

Research Practicum 2020-21

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Investigate and Improve the Therapeutic Window of an Organic Fungal Treatment

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Abstract:

Fungi based microbial plant diseases are among the major causes of agricultural crop loss on a global scale, and threatens the worldwide goal of producing sufficient food to feed the growing world population and prevent famine. It is therefore imperative to control the damage caused by fungi to reduce the loss of crop production. However, agricultural fungi control has been a big challenge. The excessive application of synthetic fungicide in agriculture poses a potential threat to both human health and the environmental balance and safety. Copper based organic fungicide has shown to be effective in controlling a wide range of fungal diseases on fruit trees and vegetables, although it would often result in the damage of the plants due to its narrow therapeutic window. Therefore, if the therapeutic window of copper fungicide can be significantly improved, it could provide an efficient fungal treatment that is organic and can be widely used. The study discussed here investigated the impact of copper sulfate, when used as a fungicide, on the growth of a fungal mycelium, mung bean sprout, and young celery. The results indicate that there are optimal concentrations of copper sulfate when used to treat fungi and the timing of application during the stages of the plant growth is critical to maximize the therapeutic window. The study also revealed an under-noticed new observation of potential growth promotion for fungi if copper fungicide is not used properly. These results paved a way to further improve the therapeutic window of copper fungicide through additional explorations such as formulation optimization.

Introduction

It is estimated that the world population will grow by approximately 30% by 2050 (Sadik). Producing sufficient food to feed and prevent starvation for a growing world population is one of the most critical issues modern human society faces. The world agriculture, however, suffers significant losses due to plant disease despite all our scientific advances of the past 200 years. Microbial diseases are some of the leading causes of crop loss on a global scale, and among them, fungi constitute the largest number of plant pathogens and are responsible for a range of serious plant diseases. One survey made several years ago in the American State of Ohio came up with the estimate that the state had one thousand diseases of plants caused by fungi, one hundred caused by viruses and fifty due to bacteria. This is consistent with the estimate that 70-80% of the microbial diseases-induced loss of crop production tonnage was caused by fungi (Moore et al.). Accordingly, there has been worldwide pressure to control the botanical damage caused by fungi to reduce the loss of crop production and subsequently minimize famine around the world.

There are >99,000 known species of fungi, including molds, yeasts, mushrooms, and polypores (Redecker et al.; Blackwell et al.). Pathogen fungi affect a wide range of agricultural plants including fruit trees, grain crops, and vegetables. In particular, most vegetable diseases are caused by fungi. They damage plants by killing cells and/or causing plant stress. There are many sources of fungal infections, such as infected seeds, soil, crop debris, nearby crops, and weeds. Fungi also spread very easily; the movement of contaminated soil, animals, workers, machinery, tools, seedlings and other plant materials can all result in the spread of fungi (Kutateladze et al.). It is known that fungi can enter plants through natural openings such as stomata and through wounds caused by pruning, insects, diseases, or other types of damages (Ausveg et al.). Because of this, fungi control has been a big challenge and synthetic antifungal agents have been widely used. However, the excessive use of the fungicides in agriculture poses a potential threat to human health, derived from the exposure of consumers and workers to pesticides through contact, inhalation, or ingestion of food or

water contaminated with pesticides. Adverse effects of such exposures could include endocrine, immunological, neurological, and carcinogenic problems, as well as premature births (Munger et al.). In addition to human health, the excessive use of the pesticide can also cause environmental problems, as accumulation of pesticides in the environment could reduce the biodiversity of soil macroorganisms and gives rise to pathogenic resistance to the pesticides (Brauer et al.; Bünemann et al.). Thus, it is imperative to find an efficient way of fungal control that is organic and leaves minimum adverse impact to human and environment.

Copper based fungicide has been one of the popular organic treatments for fungal infection. It can be used to control conditions caused by powdery mildew, downy mildew, black spot, peach leaf curl, rust, and many other diseases of vegetables and fruit trees (Brauer et al.). However, the use of copper fungicide can also cause significant damages to the plants, such as leaf burns and even the death of the plants. The narrow therapeutic window of copper fungicide has prompted many users to resort to apply highly diluted copper fungicide for treatment, which often results in ineffectiveness for fungal control. Therefore, if the therapeutic window of the copper fungicide can be significantly improved, it could provide an efficient fungal treatment that is organic and can be widely used.

The study that is discussed here is to investigate if there is an optimal concentration of copper sulfate solution that would effectively inhibit the growth of fungi while maintaining the growth of the plants. The hypothesis is that the timing of application will have a significant impact on the therapeutic window of copper-based fungal treatment. Multiple experiments on the effect of varying levels and timing of a copper sulfate solution application on fungal growth and plant growth.

Materials and Methods

Preparation of Materials

The copper sulfate solution used water as the solvent and copper sulfate and detergent as the solute. The control group was 1.5 grams of detergent into 378 mL of water. A 1g/Gal (grams per gallon) of copper sulfate solution would use 1.5 grams of

detergent, 0.1 grams of copper sulfate, and 378 mL of water. A 3g/Gal of copper sulfate solution would use 1.5 grams of detergent, 0.3 grams of copper sulfate, and 378 mL of water. A 10g/Gal of copper sulfate solution would use 1.5 grams of detergent, 1 gram of copper sulfate, and 378 mL of water. The same proportions would be used for 30g/Gal and 100g/Gal.



