Biocontrol of fungal plant pathogens in cannabis using *Bacillus amyloliquefaciens* I113

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Introduction

- Since the legalization of cannabis in 2018 fungal pathogens have been significantly undermining cannabis yield, with losses as high as 30%[1]
- Furthermore, fungal pathogens in cannabis can infect humans if not managed correctly by producing mycotoxins which can result in fever, pneumonia, and neutropenia[2, 3]
- Fungal pathogens are often very difficult for growers to manage due to the lack of registered fungicides available for use on cannabis[1]
- Additionally, fungicide residue on cannabis can be detrimental to human health as it has been shown to contain endocrine disruptors and hepatoxic compounds[3]
- *Bacillus amyloliquefaciens* (I113), a biocontrol agent, offers a promising solution to reduce fungal infections in cannabis due to its strong antifungal metabolite production
- From previous trials I113 is known to inhibit *Fusarium*, *Alternaria*, *Colletotrichum*, *Septoria* and *Botrytis* fungal species, all of which are common fungal pathogens in cannabis
- I113 has also shown to be an effective bio-stimulant in other field trials with crops such as squash, pumpkins, and tomatoes

Objectives

- To identify the most common fungal pathogens affecting outdoor cannabis production where the trial was conducted
- To determine the *in vitro* anti-fungal activity of I113 against common fungal isolates found in cannabis
- To determine the biocontrol capabilities of I113 on fungal pathogens in cannabis grown outdoors

Methods

Inhibition Assays

- A colonization assay was performed to determine the fungal pathogens that had infected this crop of cannabis
- Fungi were isolated and identified by sequencing
- Fungal isolates were plated on 1:1 PDA-NA and grew until they reached a diameter of ~3cm
- 10µL of I113 along with an NB control were plated on opposite sides of the fungal isolates

Metabolite Screening

- Metabolites were harvested from I113
- High Performance Liquid Chromatography (HPLC) was used to identify metabolites

Field Trail

- I113 was inoculated in nutrient broth and grown for 48 hours on a shaker at 150 rpm and 30°C
- Culture was diluted to 3% and sprayed weekly from June 20th, 2023, to August 18th, 2023
- Disease scores were recorded weekly from August 1st, 2023, to September 9th, 2023, using a scale of 1-4

Results

Inhibition Assays

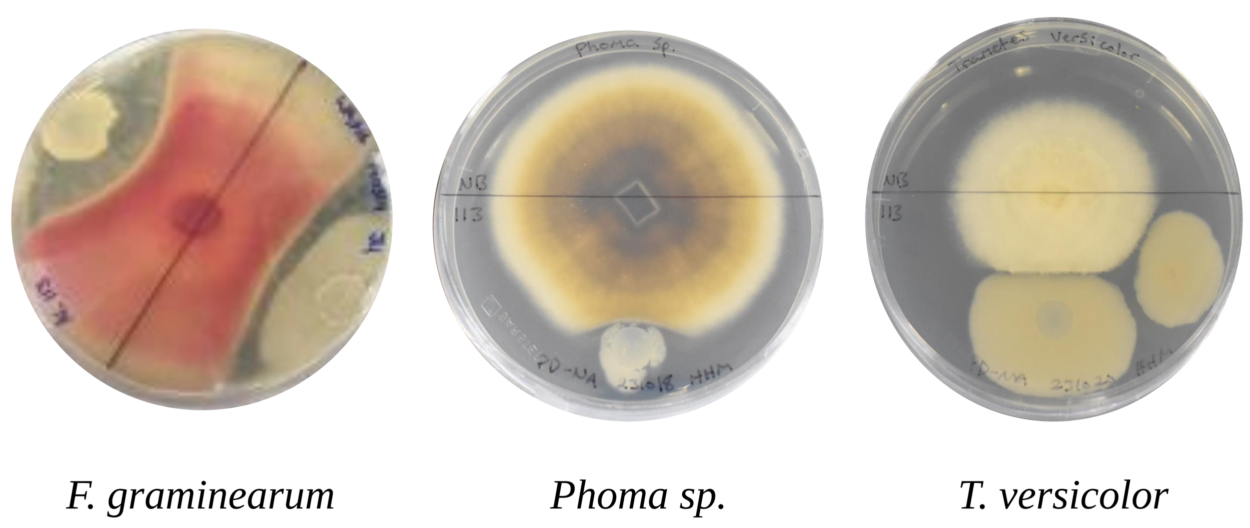
- *Fusarium*, *Phoma*, *Sarocladium*, and *Trametes* fungal species were found to be the main fungal pathogens infecting this crop of cannabis
- I113 is a good biocontrol agent (**Table 1**) as it shows strong inhibition towards *Fusarium*, and moderate inhibition towards *Phoma* and *Trametes* (**Figure 1**)
- I113 produces antifungal metabolites to inhibit the growth of fungal pathogens (**Table 2**)

Functional Traits	I113
Fusarium Inhibition	++++
Phoma sp.	+
Sarocladium strictum	Unknown
Trametes versicolor	++

Table 1. Functional; traits of I113. Scoring scale: (-) negative, (+) weak positive, (++) positive, (+++) high positive, (++++) strong positive.

Metabolite	Mode(s) of Action
Fengycins	Induced systematic resistance Membrane pore formation
Surfactins	Induced systematic resistance Membrane pore formation
Iturins	Membrane Pore Formation

Table 2. Mode of action of 3 cyclic lipopeptides produced by I113[4]. Responsible for the antifungal properties of I113.



