**The Effects of Salinity and Temperature on the Germination**

**Rate of *Raphanus sativus* Seeds**

Noah Banick and Martin Bault

Macomb Mathematics Science Technology Center

Biology 1

Section 9A

Mr. Acre/Mr. Estapa/Mrs. Gravel

21 May 2015

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**Introduction**

Around the world, approximately 750 million people are lacking fresh water.

96.5 % of surface water is salt water (Perlman). Some of that precious water is wasted watering crops. The aim of the experiment was to find the impact of using seawater instead of fresh water for farming.

 The experiment’s goal was to germinate *Raphanus sativus* seeds using varying amounts of salinity and temperature. Three different solutions were mixed with varying levels of salinity.  Sets of ten seeds were then placed into plastic bags in between sheets of paper towel. The seeds were then watered with 20 ml with one of three solutions, and placed into one of three incubators (“Seed Starting: The Baggie Method”).

 As temperature rises, the germination process of *Raphanus sativus* seeds becomes quicker. As temperature lowers, the process slows down. The temperature should not reach beyond 35 ℃ or 18 ℃ because the seed will die due to the extreme cold or heat (Gate).

When varying temperature levels are the only variable, generally results in different germination rates. The three temperatures chosen (30 ℃, 27 ℃, and 24 ℃) are in the prime range for *Raphanus sativus* germination, and are still spread enough to have differences notable in the data (Baskin).

When a seed has a higher salinity than the water outside the seed, then inside the cell will begin to shrivel because osmosis moves liquid from low salinity levels to high (Weinstein-Crowe). When salinity levels increase germination rates decrease because the seeds, even though they are being watered, have a lack of water in the actual seed due to osmosis.

Since the experiment aimed to germinate the seeds using abnormal factors, it was decided to measure in the average days it took the seeds to germinate, in order to see reliable results. There are limited ways to measure data regarding seed germination and average days to germinate was the best option to record measurements.

**Problem Statement**

Problem:

To determine the effect of varying salinity levels incubated at different temperatures on the germination rate of *Raphanus sativus* seeds.

Hypothesis:

If *Raphanus sativus* seeds are treated with varying salinity levels with different incubation temperatures, than lower concentrations of NaCl will slow the germination rate and higher concentrations will cause the seeds to die. In addition, higher incubation temperature will increase the germination rate.

Data Measured:

Data will be measured in germination rate or the amount of time it takes for the seed to germinate. The amount for NaCl was; 3% for the high, 2% for the standard, and 1% for the low.  In addition the incubation temperatures were; 30℃ for the high, 27℃ for the standard, and 24℃ for the low.  The levels of each variable were chosen by existing standards found in prior experiments. In order to analyze the data collected, a 2 factor DOE was used.

**Experimental Design**

Materials:

1 paper towel roll

20 g NaCl

50 Ziploc bags (16.51 x 14.92)

1 L water

500 *Raphanus Sativus* seeds

Calculator

1 ml dropper

100 ml graduated cylinder

500 ml beaker

Scoopula

Tweezers

Incubator set at 27 ℃

Incubator set at 24 ℃

Incubator set at 30 ℃

20 oz. bottle (3)

Plastic cup

Procedures:

*Making the solutions:*

1. Mix 396 ml water with 4.62 g salt in the beaker.

2. Stir until the salt is completely dissolved.

3. Mix 388 ml water with 13.85 g salt in the beaker.

4. Stir until the salt is completely dissolved.

5. Mix 196 ml water with 4.62 g salt in the beaker.

6. Stir until the salt is completely dissolved.

*Preparing the seeds:*

7. Fold paper towel sheet in half.

8. Place ten radish seeds on the folded paper towel sheet using the tweezers.

*Watering the seeds:*

9. Water ten sets of ten seeds with 20 ml of 1% salt solution.

10. Place paper towel into plastic bag.

11. Put the sets into an incubator set at 24 ℃.

12. Water ten sets of ten seeds with 20 ml of 1% salt solution.

13. Place paper towel into plastic bag.

14. Put the sets into an incubator set at 30 ℃.

15. Water ten sets of ten seeds with 20 ml of 3% salt solution.

16. Place paper towel into plastic bag.

17. Put the sets into an incubator set at 24 ℃.

18. Water ten sets of ten seeds with 20 ml of 3% salt solution.

19. Place paper towel into plastic bag.

20. Put the sets into an incubator set at 30 ℃.

21. Water ten sets of ten seeds with 20 ml of 2% salt solution.

22. Place paper towel into plastic bag.

23. Put the sets into an incubator set at 27 ℃.

******

Germinated seeds in bag

Figure 1. Bag of Germinated Seeds

 Figure 1, above shows a bag of seeds used in the experiment.