Figure 1: Solutions of Copper Sulfate

Fungal Mycelium Experimental Setup

The growth medium used to cultivate the *Pleurotus eryngii* mycelium was single walled corrugated fiberboard. The fiberboard was cut into 24 rectangles of 9 cm by 12 cm and then completely immersed in water for 1 hour. After the fiberboard pieces were soaked through, excess water was removed so that water did not drip off when vertically.

The *Pleurotus eryngii* was cut into small rectangular slices. These slices were approximately 1 cm by 1 cm but were no more than 1 mm in height. The slices were placed onto the corrugated layers of the fiberboard and covered approximately $\frac{1}{6}$ of the surface area on one side. The amount on each layer did not have to be exact since growth would be measured relative to the initial surface area covered. The varying concentrations of the copper sulfate solution were applied to the respective groups. The fiberboard was placed into sanitized polypropylene containers that are commonly used in storing food.



Figure 2: Example of Fungal Mycelium Setup

Design of Algorithms

The *Pleurotus eryngii* used in this experiment represented pathogenic fungal mycelium. Each level of the independent variable had its own container with 4 corrugated layers, with a flat wall above and below the corrugated layer to separate them. This was the same for all 6 levels and they were stored in a large box in a dark closet. The temperature was kept at $70^{\circ}\text{F} \pm 2^{\circ}\text{F}$ to ensure favorable growing conditions for the fungal mycelium. Each day, the containers were opened to allow an exchange of air and to prevent the CO_2 buildup from stunting the mycelium growth.

Collection of Datasets

To measure the growth of the mycelium, an image was taken for each layer that had grown mycelium (this included layers that did not initially have the slices of the *Pleurotus eryngii*). It was critical to have even lighting when images of the mycelium were taken and ensure the edges of the fiberboard layers were parallel with the edges of the photo. Measurements were taken once every two days, starting at the beginning of the experiment. The experiment lasted seven days, with measurements taken on day 1, 3, 5, and 7.

Images to Quantitative Growth

The images were cropped so the edges of the fiberboard layer was the edge of the image. Contrast on the images were increased using IrfanView, a graphic viewing

program. Using the batch conversion software in IrfanView, the contrast was set to 127, which limited the amount of colors in the image. The images with increased contrast were put into Image Color Extract PHP, a software program that pulls the most common colors out of an image file and presents the percentages of each one. The growth of the mycelium was measured by 100 minus the percentage of black. Multiple layers that had grown mycelium for a single trial would be summed.

Mung Bean Sprout Experimental Setup

Mung beans were germinated by soaking dry mung beans at room temperature for 24 hours. One hundred germinating mung beans were randomly selected and placed into polypropylene cups. The bottoms were pre-drilled with small holes to drain out the water. Each day, the beans were rinsed with water and covered with a damp paper towel. After three days, they were sprayed with their respective concentrations of the copper sulfate solution and covered with aluminum foil and placed in the dark.

Measurements of the bean sprout growth was done by taking a random sample of 21 mung beans from each cup and measuring the length of the sprouts before treating them with the copper sulfate solution. After 2 days, measurements were taken again and the percent growth was calculated.



Figure 3: Mung Bean Sprout Setup

Celery Experimental Setup

Young celery plants (approximately 3 weeks after germination) were used in this experiment. Polypropylene boxes (previously holding bean curd/tofu before being

sanitized and reused) were filled with soil and had young celery plants transplanted into them. Each level of the independent variable had one box and in each box were 12 plants. The plants were initially watered with MicroGrow fertilizer (1 teaspoon/gallon) and sprayed with their respective concentrations of the copper sulfate solution. The celery plants had the fungicide solution reapplied three days later and were lightly watered every other day. The plants were kept at room temperature and under red/blue lights for 12 hours per day.

Initial measurements of height were taken for each plant, and one week later, measurements were taken again and the percent growth was calculated.



Figure 4: Celery Experimental Setup

Analysis Methods

The percent growth was calculated by dividing the measured amount of growth on a specific day over the initial area covered (fungal mycelium) or over the initial length (bean sprouts) or height (celery). Subtracting one from this value would give the percent increase of the growth.

$$\% \text{ increase} = 100 * \frac{\text{new measurement} - \text{initial measurement}}{\text{initial measurement}}$$

ANOVA tests were conducted to determine if there was a significant difference between the levels of the independent variable. If the p value was less than 0.05, there

would be a significant difference, and individual t-tests were performed between each two levels.

5 t-tests were conducted between each combination of a pair of levels. The results of the t-tests would determine whether the level(s) that were significantly different from the others. The least group that was significantly different would be used as the lower bound for the therapeutic window of the copper-based fungicide.

Results

The raw data collected for the fungal mycelium growth experiment is presented in Tables 1-6. The calculated data collected for the fungal mycelium growth experiment is presented in Tables 7-12. The values from the calculated data tables were taken and averaged in the calculated data table of average mycelium growth (Table 13). The Day 5 values from the calculated data tables (Tables 7-12) of mycelium growth were transferred to the Day 5 summative data table (Table 14). The result of the ANOVA test p value was less than alpha (Table 15) so t-tests between group A and each other group had to be conducted (Table 16). This was done again for Day 7 with the same process. For Day 7, a summative data table was made (Table 17), a ANOVA test was conducted which resulted in a p value less than alpha (Table 18) and t-tests between group A and each other group was conducted again (Table 19).

