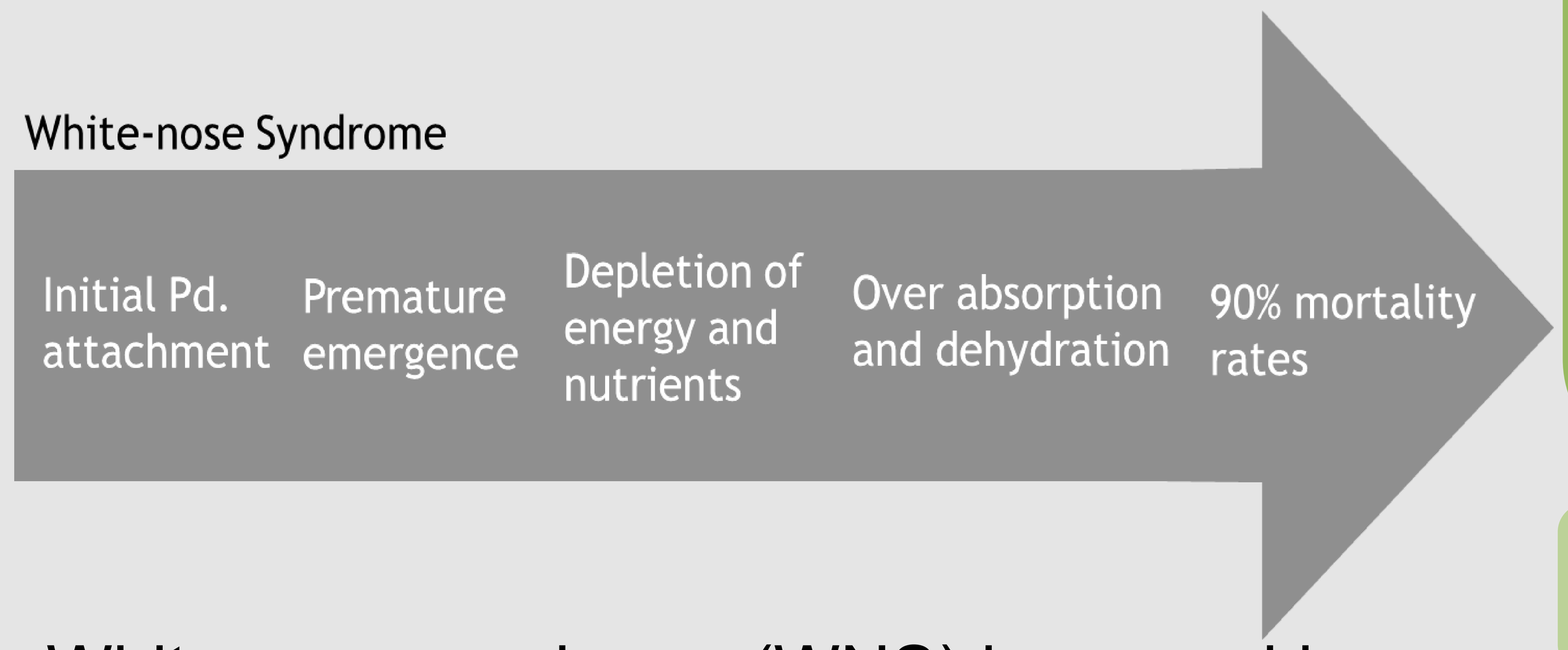
The Quantitative Effect of Colchicine on the Inhibitory Growth of *Pseudogymnoascus destructans*

Introduction



- White-nose syndrome (WNS) is caused by the fungus, *Pseudogymnoascus destructans* (*Pd*)
- *Pd* is a psychrophilic fungus, and grows best in temperatures similar to that of a bat hibernaculum
- Certain bacteria applied to infected bats while in a lab setting can help recovery from white-nose syndrome (Zhang, T. 2015)
- A compound that is easily accessible and easily applied needs to be discovered that could inhibit the growth of *Pseudogymnoascus destructans*
- A mitotic inhibitor could limit the number of organisms affected, and most likely still inhibit any growth of *Pd* (Chaturvedi, V. 2010)
- The problem trying to be answered in this study is if mitotic inhibitors can be used to hinder the growth of *P. destructans* in a lab setting
- Colchicine is the mitotic inhibitor used in this study

