BIOLOGY

The Effect of Indole-3-Acetic Acid (IAA) Concentration on The Rate of Root Growth of *Raphanus Sativus*

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Research Question

How does indole-3-acetic acid (IAA) concentration (0.010, 0.060, 0.100, 0.600, 1.000 ±0.001 g cm^{-3}) affect the rate of root growth (cm 24 ±1 h^{-1}) of *Raphanus sativus.* (Radish)

Aim

To investigate the effect of IAA concentration (0.010, 0.060, 0.100, 0.600, 1.000 ±0.001 g cm^{-3}) on the rate of root growth (cm 24 ±1 h^{-1}) of *Raphanus sativus.*

Hypothesis

Hypothesis: If the concentration of IAA increases, then the root will grow at a lower rate compared to without IAA. This is Because IAA is a plant growth hormone responsible for the regulation of growth by causeing increase in the shoot growth rate and decrease in the root growth rate.

Null hypothesis H_0: All concentrations have equal mean root growth.

Background

Indole-3-acetic acid (IAA) is the most common plant hormone of the auxin class, responsible for growth regulation (National Center for Biotechnology Information, 2019)[1]. Plants, bacteria, and fungi can synthesise IAA from tryptophan and it could also be synthesised artificially.

Plants have both phototropism and gravitropism properties, which means the natural response of plants towards light and gravity. Coleoptile grows towards the light and opposite towards the gravity direction. In the case of root growth, it is not influenced by light as it normally is underground, and it grows towards the direction of gravity. This is due to the properties of auxin. Auxin accumulates in the dark and the tip of the root and promotes elongation of the cell where it accumulates. However, IAA inhibits cell elongation in the roots. In plants, auxins are normally synthesised in the stem, root, and buds and promote the elongation of the cells in the shoot and inhibit the elongation of cells in the root. Which is qualitatively shown as stem growth, cell division, differentiation and fruit development, and decrease in root growth (Barrington, 2019)[2].

Plants have different types of root systems; the two main types are the tap root system and fibrous root systems. (Boundless, 2019)[3] Taproot systems have a main root that grows downwards, and the fibrous root system has many roots that grow in every direction. To measure the root growth accurately, a tap root system is preferred. Moreover, there are two types of tap root systems, deep feeder and surface feeder. The Deep feeder tap root system

[1] National Center for Biotechnology Information. (2019, April 30). *PubChem Database*. Retrieved from Indole-3-acetic acid: https://pubchem.ncbi.nlm.nih.gov/compound/Indole-3-acetic-acid

[2] Barrington, E. J. (2019, April 28). *The Hormone Of Plants*. Retrieved from Britannica: https://www.britannica.com/science/hormone

[3] Boundless. (2019, May 23). *30.3A: Types of Root Systems and Zones of Growth*. Retrieved from Biology LibreTexts: https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_General_Biology_(Boundless)/30%3A_Plant_Form_and_Physiology/30.3%3A_Roots/30.3A%3A__Types_of_Root_Systems_and_Zones_of_Growth

has an elongated primary root which penetrates further into the soil (Manisha, 2019)[4]. Therefore, the best type of root should be deep feeder tap root system. Examples of deep feeder tap root species include *Vigna Radiata, Raphanus sativus, Daucus carota, Brassica rapa.*

During the research, the auxin area and plant growth was being focused. Most studies found was about how different auxins and different conditions affect the root growth of certain plants. As the research question was being finalized, the area of research narrowed down to common artificial plant growth hormones in the auxin class, and the common concentrations of the auxins used in various studies. All in all, the research question has finalized with the most common auxin: Indole Acetic Acid (IAA) and a large range of concentrations (0.01, 0.06, 0.10, 0.60, 1.00 g cm^{-3}) which is not commonly found in studies. Therefore, the maximum effect of IAA and its range of effectiveness can be discovered.

Preliminary Practical Work

During the preliminary study, I have discovered many problems with my original method. My first method was to grow V*igna radiata* (mung bean) in a straw that was sealed at the bottom. An additional piece of tape was looped into a circle and put into the straw. This prevents the mung bean from falling to the bottom (See Appendix2). *Vigna radiata* was chosen as it is small enough fit into the straw, and large enough to stay on the looped tape. The straws were then filled with IAA solutions and the beans were put into the straws. Ideally, the root should grow straight downwards. However, in reality, the roots grew in every direction, some of them coiled up together, some of them fell down the bottom, hence, the results were impossible to attain (See Appendix1). Additionally, the setup was slow, and the looped tape was not stable enough to hold the seeds while they grew. Therefore, the first method was rejected. Then, I researched methods that other studies have used. I found a guidance for school experiments about IAA and seed germination (Science&Plants for Schools, 2019)[5], so I decided to improve the method to suit my investigation. The method was to plant the seeds on filter papers in petri dishes. So that with the adhesive property of water, the seeds will stick. However, *Vigna radiata* is too heavy to stick on the filter paper, so a lighter and smaller plant with taproot is chosen, *Raphanus sativus*. The result was promising (See Appendix 5). Additionally, it was decided to measure the root length and replenish the water and IAA solution every day instead of waiting for 5 days, which is predicted to cause dehydration. Since the petri dishes were going to open anyways, it was decided to use a string and a calliper for the measurement of root length instead of using an acetate grid which could cause extra trouble doing mathematical calculations. Another issue when measuring the root length is as the plant grows, the seed coat separates from the plant, so it is hard to determine which part is the root. The method in this investigation is to measure from the first turning point above the root hair. This point has been decided judging by where the seed coats fell off in the preliminary practical works.