The raw data collected for the mung bean sprout growth experiment is presented in Tables 20 and 21. The calculated data collected for the average mung bean sprout growth experiment is presented in Table 22. The values from the raw data table of the final mung bean length were transferred to the summative data table (Table 23). The result of the ANOVA test and the t-tests between group A and each other group or shown (Table 24 and 25).

The raw data collected for the celery plant growth experiment is presented in Tables 26 and 27. The calculated data collected for the average celery plant growth experiment is presented in Table 28. The values from the raw data table of the final celery plant length were transferred to the summative data table (Table 29). The result

of the ANOVA test was that the p value was greater than the critical value (Table 30), so there was no need for a t-test to be conducted between the groups.

Note:

Group A = Control

Group B = 1 gram of CuSO_4/Gal

Group C = 3 gram of CuSO_4/Gal

Group D = 10 gram of CuSO_4/Gal

Group E = 30 gram of CuSO_4/Gal

Group F = 100 gram of CuSO_4/Gal

Table 1: Raw Data Table of Mycelium Growth Over Time (Control Group)

	Day 1	Day 3	Day 5	Day 7
A1	15.7	21	37.2	46
A2	17.3	20.4	33	46.8
A3	14.1	18.1	44.2	57.9
A4	15.5	17	34.3	40.7
Average	15.65	19.125	37.175	47.85

**Table 2: Raw Data Table of Mycelium Growth Over Time by Copper Sulfate
Concentration of 1 g/Gal**

	Day 1	Day 3	Day 5	Day 7
B1	17.6	21.3	30.4	36.6
B2	15.9	21	39.6	56.7
B3	17.6	20.9	28.1	53.2
B4	14.6	18.2	28.6	51.1
Average	16.425	20.35	31.675	49.4

**Table 3: Raw Data Table of Mycelium Growth Over Time by Copper Sulfate
Concentration of 3 g/Gal**

	Day 1	Day 3	Day 5	Day 7
C1	16.9	20	29	49.7
C2	13.9	20.9	46.5	53.3
C3	18.2	21.7	36.6	60.1
C4	15.8	17.4	46.2	48.9
Average	16.2	20	39.575	53

**Table 4: Raw Data Table of Mycelium Growth Over Time by Copper Sulfate
Concentration of 10 g/Gal**

	Day 1	Day 3	Day 5	Day 7
D1	17	18.9	39.8	83.3
D2	16.8	19.1	39.5	84.7
D3	19.3	22.8	28.9	60.4
D4	17.2	21.1	36	46.3
Average	17.575	20.475	36.05	68.675

**Table 50: Raw Data Table of Mycelium Growth Over Time by Copper Sulfate
Concentration of 30 g/Gal**

	Day 1	Day 3	Day 5	Day 7
E1	14.8	15.2	25.6	41.7
E2	13.6	14.6	21	51.5
E3	16.4	18.1	24.8	33.7
E4	14.2	16.9	18.5	32.4
Average	14.75	16.2	22.475	39.825

**Table 6: Raw Data Table of Mycelium Growth Over Time by Copper Sulfate
Concentration of 100 g/Gal**

	Day 1	Day 3	Day 5	Day 7
F1	16.9	17	17	18.3
F2	17.8	17.9	17.9	20.4
F3	15	15.1	17.8	25.6
F4	13.6	13.6	13.6	13.6
Average	15.825	15.9	16.575	19.475

**Table 7: Calculated Data Table of Mycelium Growth Over Time (% of Day 1)
(Control Group)**

	Day 1	Day 3	Day 5	Day 7
A1	0.00	33.76	136.94	192.99
A2	0.00	17.92	90.75	170.52
A3	0.00	28.37	213.48	310.64
A4	0.00	9.68	121.29	162.58
Average	0.00	22.43	140.62	209.18

**Table 8: Calculated Data Table of Mycelium Growth Over Time (% of Day 1) by
Copper Sulfate Concentration of 1 g/Gal**

	Day 1	Day 3	Day 5	Day 7
B1	0.00	21.02	72.73	107.95
B2	0.00	32.08	149.06	256.60
B3	0.00	18.75	59.66	202.27
B4	0.00	24.66	95.89	250.00
Average	0.00	24.13	94.34	204.21

**Table 9: Calculated Data Table of Mycelium Growth Over Time (% of Day 1) by
Copper Sulfate Concentration of 3 g/Gal**

	Day 1	Day 3	Day 5	Day 7
C1	0.00	18.34	71.60	194.08
C2	0.00	50.36	234.53	283.45
C3	0.00	19.23	101.10	230.22
C4	0.00	10.13	192.41	209.49
Average	0.00	24.52	149.91	229.31

**Table 10: Calculated Data Table of Mycelium Growth Over Time (% of Day 1) by
Copper Sulfate Concentration of 10 g/Gal**