Hypothesis

If this mitotic inhibitor is applied to the fungus, then the growth of the fungus may slow and decay could occur. It is expected that higher concentrations of Colchicine applied to wild *P. destructans* isolates will cause the growth of the fungus to be inhibited. If Colchicine can stop the growth of this fungus, it could be applied to bats that have *Pseudogymnoascus destructans* attached to them.

Materials and Methods

- Fungal samples were taken in the Long Island area (2018-2019)
- Incubated at 6°C at the DEC Pathology Lab in Delmar, New York
- Five rounds of testing, grown on Yeast Peptide Dextrose agar plates
- The initial test had three agar plates with *P. destructans*; Control (with no Colchicine), 15mL (diluted at 0.5mg Colchicine/1mL PBST), and 30mL (diluted at 0.5mg Colchicine/1mL PBST)
- The second and third rounds of testing ran similarly, with replicate dilutions
- The fourth and fifth test were run likewise with a control, 15mL (diluted as 1mg Colchicine/1mL PBST), and 30mL (diluted 2mg Colchicine/1mL PBST)
- Plates were incubated for two weeks, and were checked every few days
- The growth was photographed and quantified based on area, using a grid pattern
- All materials were submerged in a 5% bleach solution for 10 minutes

Results

Wibbelt, 2010

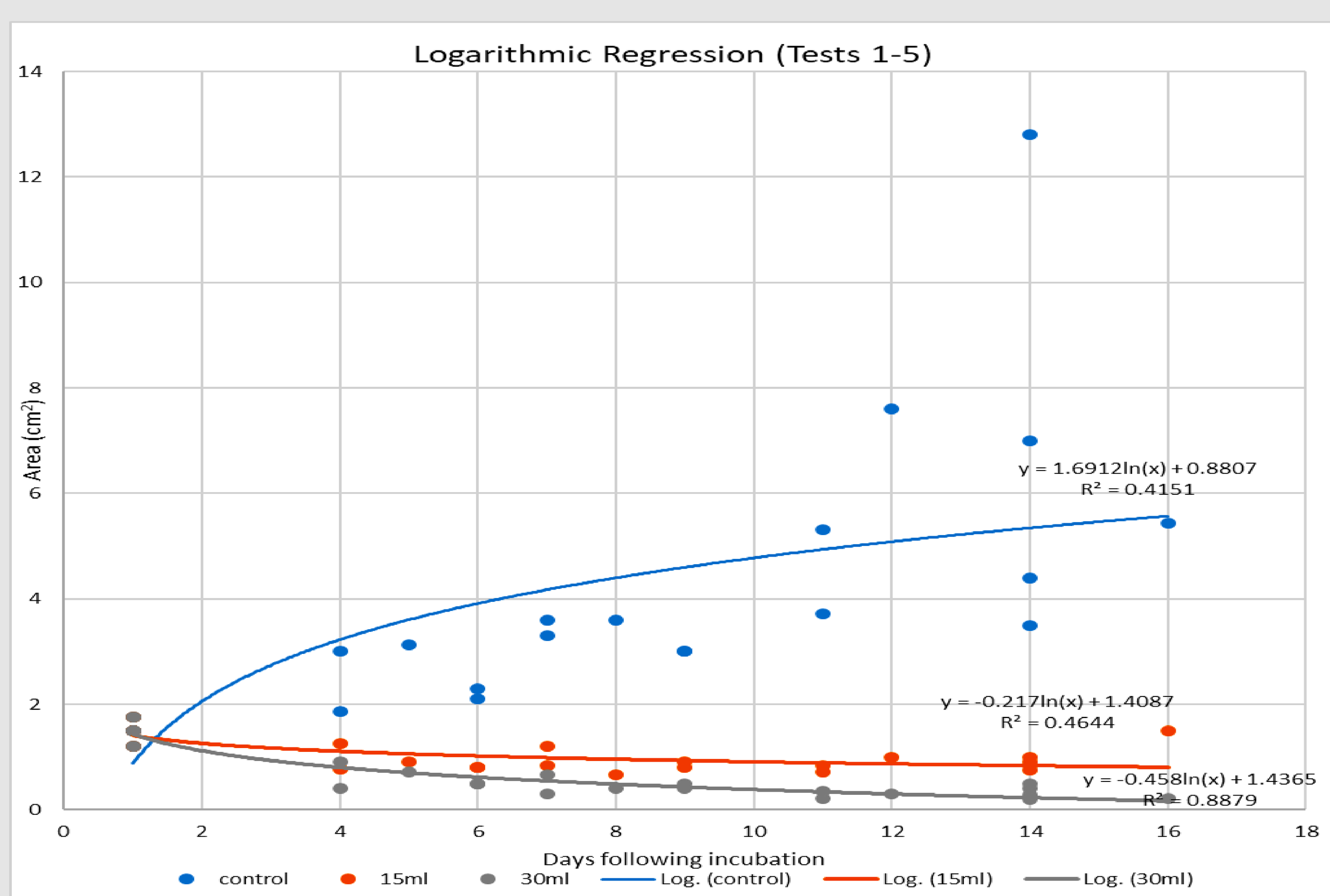
SUMMARY

Groups	Count	Sum	Average Area (cm^2)	Variance	Standard Deviation	Standard Error
Control	23	86.11	3.74391304	6.796024901	2.60691866	0.5435801169
15mL	23	23.94	1.04086956	0.100271936	0.3166574439	0.06602764137
30mL	23	15.2	0.660869565	0.233135573	0.4828411469	0.1006793388