*Observations:*

24. Let the bags of seeds sit until all seeds have germinated.

25. Record the number of seeds that germinate each day.

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A seed at the initial stage of germination

Figure 2. Germinated Seed

 Figure 2, above shows an example of a seed that has germinated.

26. Calculate the average amount of time it took for a seed to germinate in each solution.

**Data and Observations**

Data:

Table 1

Design of Experiment Values

|  |  |
| --- | --- |
| Salinity (%) | Temperature ( ⁰ C) |
| - | Standard | + | - | Standard | + |
| 1 | 2 | 3 | 24 | 27 | 30 |

Table 1 shows the two variables used in the experiment (Incubation Temperature and Percentage of Salinity in the Solutions used). The incubation temperatures were set at the optimal point for the fastest rate of germination. The salinity percentages were chosen to have the greatest effect without killing the seeds entirely.

Table 2

Data Collected

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | 3 %Solution30 °CIncubator(Days) | 3 %Solution24 °CIncubator(Days) | 2 %Solution27 °CIncubator(Days) | 1 %Solution30 °CIncubator(Days) | 1 %Solution24 °CIncubator(Days) |
| T1 | 10.600 | 9.850 | 7.800 | 12.700 | 8.950 |
| T2 | 12.250 | 9.100 | 7.700 | 10.650 | 12.400 |
| T3 | 10.950 | 8.900 | 8.600 | 9.550 | 6.450 |
| T4 | 10.700 | 8.850 | 8.800 | 8.950 | 10.650 |
| T5 | 10.050 | 9.00 | 8.200 | 8.570 | 8.900 |
| T6 | 10.350 | 9.850 | 8.500 | 8.100 | 8.100 |
| T7 | 9.850 | 9.800 | 10.300 | 10.050 | 9.300 |
| T8 | 10.150 | 9.250 | 9.800 | 10.950 | 9.400 |
| T9 | 10.800 | 7.900 | 8.300 | 10.400 | 9.900 |
| T10 | 9.650 | 9.330 | 11.960 | 9.150 | 8.650 |
| Average | 10.535 | 9.183 | 8.996 | 9.907 | 9.270 |

Table 2 represents how many days the seeds in each trial took to germinate and the averages of the trials. The data is measured in days for seeds to germinate.

Observations:

Table 3

Observations

|  |  |
| --- | --- |
| Date | Observations |
| 3/15/2015 | Seeds that were placed in bags were not spaced exactly the same. |
| 3/17/2015 | Red dots/spots on seeds have formed in the + and - temps. About one in ten are like this. Few have germinated. |
| 3/18/2015 | The seeds that were turning red/pink yesterday have darkened. They still have not germinated. |
| 3/19/2015 | Two seeds in the -,- packets were dead due to being overwhelmed by fungus. |
| 3/23/2015 | Any seed completely covered in the pink fungus we have deemed deceased in fear of it tainting results. |
| 3/31/2015 | The seeds have started smelling of decomposition. It is believed to be a result of the fungus. |

 Table 3 contains notes on daily observations and anything that was not as expected. The amount of fungus and the smell were the most significant unexpected factors.



An infected seed as noted in Table 3 on day 7 of experiment

Figure 3. Deceased Seed

Figure 3. Above, shows an infected seed. The seed has a purple/black look to it which is from an unknown fungus growing on the seed.

**Data Analysis and Interpretation**

 A two-factor Design of Experiment (DOE) was used to analyze data in this experiment. It tested how two variables and their interactions affect a response variable. The experiment tested how many days it would take for *Raphanus sativus* seeds to germinate while being affected by different levels of salinity and different incubation temperatures.

 The data was collected by putting ten watered (with varying salinity levels) seeds surrounded by a paper towel sheet into a Ziploc bag and inserting it into a certain incubator (each trial had a different amount of salinity and a different incubation temperature). Pictures were taken to document what seeds looked like for all of the trials. The data was reliable due to the fact that each set of seeds was set up the same and nothing was different from the first set to the last.