	Day 1	Day 3	Day 5	Day 7
D1	0.00	11.18	134.12	390.00
D2	0.00	13.69	135.12	404.17
D3	0.00	18.13	49.74	212.95
D4	0.00	22.67	109.30	169.19
Average	0.00	16.42	107.07	294.08

**Table 11: Calculated Data Table of Mycelium Growth Over Time (% of Day 1) by
Copper Sulfate Concentration of 30 g/Gal**

	Day 1	Day 3	Day 5	Day 7
E1	0.00	2.70	72.97	181.76
E2	0.00	7.35	54.41	278.68
E3	0.00	10.37	51.22	105.49
E4	0.00	19.01	30.28	128.17
Average	0.00	9.86	52.22	173.53

Table 12: Calculated Data Table of Mycelium Growth Over Time (% of Day 1) by Copper Sulfate Concentration of 100 g/Gal

	Day 1	Day 3	Day 5	Day 7
F1	0.00	0.59	0.59	8.28
F2	0.00	0.56	0.56	14.61
F3	0.00	0.67	18.67	70.67
F4	0.00	0.00	0.00	0.00
Average	0.00	0.46	4.96	23.39

Table 13: Calculated Data Table of Average Mycelium Growth Over Time (% of Day 1) by Varying Concentrations of Copper Sulfate Fungicide

	Day 1	Day 3	Day 5	Day 7
A	0	22.43	140.62	209.18
B	0	24.13	94.34	204.21
C	0	24.52	149.91	229.31
D	0	16.42	107.07	294.08
E	0	9.86	52.22	173.53
F	0	0.46	4.96	23.39

Table 14: Summative Data Table for Day 5 Mycelium Growth (% of Day 1) by Varying Concentrations of Copper Sulfate Fungicide

	A	B	C	D	E	F
Mean	140.62	94.34	149.91	109.3	52.22	4.96
SD	52.226	39.439	76.336	40.043	17.490	9.147
Var	2727.596	1555.458	5827.222	1603.402	305.907	83.674
n	4	4	4	4	4	4

Table 15: Statistical Table Describing Results of ANOVA Test for Day 5 Mycelium Growth

P-Value	Alpha
0.00196702	0.05

Table 16: T-test Results for Day 5 Mycelium Growth

T-test Comparison	P Value
A-B	0.1053
A-C	0.4241
A-D	0.1749
A-E	0.0185
A-F	0.0062

Table 17: Summative Data Table for Day 7 Mycelium Growth (% of Day 1) by Varying Concentrations of Copper Sulfate Fungicide

	A	B	C	D	E	F
Mean	209.18	204.21	229.31	294.08	173.53	23.39
SD	68.853	68.584	39.013	120.416	77.052	32.083
Var	4740.801	4703.729	1521.984	14500.014	5937.076	1029.297
n	4	4	4	4	4	4

Table 18: Statistical Table Describing Results of ANOVA Test for Day 7 Mycelium Growth

P-Value	Alpha
0.00184915	0.05

Table 19: T-test Results for Day 7 Mycelium Growth

T-test Comparison	P Value
A-B	0.4609
A-C	0.3169
A-D	0.1390
A-E	0.2581
A-F	0.0035

Table 20: Raw Data Table of Initial Mung Bean Sprout Length (in mm)

Bean Sprout	A	B	C	D	E	F
1	11	18	15	19	18	18
2	17	19	18	20	20	23
3	20	16	20	24	23	17
4	21	18	21	25	38	23
5	20	19	16	18	28	19
6	15	21	17	19	25	20
7	16	15	18	31	24	19
8	19	25	20	20	20	18
9	22	18	16	21	21	21
10	20	17	18	21	22	22
11	18	20	15	24	21	19
12	18	17	15	24	22	18
13	21	18	15	20	18	20
14	10	17	17	26	24	19
15	18	16	15	25	19	18
16	16	19	16	24	21	19
17	21	16	16	20	17	21
18	15	18	17	30	23	22
19	21	17	21	21	23	21
20	19	18	18	24	24	17
21	16	13	18	23	26	22

Table 21: Raw Data Table of Final Mung Bean Sprout Length (in mm)

Bean Sprout	A	B	C	D	E	F
1	49	46	32	35	28	20
2	42	49	42	34	26	24
3	51	51	41	28	24	19
4	54	57	42	21	27	23
5	50	47	31	31	24	19
6	39	45	41	28	28	22
7	43	41	33	32	25	20
8	41	33	36	35	25	18
9	42	49	29	32	23	20
10	46	39	28	24	23	22
11	35	33	41	25	22	18
12	33	33	29	27	20	16
13	37	43	33	29	21	23
14	41	38	28	31	23	21
15	44	38	30	25	24	21
16	38	36	41	27	21	24
17	49	40	36	27	23	19
18	28	34	41	28	42	18
19	37	35	31	24	27	17
20	36	36	31	26	26	16
21	34	41	32	28	27	14

Table 22: Calculated Data Table of Average Mung Bean Sprout Growth (in %)

A	B	C	D	E	F
132.353	130.400	101.105	24.635	10.901	0.481

Table 23: Summative Data Table Of Mung Bean Sprout Final Length

	A	B	C	D	E	F
Mean	41.381	41.143	34.667	28.429	25.190	19.714
SD	6.719	6.762	5.228	3.789	4.501	2.741
Var	45.148	45.729	27.333	14.357	20.262	7.514
n	21	21	21	21	21	21