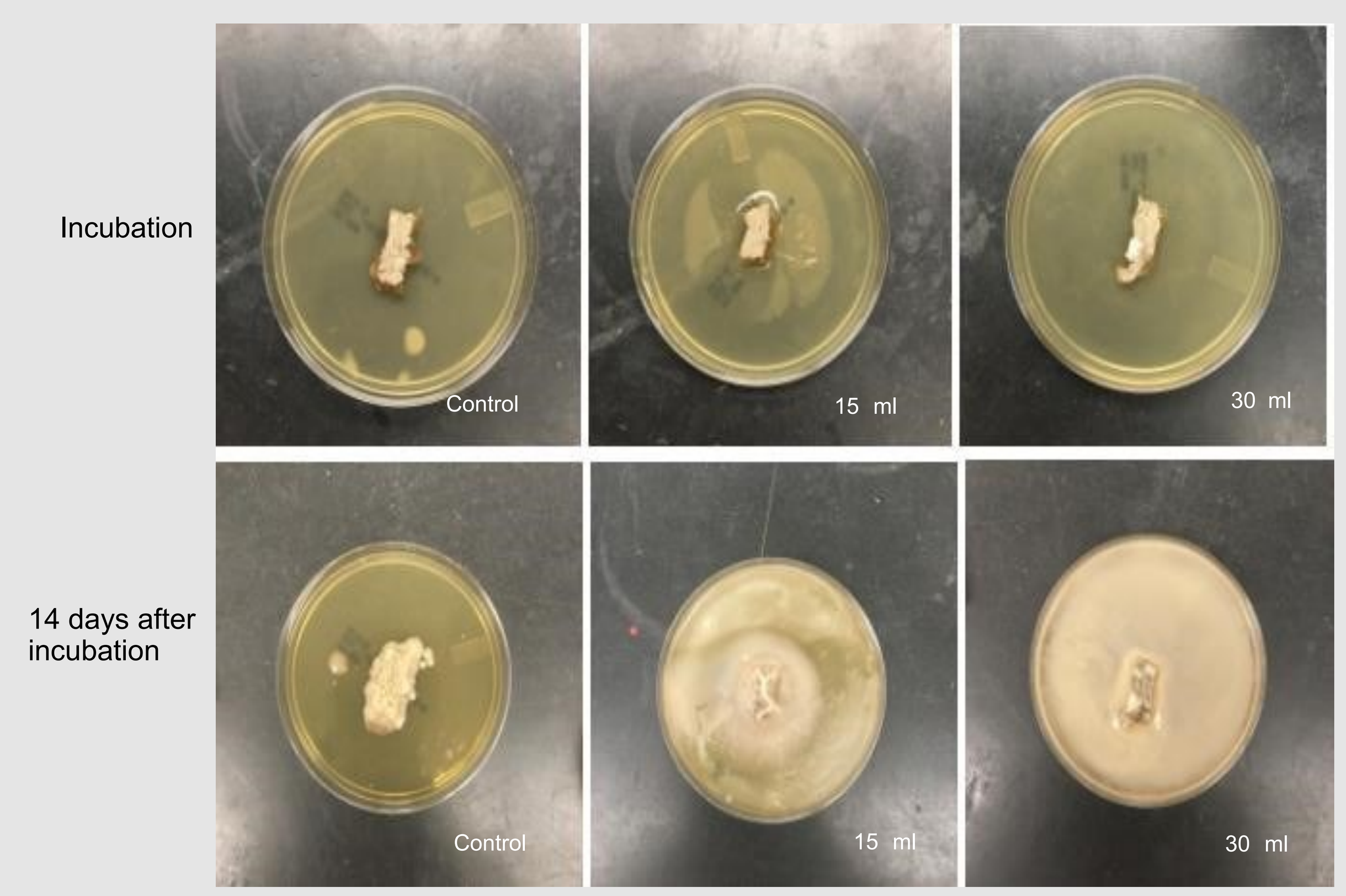
ANOVA

Source of Variation	Sum of Squares	Degrees of Freedom	Mean of Squares	*F*	*P-value*	*F crit*
Between Groups	129.9960087	2	64.9980043	**27.35056619**	**0.00000000223087637**	**3.135917934**

ANOVA Single Factor results. F-critical is lower than F-value, and p-value is lower than 0.5 shows it is able to reject the null hypothesis (Hogan, 2019).

