substrate (no LG) | Organic substrate with LG

Preliminary results: soil amendment with lemongrass oil (LG) in an organic substrate suppressed SDS root rot and foliar symptoms (plants transferred to water for symptoms observation only)

Implications

- Plant essential oils have the potential to become an environmentally- friendly option for managing fungal diseases of plants
- Current limitations to their use include active compound volatility, lack of an effective delivery system to plant roots and unknown impacts on beneficial microorganisms
- Future research will test nanoencapsulation and combination of essential oils with synthetic fungicides as approaches to offset these limitations

Acknowledgements

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