Table 24: Statistical Table Describing Results of ANOVA Test for Mung Bean Growth

P-Value	Alpha
< 0.0001	0.05

Table 25: T-test Results for Mung Bean Growth

T-test Comparison	P Value
A-B	0.4547
A-C	0.0004
A-D	< 0.0001
A-E	< 0.0001
A-F	< 0.0001

Table 26: Raw Data Table of Initial Celery Plant Height (in cm)

Celery Plant	A	B	C	D	E	F
1	7.5	7.5	8	9	6.5	8.5
2	6	11	7	7.5	7.5	8
3	7	7	8	7	7	6.5
4	5.5	8	9	7	7	6.5
5	5.5	7.5	7.5	9.5	7.5	6.5
6	6	9	7.5	7.5	7.5	6
7	8	6	7	8.5	7	6.5
8	10	6	8	8	7	7.5
9	8	7	6	8	8	10.5
10	6.5	7.5	6.5	6.5	6	9.5
11	6	7	9	8.5	7.5	6.5
12	6.5	6.5	7	8	11	6.5

Table 27: Raw Data Table of Final Celery Plant Height (in cm)

Celery Plant	A	B	C	D	E	F
1	10	14	13	13.5	6.5	8.5
2	6.5	11	10	10.5	10	8.5
3	12	8	11	9	10.5	8.5
4	10	7.5	11	9.5	10	10.5
5	6.5	12	10	10.5	8	10
6	7	7.5	11.5	12	8.5	6.5
7	13	8.5	11.5	11	7.5	9.5
8	7	7.5	10	7.5	12	6.5
9	8	11.5	9.5	7.5	10	8.5
10	9.5	10	9.5	7.5	9	10.5
11	12.5	11.5	12	11.5	8	7
12	9	11	11	9.5	18.5	7

Table 28: Calculated Data Table of Average Celery Plant Growth (in %)

A	B	C	D	E	F
34.545	33.333	43.646	25.789	32.402	14.045

Table 29: Summative Data Table Of Celery Plant Final Length

	A	B	C	D	E	F
Mean	9.250	10.000	10.833	9.958	9.875	8.458
SD	2.241	2.072	1.027	1.831	2.973	1.406
Var	5.021	4.292	1.056	3.352	8.839	1.977
n	12	12	12	12	12	12

Table 30: Statistical Table Describing Results of ANOVA Test for Celery Plant Growth

P-Value	Alpha
0.140569	0.05

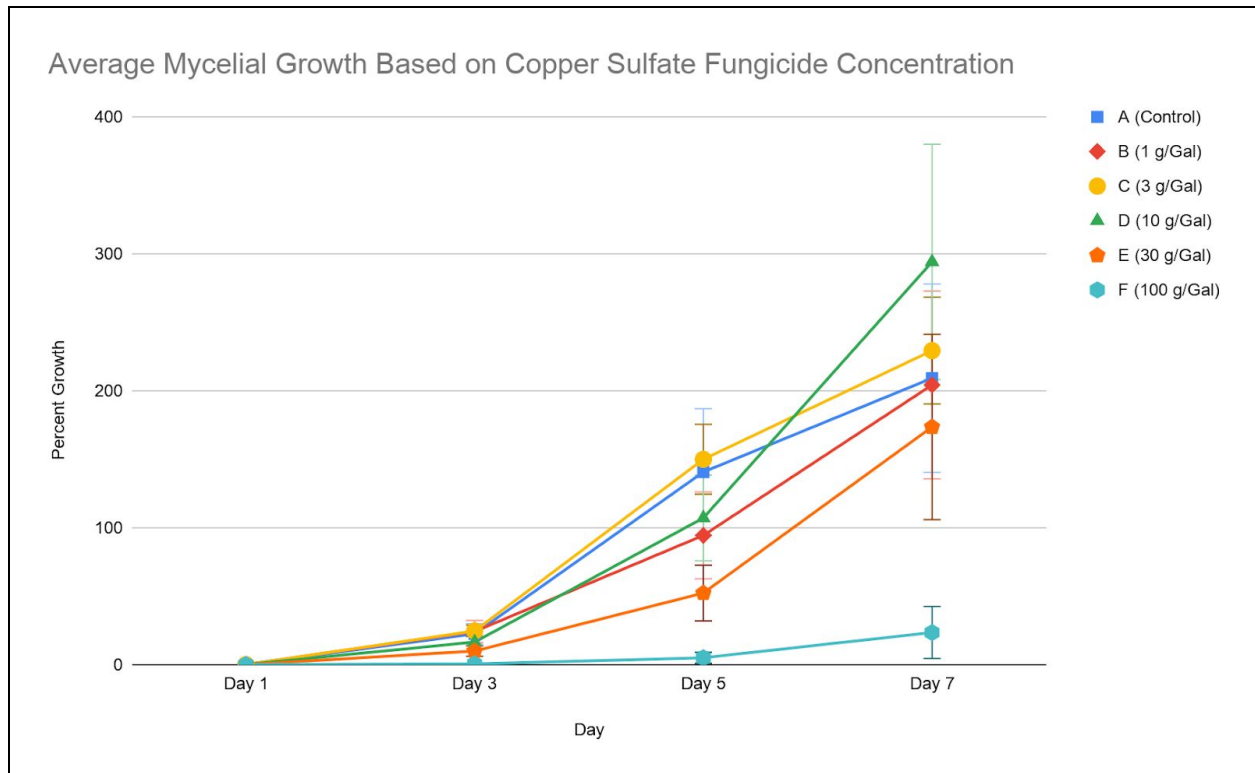


Figure 5: Graph of Average Mycelial Growth Based on Fungicide Concentration

The data presented here had a standard deviation of 68.853 for group A, 68.584 for group B, 39.013 for group C, 120.416 for group D, 77.052 for group E, and 32.083 for group F.

