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# EFFECTS OF HYDROGEN PEROXIDE ON SEED GERMINATION OF WISCONSIN FAST GROWING PLANTS [*BRASSICA RAPA*]

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## ABSTRACT

Hydrogen peroxide is a common pesticide used in agriculture in the United States. However, there are concerns about the ecological effects of hydrogen peroxide on seed germination and growth. Previous research found that hydrogen peroxide prevents oxidative stress which plays a significant role in plant development (Wojtyla et al. 2016). Another article found that hydrogen peroxide behaves as a signaling molecule that mediates biochemical pathways (Nurnaeimah et al. 2020). We used this research to inform our experiment in determining the detrimental hydrogen peroxide concentration on seed germination. In this study, we conducted an experiment to observe the germination of Wisconsin Fast Growing seeds exposed to three different concentrations of hydrogen peroxide: 5 mL, 15 mL, and 30 mL. We conducted an analysis of variance test on our results. We found that at 30 mL, seed germination and growth were significantly inhibited. This suggests that high concentrations of hydrogen peroxide are harmful to seed germination and growth.

*Keywords: Wisconsin fast plants, seed germination, hydrogen peroxide, Brassica rapa*

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## INTRODUCTION

Agriculture is a major industry in the United States and is the primary source of nutrition for most people (Lindwall 2019). As of 2017, there are 2.04 million farms across the United States (Farmland Information Center 2017). Agriculture has many negative side effects on wildlife such as loss of habitat, wildlife depredation on crops, and competition for rangeland. Agriculture methods produce pollutants that are released into the environment. As defined by the Natural Resources Defense Council, agricultural pollution “is the contamination we release into the environment as a by-product of growing and raising livestock, food crops, animal feed, and biofuel crops”

(Lindwall 2019). Agriculture is necessary for producing food, clothing, and jobs for people, however the practices associated with agriculture must be modified to protect the environment.

Hydrogen peroxide has been used in agriculture for pest control and sterilization in water systems. Hydrogen peroxide is a weak acid and has a pH of about 3.5. It functions to control plant bacteria and fungi in the water systems of livestock. At low concentrations, hydrogen peroxide has been found to increase growth in plants, especially in the germination period (Nurnaeimah et al. 2020). However, recent research shows that high concentrations of hydrogen peroxide is toxic and damaging to cells (Wojtyla et al. 2016). This effect is

especially important in seed germination, in which high concentrations of hydrogen peroxide were found to inhibit seed germination. Seed germination is extremely vital to the survival of plants and indicates proper development into mature plants. We wish to provide evidence that inhibition of seed germination due to agricultural pollution can lead to a lower lifespan, and improper development, which can have negative effects on the overall ecosystem (Barba-Espin et al. 2010).

The purpose of this research project is to determine the impact of different concentrations of hydrogen peroxide on seed germination of Wisconsin fast plants. Thus, we are analyzing the effects of pollution on plant species. We hypothesize that seed germination will be delayed or shortened by hydrogen peroxide, a toxin often found in pesticides used in agriculture.

## METHODS AND MATERIALS

We developed our methods from the article published by Sandra Slutz on the Science Buddies website (Slutz n.d.). The materials/equipment needed for this experiment are as follows: hydrogen peroxide (3% concentration), sandwich baggies, Wisconsin Fast Growing Seeds, and paper towels. First, we created hydrogen peroxide solutions of varying concentrations to test on the Wisconsin fast plants. We prepared hydrogen peroxide solutions of low hydrogen peroxide (5mL), medium hydrogen peroxide (15mL), high hydrogen peroxide (30mL) and a control solution of water. We then placed 10 Wisconsin fast plant seeds in sandwich bags with paper towels moistened by the differing solutions. The sandwich bags with the

differing concentrations were placed on a flat surface with direct sunlight for a couple of days, or until all concentrations have fully germinated seeds. We measured the seeds every day and monitored for the development of the cotyledon. In the Wisconsin Fast Growing Plant Seed Booklet, it states that the seeds take around 24-48 hours to emerge, and from 48-72 to develop the cotyledons. For our purposes, we will determine that a plant has fully germinated normally if the seeds successfully develop the cotyledon in 48-72 hours. Those that germinated were planted in soil and sprayed with their respective hydrogen peroxide concentration solutions.

Growth was compared among the treatment groups by using an analysis of variance test (SYSTAT 10, SPSS Inc., Chicago, IL).

## RESULTS

A preliminary test was run on the Wisconsin Fast Plants with the varying hydrogen peroxide samples to determine how quickly germination occurred. Once this was done, the experiment was reset, and measurements were taken of seed germination over a course of 95 hours. We took measurements of each seed (root and/or stalk) to determine the mean of growth. Table 1 includes this data at 70 hours. Table 2 displays the data at 90 hours. The calculated means for each group over the 4 days are included in Table 3. The data from Table 3 was used to make Figure 1. The graph displays the relationship between seed germination over time per group. Significant differences in seedling growth were found among treatment groups at 70 and 95 hours (Figure 1; Tables 4-7).