F. graminearum *Phoma sp.* *T. versicolor*

Figure 1. Inhibition assays plates with cannabis isolates and I113. Qualitative analysis of assays determined I113 functional traits.

Field Trial

- 40% of fungal isolates from this crop of cannabis were *Fusarium*, the next most abundant fungal pathogen only comprised 10% of the isolates
- I113 reduced disease by an average of 38% during the cannabis vegetative stage with 49% of treated plants having a healthy disease score (**Figure 2&3**)
- Through the vegetative stage to the end of the pre-flowering stage, I113 reduced disease by an average of 28% with 37% of treated plants having a healthy disease score (**Figure 2)**
- Over the whole growing season I113 reduced disease by an average of 20% (**Figure 2&3**)
- The disease characteristics did not vary significantly between the control and treated replicates

Figure 2. Comparison of disease in control and treated cannabis plants. (Top) 6 weeks after treatment began (Bottom) 2 Weeks after budding started.

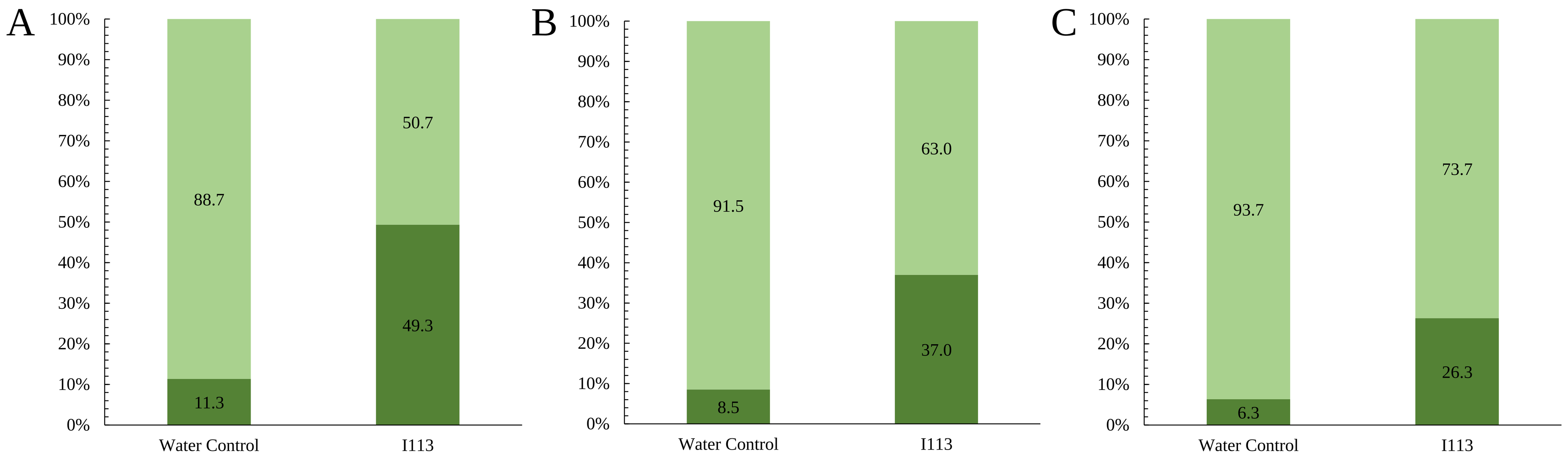


Figure 3. Ratio of healthy (2 or under, dark green)) to unhealthy (3 or over, light green) disease scores. (A) Vegetative stage (B) Vegetative and pre-flowering stage (C) Entire growing season.

Conclusions

- The most common fungal pathogen in this crop of cannabis is *Fusarium* with the most common species being *Fusarium* equiseti
- *In vitro* I113 is effective at inhibiting common fungal pathogens such as *Fusariam,* while still moderately inhibiting less common pathogens such as *Phoma* and *Trametes*
- I113 was effective at reducing disease incidence and severity in cannabis plants
- As the growing season progressed treated plants became increasingly more diseased

Future Directions

- Confirm the effectiveness of I113 at supressing fungal pathogens in cannabis, specifically how to reduce pathogens near the end of the growing season
- Continue to build a database of common fungal pathogens in cannabis to ensure the application of biocontrol treatments targets the most plentiful and harmful pathogens
- Explore the possibility of I113 working as a bio-stimulant in cannabis
- Run trials to determine if I113 could act as either a biocontrol agent or bio-stimulant in closely related crops such as hemp or hops

References

1. Punja, Z.K. (2021). Emerging diseases of Cannabis sativa and sustainable management. Pest Management Science, 77(9), 3857-3870. https://doi.org/10.1002/ps.6307
2. Gwinn, K.D., Hansen, Z., Kelly, H., & Ownley, B.H. (2022). Diseases of *Cannabis sativa* Caused by Diverse *Fusarium* Species. Frontiers in Agronomy, 3(796062). https://doi.org/10.3389/fagro.2021.796062
3. Montoya, Z., Conroy, M., Vandan Heuvel, B.D., Pauli, C.S., & Park, S. (2020). Cannabis contaminants Limit Pharmacological Use of Cannabidol. Frontiers in Pharmacology, 11(571832). https://doi.org/10.3389/fphar.2020.571832
4. Falardeau, J., Wise, C., Novitsky, L., & Avis, T.J. (2013). Ecological and Mechanistic Insights Into the Direct and Indirect Antimicrobial Properties of *Bacillus subtilis* Lipopeptides on Plant Pathogens. Journal of Chemical Ecology, 39, 869-878. https://doi.org/10.1007/s10886-013-0319-7