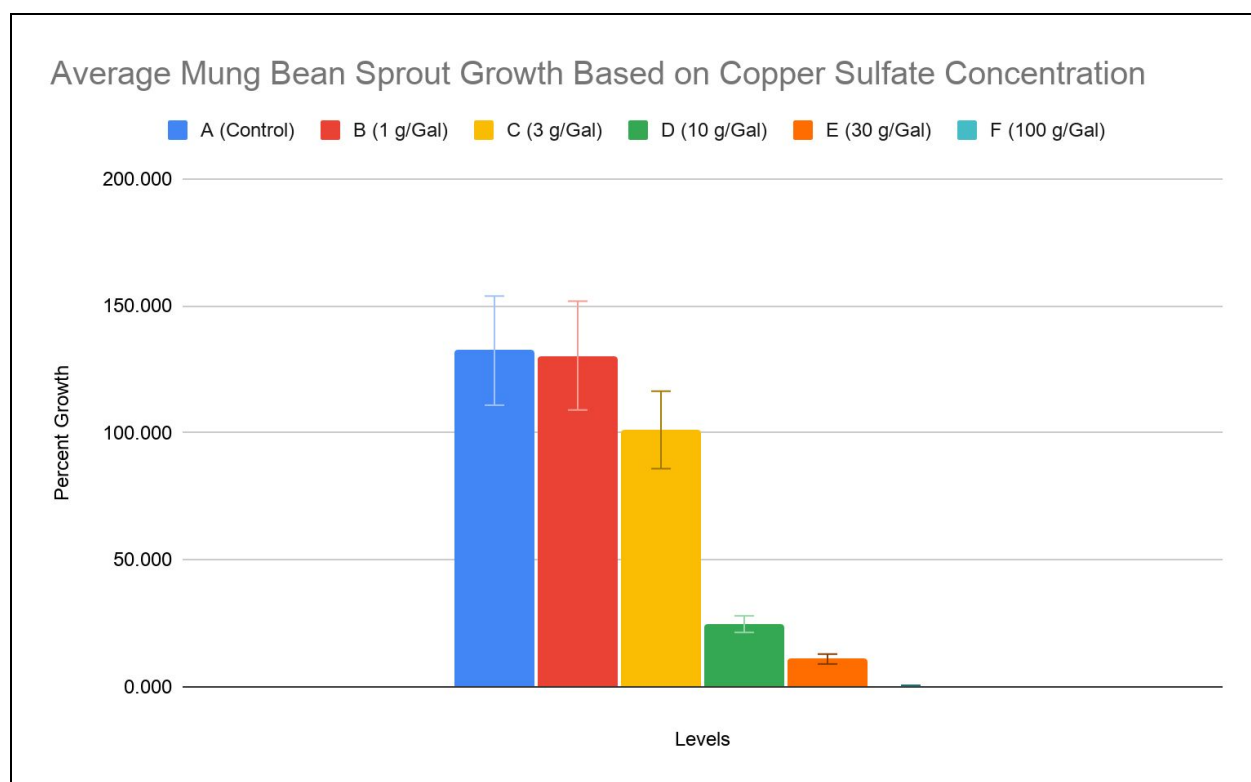


Figure 6: Graph of Average Mung Bean Sprout Growth Based on Copper Sulfate Concentration

The p value found for a significant difference between groups is 0.0001, and the alpha value is 0.05. Note: the value of F is 0.481%. The data presented here had a standard deviation of 21.49 for group A, 21.43 for group B, 15.25 for group C, 3.281 for group D, 1.948 for group E, and 0.0669 for group F.