*Table 1. Seed germination progress in mm at 70 hours.*

	Control	5 mL	15 mL	30 mL
Seed 1 (mm)	5	13	0	3
Seed 2 (mm)	5	15	5	4
Seed 3 (mm)	7	12	8	4

Seed 4 (mm)	5	11	7	4
Seed 5 (mm)	4	11	9	3
Seed 6 (mm)	4	10	10	2
Seed 7 (mm)	8	13	7	4
Seed 8 (mm)	7	10	8	3
Seed 9 (mm)	5	12	7	1
Seed 10 (mm)	5	15	5	1

\*Seeds were numbered from left to right.

Table 2. Seed germination progress in mm at 95 hours.

	Control	5 mL	15 mL	30 mL
Seed 1 (mm)	20	13	7	5
Seed 2 (mm)	9	15	10	5
Seed 3 (mm)	20	15	9	4
Seed 4 (mm)	5	14	9	7
Seed 5 (mm)	12	13	13	8
Seed 6 (mm)	20	15	13	6
Seed 7 (mm)	18	14	11	8
Seed 8 (mm)	20	12	10	9
Seed 9 (mm)	20	13	13	5
Seed 10 (mm)	10	15	10	3

Table 3. Mean ( $\pm$  95% CI) seed germination mm over 4 days.

	0 hr	24 hr	36 hr	70 hr	95 hr
Control (mm)	0	0	0	5.5 $\pm$ 0.97	15.4 $\pm$ 4.15
5 mL (mm)	0	0	0	12.2 $\pm$ 1.30	13.9 $\pm$ 0.79
15 mL (mm)	0	0	0	6.6 $\pm$ 2.00	10.5 $\pm$ 1.44
30 mL (mm)	0	0	0	2.9 $\pm$ 0.86	6 $\pm$ 1.39