 Each trial was repeated ten times along with the standard which was also repeated ten times, ensuring that the data collected was more reliable as a whole. The averages of each trial and the standard were taken to represent each trial and standard, making it easier to interpret the data. Table 1 shows all of the trials along with their averages.

Table 4

Factors and Values

|  |  |
| --- | --- |
| Salinity (%) | Temperature ( ⁰ C) |
| - | Standard | + | - | Standard | + |
| 1 | 2 | 3 | 24 | 27 | 30 |

Table 4 shows the highs, lows, and standards per each variable (Salinity and Temperature). In the research, the standards were only paired with the standards, while the high and lows were paired with each other in the trials. The values were chosen because their amounts were enough to affect, but not kill *Raphanus sativus* seeds in germination.

Table 5

Averages

|  |  |
| --- | --- |
| Trials | Averages |
| Salinity | Temp. |
| (+) | (+) | 10.535 |
| (-) | (-) | 9.27 |
| (+) | (-) | 9.183 |
| (-) | (+) | 9.907 |
| Grand Average | 9.72375 |

Table 5 shows the averages for each trial along with the grand average. The data represents the number of days it took for the seeds to germinate in total.

Table 6.

|  |
| --- |
| Effect of Salinity |
| -1% | -3% |
| 9.27 | 10.535 |
| 9.907 | 9.183 |
| Avg =9.5885 | Avg =9.859 |

Effect of Salinity

Effect Value = .2705

 Figure 4. Effect of Salinity

Table 6 shows the average of both high trials, and both low trials in regards to salinity. Both averages are very similar, but the two trials that had 3% salinity solution took roughly .2705 days more to germinate, as seen in Figure 4 which shows a positive effect value. The effect value is not statistically significant as it did not pass the test of significance. The effect value represents the difference in days from the high salinity value compared to the low value. This means (disregarding the temperature’s effect and the interaction effect) that, on average, *Raphanus sativus* seeds that are treated with 3% salinity solution will take .2705 less days than seeds germinated with a 1% solution. The effect value was found by subtracting the low value of salinity from the high value to get the effect of .2705.

Table 7

Effect of Temperature

|  |
| --- |
| Effect of Temperature |
| (24°C) | (30°C) |
| 9.27 | 10.535 |
| 9.183 | 9.907 |
| Avg =9.2265 | Avg =10.221 |

Effect Value = .9945

 FFigure 5. Effect of Temperature

Table 7 shows the average of both high trials, and both low trials in regards to temperature. The averages are further apart in comparison to Table 6. The 30 ℃ average took roughly .9945 more days to germinate than the 24 ℃ average, .9945 being the effect value. This value is not statistically significant, but is practically significant as roughly one day is the difference between the two averages. This means (disregarding the other effect value and the interaction effect) that *Raphanus sativus* seeds that are inserted into 30 ℃ incubators take about a day longer to germinate than seeds placed into 24 ℃ incubators on average. The value of .9945 was determined by subtracting the low value from the high value of temperature.

Table 8

Interaction Effect

|  |  |
| --- | --- |
|  | Salinity (%) |
| (-) 1 | (+) 3 |
| Temperature (⁰ C) | Line Segment Solid | (+) 30 | 9.907 | 10.535 |
| Line Segment Dotted | (-) 24 | 9.27 | 9.183 |

Effect of Salinity and Temperature

Figure 5. Effect of Salinity and Temperature

Table 8 shows the averages of the trials and how they correspond to Figure 6. The interaction effect (.3575) is the interaction between the two variables (Salinity Percentage and Incubation Temperature).  The dotted line segment’s slope is .0435 and the solid line segment’s slope is .314.  Since the slopes do not intersect there is a low interaction between the two variables. The interaction effect is not statistically significant, as .3575 x 2 < 8.52 (the difference between highest and lowest standard is 4.26 and once doubled makes 8.52). The interaction effect represents how the two variables were affected by one another. The interaction effect was found by subtracting the -,- from the +,- and dividing that by two, repeating the same process but with the -,+ and +,+ respectively, then combining the two for the final value.