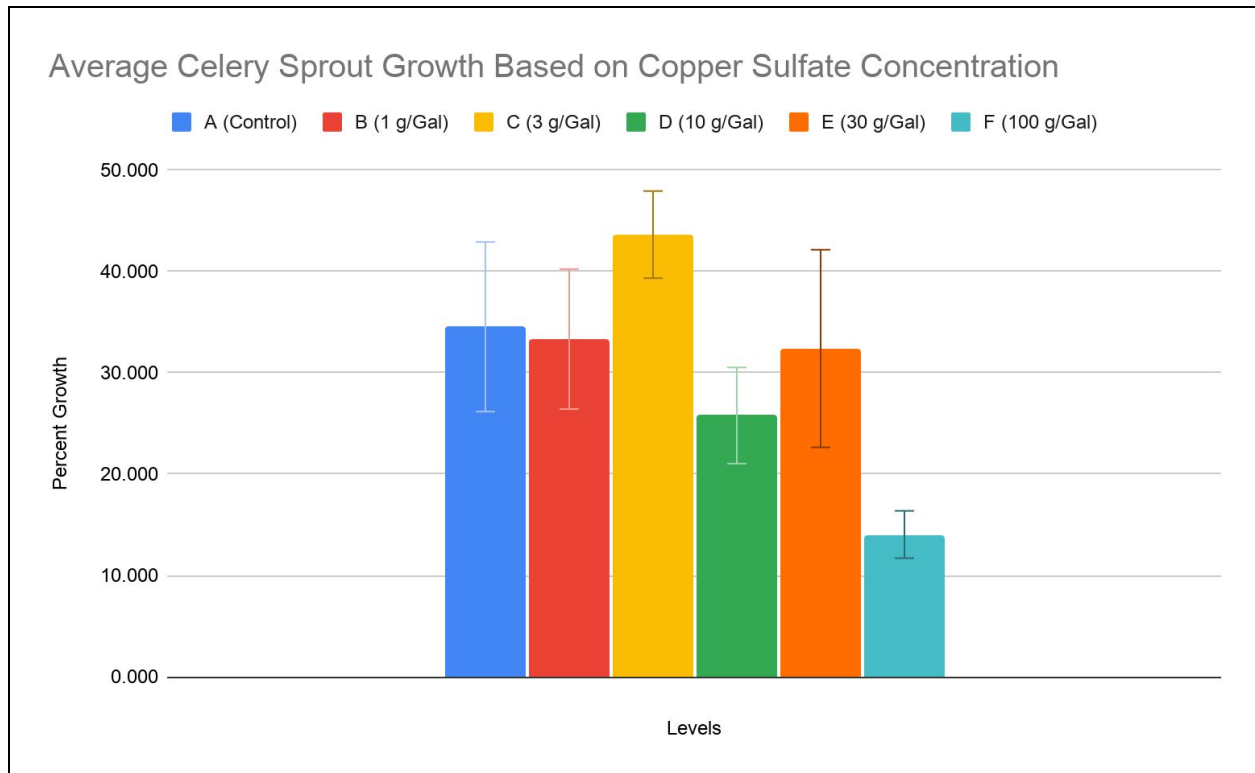


Figure 7: Graph of Average Celery Plant Growth Based on Copper Sulfate Concentration

The p value found for a significant difference between groups is 0.141, and the alpha value is 0.05. The data presented here had a standard deviation of 8.368 for group A, 6.905 for group B, 4.298 for group C, 4.742 for group D, 9.755 for group E, and 2.335 for group F.

Discussion

Summary of Results and Conclusions

On day 5 of the fungal mycelium experiment, significant inhibition of growth was observed for 30 and 100 g/Gal treatment groups. While the 100 g/Gal treatment group continued to exhibit near complete inhibition of growth on day 7, the 30 g/Gal treatment group started to show signs of growth recovery.

It is intriguing to note that the group which displayed the best signs of growth was the treatment group of 10 g/Gal. It had grown 294.08% of its initial surface area which I found to be quite unexpected (Table 10). It was predicted that the control group would have the best growth, since fungicide was not applied to it, but group D (10g/Gal) had grown over 1.4 times more than the control group (Table 13). Although the t-test gave a p value of 0.139, which fails to reject the null and do not support the alternative hypothesis, deeming the difference between group A and group D insignificant, this should not be a statistic that is completely overlooked. Copper is an essential nutrient for most living organisms. However, its dual role in essentiality and toxicity made it crucial to maintain copper intracellularly, but only at a tolerable level (Cervantes and Gutierrez-Corona). It is commonly thought that applying any concentration of a copper sulfate fungicide was beneficial to a plant's survival as long the plant itself was not harmed due to the overwhelming amount of copper in the environment, but this experiment shows that might be false. A fungi's growth may also be supplemented along with the plant's if the fungicide concentration is not high enough. This is why it would be important to find a therapeutic window for a copper-based fungicide which is able to be tolerated by the plant it is being applied to, but effective in inhibiting fungal growth.

In the mung bean (*Vigna radiata*) sprout experiment, the bean sprouts were in a stage of very active growth. The fragility of the sprouts can clearly be seen from the difference in the amount of growth between the control group and group F (100g/Gal). Group B has growth similar to that of the control group, but groups C, D, E, and F, all have noticeably less growth when compared to the control group. The table of p values calculated from t-tests (Table 22) supports the observation that groups C-F all have significantly lower growth amounts than the control.

In the experiment on young celery plants (*Apium graveolens*), which were approximately 3 weeks old, the plant's increased resistance to copper toxicity was evident. Although the average height of the celery plants in group F seemed shorter qualitatively, the ANOVA test revealed there was no significant difference (Table 27) in the growth of the plants.

From the mung bean sprout experiment, there is no window where the copper sulfate allows the growth of the sprouts while inhibiting the growth of fungi. However, the experiment conducted on how fungicide affects the growth of young celery plants shows that concentrations of a copper sulfate solution from 0-30 g/Gal has an insignificant effect on the growth of the plant. At the highest concentration of 100 g/Gal, a trend towards inhibition was noticed. Between this experiment and the experiment on fungal mycelium, a therapeutic window is presented. This emphasizes the importance of the timing of an application of fungicide to plants. If applied when the plant is sprouting, it may prevent fungal infections, but it would likely harm/kill the plant, and if applied when the plant is already fully grown, the plant may already be infected, in which case the plant would likely die.