## Seed Growth vs. Time

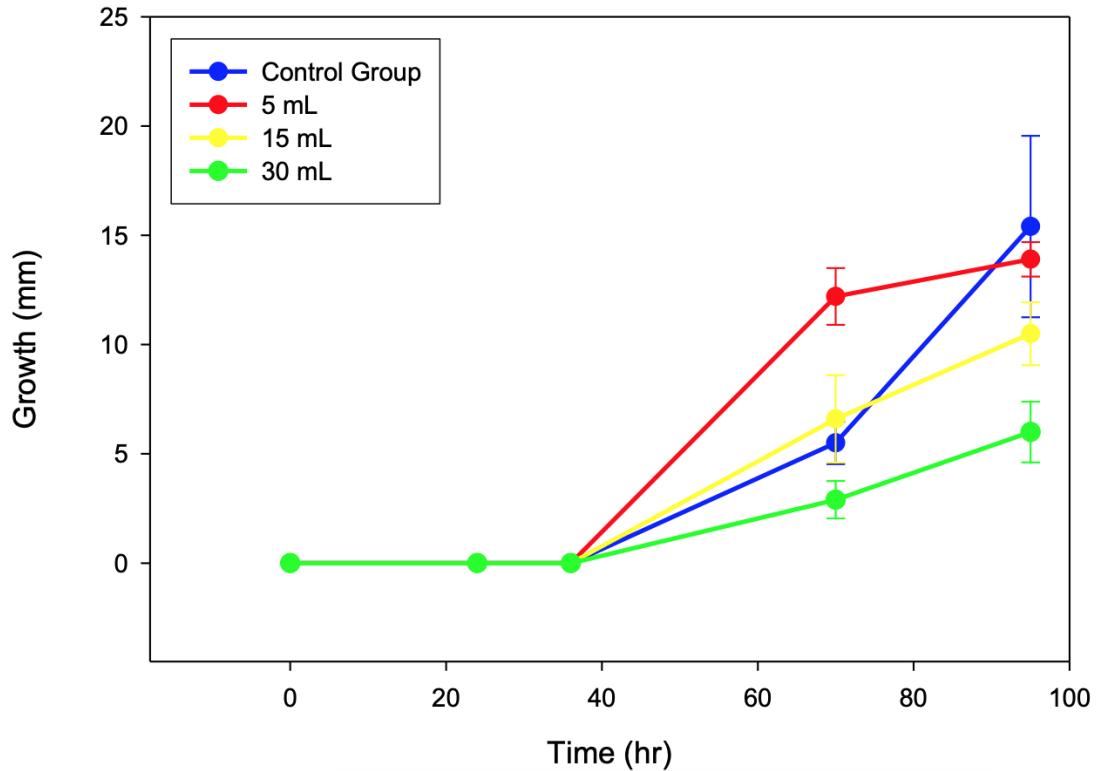


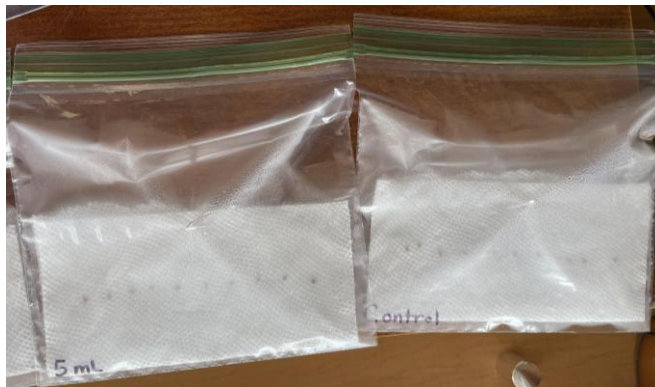
Figure 1. Seed growth (means  $\pm$  95% CI) of a control group and three treatment groups with different levels of added hydrogen peroxide (as indicated) vs. time.



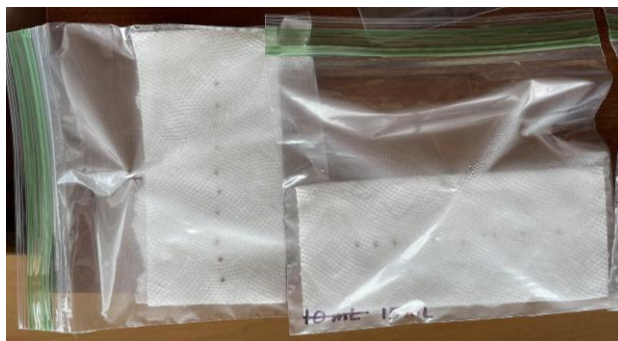
Figure 2. Experiment setup of the control group and 5mL Hydrogen Peroxide samples on Day 1, April 11th, 2022.



*Figure 3. Experiment setup of 15mL Hydrogen Peroxide sample and 30mL Hydrogen Peroxide sample on Day 1, April 11th, 2022.*



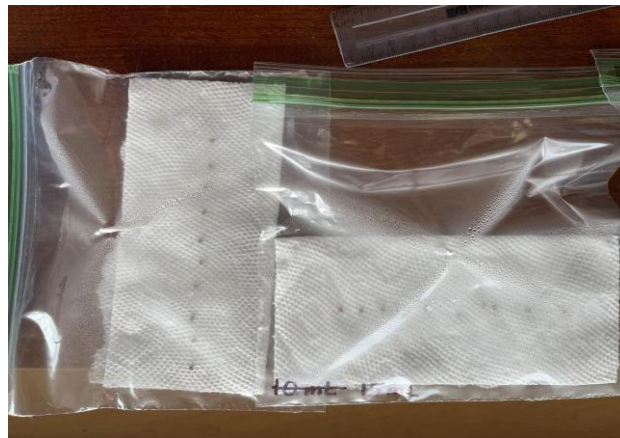
*Figure 4. Experiment setup of the control group and 5mL Hydrogen Peroxide sample on Day 2, April 12th, 2022.*



*Figure 5. Experiment setup of 15mL Hydrogen Peroxide sample and 30mL Hydrogen Peroxide sample on Day 2, April 12th, 2022.*



*Figure 6. Experiment setup of the control group and 5mL Hydrogen Peroxide sample on Day 3, April 13th, 2022.*



*Figure 7. Experiment setup of 15mL Hydrogen Peroxide sample and 30mL Hydrogen Peroxide sample on Day 3, April 13th, 2022.*



*Figure 8. Experiment setup of control group on Day 4, April 14th, 2022.*



*Figure 9. Experiment setup of 5mL Hydrogen Peroxide sample on Day 4, April 14th, 2022.*



*Figure 10. Experiment setup of 15mL Hydrogen Peroxide sample on Day 4, April 14th, 2022.*



*Figure 11. Experiment setup of 30mL Hydrogen Peroxide sample on Day 4, April 14th, 2022.*



*Figure 12. Experiment setup of control group on Day 5, April 15th, 2022.*



*Figure 13. Experiment setup of 5mL Hydrogen Peroxide sample on Day 5, April 15th, 2022.*



*Figure 14. Experiment setup of 15mL Hydrogen Peroxide sample on Day 5, April 15th, 2022.*



Figure 15. Experiment setup of 30mL Hydrogen Peroxide sample on Day 5, April 15th, 2022.

Table 4. Summary of data for analysis of variance test on seed germination progress at 70 hours.