Figure 7. Scatter Plot of Standards

Figure 7 shows the standards as they range from high to low, but in random order, with the Y axis being represented as the average number of days it took for a seed to germinate. The highest standard shown took 11.96 days to germinate, and the lowest took 7.7 days to germinate, meaning there was a range of standards of 4.26 days. There is a slight upward trend among the standards; the variance in the data was low enough to continue the experiment.

Legend:

**I** - Interaction Effect

S - Effect of Salinity

**T** - Effect of Temperature

8.52

Figure 8. Dot Plot of Effects

Figure 8 shows the effects as they are compared to the fences. The fences (-8.52 and 8.52) are shown at the end of the axis, with all of the data being in the middle. It could mean that the effects had little value compared to the standards, or that the standard’s trials were not properly conducted and had too much variance. Because it is believed all of the trials were conducted as properly as possible, it can only be inferred that the effects had little value compared to the range of standards.

Parsimonious Prediction:

y = 9.72375 + “noise”

Figure 9. Parsimonious Prediction

The range of standards for this data set was 4.26 and when doubled was 8.52, making ±8.52 the fence for significant data. Since no data passed the test of statistical significance the parsimonious prediction is y = 9.72375 + “noise”. Because nothing was statistically significant the interpolated equation would be the same as the parsimonious prediction equation of y = 9.72375 + “noise”. Since none of the data passed the test it can be assumed that the data could be achieved again.

Interpretation:

None of the data passed the test of statistical significance. But it is believed that the independent variables still had an effect on the germination of the seeds. In a constant test ran by one of the researcher after all data was collected, 50 seeds all germinated after 5 days, proving that either salinity or incubation temperature had a large effect on the germination of the seeds. These results did not show in the experiment conducted because the difference between our “+,” “standard,” and “-“ effects are thought to not be great enough.

**Conclusion**

It was hypothesized that salinity would negatively affect the germination rate or kill the seed and higher temperatures would increase germination. The hypothesis was true in that temperature increased germination and salt slowed it, but no seeds died due to the salinity levels. Therefore the hypothesis could not be fully accepted.

Many people on earth lack drinking water, and a large portion of freshwater is used in farming. The experiment was intended to help these people by saving their drinking water by using salt water, a readily available resource, to water crops. The purpose of the temperature variable was to determine in what regions the seeds could be planted and in what seasons. The salinity variable’s intention was to see what solution percentages could be used. To test this, sets of ten seeds were placed into Ziploc bags along with paper towel. Next the sets were watered with their specific solution and put into incubators according to their trial. The “+,-” trial had the fastest germination rate of 9.183 days, and the slowest trial was the “+,+” with an average of 10.535 days. This suggested that temperature had more of an effect than salinity. Although the data suggested there was little significance of the variables used, in a constant trial, the seeds took on average five days to germinate, proving either salinity and/or temperature greatly affected germination rate. Salinity negatively affected the seeds because when a seed is exposed to salt, osmosis pulls water out of the seed instead of drawing it in. In this case not all of the water was taken out but seeds were not getting as much water as needed for faster germination.

From all of the research articles on *Raphanus sativus* seeds and germination, only a small group involve salinity levels, and a bit more than that involved temperature as variables. “The more salt in the water, the fewer and less healthy the plants were, to the point where the seeds would not germinate at all” (Snyder). Although the article has to do with plants instead of germination, the comparison between it and this experiment is the salinity factor. The data collected follows data collected in similar experiments.

There were three major flaws in the experimental design. First, the difference between salinity levels in our solutions was not great enough to have a noticeable difference in germination rate. In addition, data was not collected over weekends, to account for this issue, seeds germinated over the two day weekend was averaged to increase accuracy. Finally, the seeds were not evenly spaced in the bags because the water would push the seeds when poured into the bag. Throughout the duration of the experiment there were no significant human errors.

 These flaws can be helpful to future research by giving an example of how to set the experiment up when it comes to salinity solutions, and placing the *Raphanus sativus* seeds in the bags. The difference between salinity solutions should be expanded to gain more of an effect on germination. Adding more variables, but not taking away the variables already used, is a way to expand the knowledge of how salinity and temperature affect the germination rate of *Raphanus sativus* seeds.

 With much experience and knowledge to gain from conducting an experiment, the most important thing learned was how to properly conduct research and write a lab report. In addition, many discoveries about how seeds germinate and different factors that affect them were made.

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