Future Studies and Research Impact

A future study on the same topic could research the effect of novel formulations of a copper-based fungicide. Having a more optimized concentration and timing of applying a copper sulfate solution allows augmented formulations to be tested, further perfecting a treatment for fungal diseases. By more extensive research on the cellular processes of pathogenic fungi, materials with certain properties could be added to the fungicide solution to evaluate their impact on the therapeutic window of fungicide. The information to be learned from this paper could be another step towards combating fungal diseases that cause global agricultural and economic losses.

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Works Cited or References

AUSVEG, A. "Fungal Diseases in Vegetable Crops." *AUSVEG*, 2009, ausveg.com.au/biosecurity-agrichemical/crop-protection/overview-pests-diseases-disorders/fungal-diseases/.

I mostly used the article as a background on the various ways fungal diseases are transmitted between vegetable crops. The article talked about how fungi can enter plants through natural openings such as stomata and through wounds. I did not use much information from the article as it was a secondary source. Instead, I researched the studies and books mentioned in the article.

Blackwell, Meredith. "The Fungi: 1, 2, 3 ... 5.1 Million Species?" *Botanical Society of America*, John Wiley & Sons, Ltd, 1 Mar. 2011, bsapubs.onlinelibrary.wiley.com/doi/full/10.3732/ajb.1000298.

This paper discusses how the technological advancements of today make it possible to apply more accurate methods to develop a stable classification of a fungi. This study found that with the molecular tools currently available and in development, discovering the world's unknown fungi species would take less than 1000 years at the rate of discovery.

Brauer, Veronica Soares, et al. "Antifungal Agents in Agriculture: Friends and Foes of Public Health." *Biomolecules*, MDPI, 23 Sept. 2019, www.ncbi.nlm.nih.gov/pmc/articles/PMC6843326/.

This paper discusses the applications of antifungals and how they impact our environment and the toxicological safety of the food we eat. It reviews the use of antifungals in agriculture worldwide, the need to develop new antifungals, and improvement of regulations regarding antifungal use.

Bünemann, E. K., et al. "Impact of Agricultural Inputs on Soil Organisms-a Review." CSIRO PUBLISHING, CSIRO PUBLISHING, 27 June 2006, www.publish.csiro.au/sr/SR05125.

This paper discusses the effect of agricultural crop treatments on macroorganisms that live in the soil. The study found that the non-target effects of microbial treatments appear to be trivial. Among the pesticides, few significant effects of herbicides on soil organisms have been documented, however. negative effects of insecticides and fungicides are more common.

Cervantes, Carlos, and Felix Gutierrez-Corona. "Copper Resistance Mechanisms in Bacteria and Fungi." *OUP Academic*, Oxford University Press, 1 June 1994, academic.oup.com/femsre/article/14/2/121/597249?login=true.

This experiment deals with the mechanisms of resistance that fungi and bacteria have to copper. Although copper is an essential micronutrient in most living organisms, it can also be toxic in higher concentrations. Using this information, creating a fungicide that could overcome these defenses should be extremely effective.

Kutateladze, L.Y., et al. "Microscopic Fungi Spread in Different Types of Soils in Western Georgia." *Annals of Agrarian Science*, No Longer Published by Elsevier, 12 Sept. 2016, www.sciencedirect.com/science/article/pii/S1512188716300604.

This paper studies microscopic species of fungi that are known to grow in soil. It was found that soils containing high fertilizer and humus content would have a large amount of biodiversity in the fungi cultures present.

Moore, David, et al. *21st Century Guidebook to Fungi*. Cambridge University Press, 2020.

This book extensively covers many topics in general fungal biology. I primarily used this source to supplement my understanding of the structural characteristics of fungal cells and how pathogenic fungi attack their host plants.

Munger, R. "Intrauterine Growth Retardation in Iowa Communities with Herbicide-Contaminated Drinking Water Supplies." *National Institute of Environmental Health Sciences*, U.S. Department of Health and Human Services, 1997, ehp.niehs.nih.gov/doi/abs/10.1289/ehp.97105308.

This study conducted a survey on drinking water supplies and found that the water system was actually contaminated with three different kinds of herbicides. This could also be a risk with the pervasive use and/or overuse of copper-based fertilizers, fungicides, and pesticides.

Redecker, Dirk, et al. "Glomalean Fungi from the Ordovician." *Science*, American Association for the Advancement of Science, 15 Sept. 2000, science.sciencemag.org/content/289/5486/1920.abstract.

This article discusses what the strong resemblance between modern arbuscular mycorrhizal fungi and fossilized fungal hyphae that dates back over 450 million years could mean. The study gives an insight into how the fungi's internal mechanisms could be retained over so many years with its favorable traits.

Sadik, N. "How to Feed the World in 2050." *FAOSTAT*, 2009, http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf.

This study researches how to deal with the rapidly growing population with the limited natural resources we have. This study acknowledges the issue of the agricultural industry not being able to produce enough food for a larger population in the future.