Summary of Data						
	Treatments					Total
	1	2	3	4	5	
N	10	10	10	10		40
$\Sigma X$	55	122	66	29		272
Mean	5.5	12.2	6.6	2.9		6.8
$\Sigma X^2$	319	1518	506	97		2440
Std.Dev.	1.354	1.8135	2.7968	1.1972		3.8908

Table 5. Results of analysis of variance test on seed germination progress at 70 hours

Result Details				
Source	SS	df	MS	
Between-treatments	461	3	153.6667	$F = 42.75116$
Within-treatments	129.4	36	3.5944	
Total	590.4	39		

The Fratio value is 42.75116. The p-value is < .00001. The result is significant at  $p < .05$ .

Table 6. Summary of data for analysis of variance test on seed germination progress at 95 hours.

Summary of Data						
	Treatments					
	1	2	3	4	5	Total
N	10	10	10	10		40
$\Sigma X$	154	139	105	60		458
Mean	15.4	13.9	10.5	6		11.45
$\Sigma X^2$	2674	1943	1139	394		6150
Std.Dev.	5.7966	1.1005	2.0138	1.9437		4.8196

Table 7. Results of the analysis of variance test on seed germination progress at 95 hours.

Result Details				
Source	SS	df	MS	
Between-treatments	522.1	3	174.0333	$F = 16.32413$
Within-treatments	383.8	36	10.6611	
Total	905.9	39		

The  $F$ -ratio value is 16.32413. The  $p$ -value is  $< .00001$ . The result is significant at  $p < .05$ .

## DISCUSSION

We hypothesized that high concentrations of hydrogen peroxide inhibit seed germination in Wisconsin Fast Growing Plants. We can accept this hypothesis through our findings. In conducting an analysis of variance test at 70 and 95 hours of growth, we saw that both of our results were significant. In Table 6, our results concluded an  $F$ -ratio value of 42.75116 and a  $p$ -value smaller than 0.00001, which is significant at the 0.05 significance level. Looking at Table 3, the highest mean value was 12.2 mm, for the 5 mL concentration group. The lowest mean value was 2.9 mm for the 30 mL concentration group. Previous hydrogen peroxide research found that low

concentrations were beneficial to agriculture (Nurnaemah et al. 2020). Therefore, the seeds with 5mL concentration of hydrogen peroxide may have had an advantage over the control group. In addition, the seeds exposed to the 30 mL concentrations of hydrogen peroxide grew the least which supports our hypothesis. Between 48 and 72 hours, the cotyledons began to appear and continue germinating, which is what we expected after reading the Wisconsin Fast Growing plants manual.

At 90 hours, we found that the control group was growing the most. In Table 7, we conducted an analysis of variance test on the germination progress at 95 hours, and we found an  $f$ -ratio value of 16.32413 and a  $p$ -value less than 0.00001 which is significant at

the significance level of 0.05. Looking at Table 3, the highest mean was 15.4mm, which is the control group, and the lowest mean was 6 mm for 30 mL. This supports our hypothesis since the 30 mL group at 95 hours has grown the least. We can also see this in Graph 1, where the control group has consistent growth, whereas the hydrogen peroxide seeds stagger a bit. Also, the group exposed to 30 mL hydrogen peroxide concentration stays consistently below all other groups growth-wise.

Looking at Figure 1, we can also visually determine how significantly different the means of growth were for each group by looking at the error bars. The error bars on the graphs display the confidence intervals, or the intervals in which the means could lie in. If the error bars for each time interval overlap, then the means are not significantly different. At 70 hours, the control group and the 15 mL group were overlapping, meaning their means were not significantly different, and their growth rates were similar. However, the 5 mL group and 30 mL group error bars were not overlapping with any other group. At 70 hours, the 5 mL concentration group grew the most, and the 30 mL concentration group grew the least.

Continuing to look at Figure 1, at 95 hours, we see that both the 5 mL and 15 mL groups overlapped with the control group. This means that both the 5 mL and 15 mL grew around the same amount as the control group. At 95 hours, we cannot determine which group grew the most, as these three groups were not significantly different from each other. However, the 30 mL group was significantly different from all the other groups and had the least amount of growth. This means that the 30 mL group, at 95 hours, still grew the least. Thus, the 30 mL group consistently had the least amount of growth.

Further research needs to be done on lower concentrations of hydrogen peroxide to determine what concentrations have a harmful effect on seed growth. 30 mL has been proven to stunt the growth of Wisconsin Fast Growing plants; however, it is still unclear if lower concentrations of hydrogen peroxide exposure have the same effect as the control group. As we stated before, looking at graph 1, at 70 hours, 5 mL concentration group grew faster than the control group, and the 15 mL group grew similarly to the control group. At 95 hours, both 5 mL and 15 mL concentrations grew in the same range as the control

group. This indicates that lower concentrations of hydrogen peroxide do not affect seed growth. This leads us to believe that concentrations of hydrogen peroxide may need to be regulated in pesticides, but not diminished completely.

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