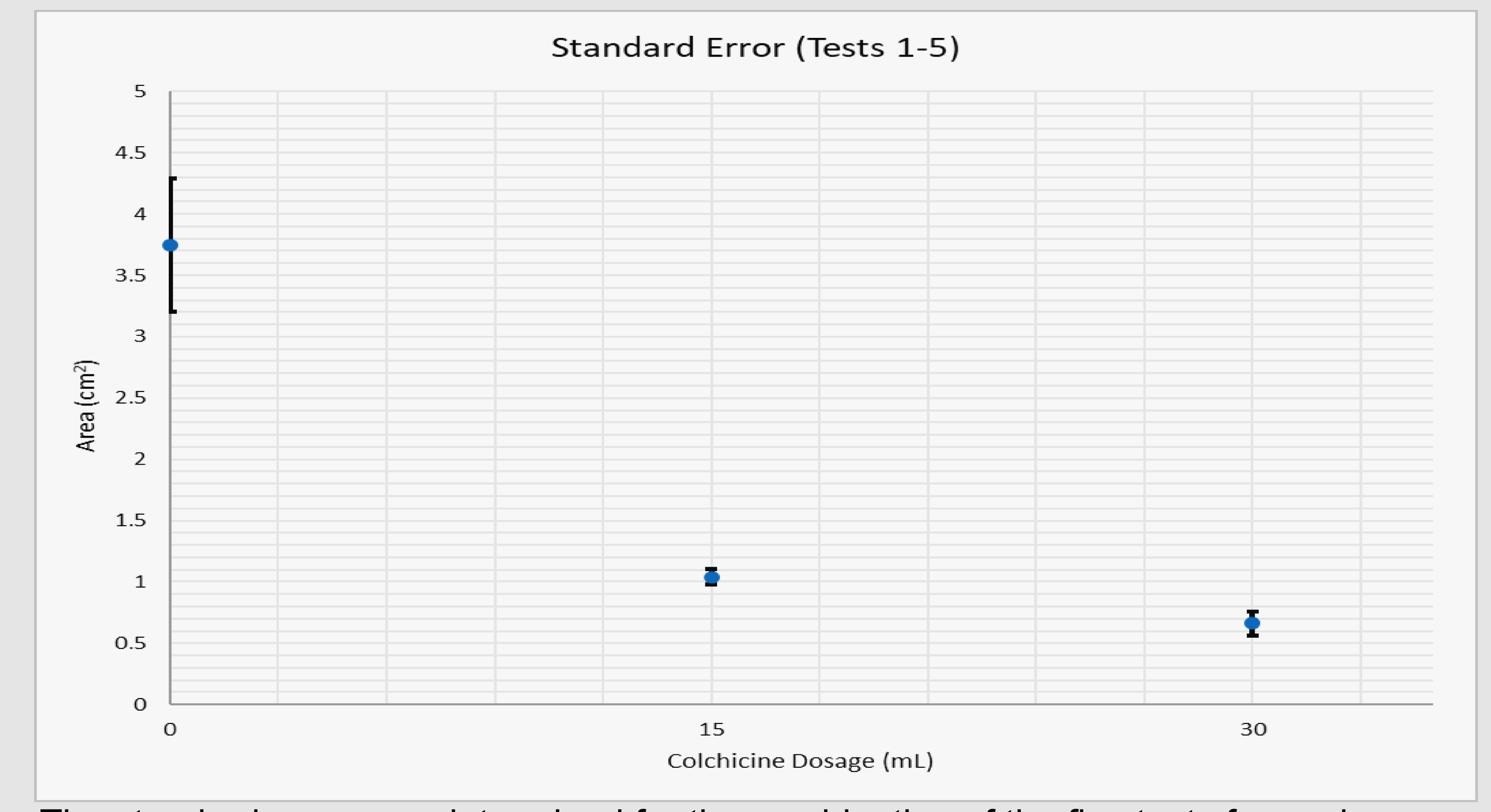


Logarithmic series for control, 15mL, and 30mL of all five tests. The control shows that in the absence of treatment, it would continue to grow given available resources over time. While *Pseudoygymnoascus destructans* is in the presence of the treatment, the growth is inhibited. The greater dose of treatment shows a greater effect of *Pd* growth (Hogan, 2019).



Before and After Test 3. Top row indicates what YPD plates looked like directly after fungus was applied to plates. Bottom row shows respective growth or inhibition following the 14-day incubation period (Hogan, 2019).

Discussion and Conclusion



The standard error was determined for the combination of the five tests for each dose applied. The dots represent the mean for the tests, and the bars show the standard error (Hogan, 2019).

- Varying doses of Colchicine effect growth
- Control: Plates continued growing
- 15mL: Initial inhibition was seen, but it began to regrow
- 30mL: Continued inhibition
- Fungal inhibition was present
- Suggests that a higher dose of Colchicine leads to greater inhibition
- ANOVA p-value: 2.23×10^{-9}
- This strong correlation shows that similar results could be found if this study were replicated
- Original hypothesis was supported

Limitations

- Little access to high amounts of fungus
- Plates had to be contained to relatively small initial areas
- Not pure cultures
- Development of other fungi
- No access to proper tools to assess spore count

Future Research

- Longer trials
- Vary Colchicine Effects
- Effect of other mitotic inhibitors
- In vivo effect

References

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