Independent Variable: IAA concentration 0.010, 0.060, 0.100, 0.600, 1.000 ±0.001 g cm^{-3}. IAA as a plant growth hormone, has the effect of decreasing root growth rate. This has been investigated by many studies such as (Shinkle & Briggs, 1984)[6] and (Bandurski & Schilze, 1977)[7]. However, most of them use small concentrations such as 0.3, 1.0, 10 μM[8]. I aim

[4] Manisha, M. (2019, April 20). *Tap Root System: definition and Types (With Diagram).* Retrieved from BiologyDiscussion.com: http://www.biologydiscussion.com/root/tap-root-system/tap-root-system-definition-and-types-with-diagram/70193

[5] Science&Plants for Schools. (2019, May 17). *Auxin investigations: the effect of indole acetic acid (IAA) on root growth in mustard seedlings.* Retrieved from Science&Plants for Schools: http://www.saps.org.uk/attachments/article/111/SAPS%20-%20Auxin%20investigations%20-%20effects%20of%20IAA%20on%20root%20growth%20-%20students%20guide.doc

[6] Shinkle, J. R., & Briggs, R. W. (1984). Auxin Concentration/Growth Relationship for Avena Coleoptile Sections from Seedlings Grown in Complete Darkness. *Plant Physiology*, 335-339.

[7] Bandurski, R. S., & Schilze, A. (1977). Concentration of Indole-3-acetic Acid and Its Derivatives in Plants. *Plant Physiology*, 211-213.

[8] 1μM IAA is roughly 0.000175 grams

to investigate the maximum effect of IAA; therefore, a high range of concentration was chosen, starting from 0.010 g cm^{-3}, up to 1.000 g cm^{-3}. Additionally, the experiment was run for 5 days, to keep the seeds from drying, 1ml of IAA solution was added to each Group of seeds.

Dependent Variable: Root growth length per 24 hours. Measurement was done about once every 24 hours to observe the effect each day.

Controlled Variables

Variable	Impact	How its controlled
Species	Different species may have different growth speed, germination speed.	All seeds used are *Raphanus sativus.*
IAA solution/water volume	Higher volume may result in more IAA absorbed than lower volume Groups.	Using a pipette, only water once about 24 hours, 1ml only.
Light intensity	Medium shading may result in faster germination rate (Yan & Cao, 2007)[9]. After germination, higher light intensity may result in faster growth of root (Aref, 2000)[10].	The plants were kept in a non-transparent box, the plants are only exposed to light when measuring.
CO_2 concentration	After germination, higher concentration of CO_2 may increase the rate of growth of root. Lower concentration of CO_2 may decrease the rate of growth of root.	All *Raphanus sativus* seeds are placed in the same environment, bought on the same day, from the same packet. And there is same quantity of seeds in each petri dish.
Temperature	Low temperature may lead to the inhibition of seed germination. While higher temperature may increase the rate of growth. Temperature that is too high may cause the enzymes in the seeds to denature.	All plants are kept in the same environment.
Petri dish size	Larger or smaller sizes of petri dishes would result in higher or lower CO_2 quantity in each petri dish. Therefore, the seeds may grow faster or slower.	All petri dishes are the same size from the same manufacturer.
Number of seeds in each petri dish	As the number of seeds in each petri dish determines the amount of carbon dioxide consumption, more seeds will consume more carbon dioxide, causing the seeds to germinate slower. Less seeds will consume less carbon dioxide, causing the seeds to germinate faster.	Each petri dish has five seeds.

Table 1 continued

Measuring time	As plants grow continuously, when measured too early, the roots might be shorter, when measured too late, the roots might be longer. If the roots are measured at	All seeds are measured at the same time of each day.

[9] Yan, X., & Cao, M. (2007). Effects of light intensity on seed germination and seedling early growth of Shorea wantianshuea. *Ying Yong Sheng Tai Xue Bao*, 23-9.
[10] Aref, I. M. (2000). *The Effects Of Light Intensity On Seed Germination And Seedling Growth Of Cassia fistula (Linn.), Enterolobium saman (Jacq.) Prain ex King. and Delonix.* Riyadh: Plant Production Department, College of Agriculture, King Saud University.

	different times in a day, then the results may not be consistent.	And the roots are measured as fast as possible.

Table 1: Controlled variables

Materials

Chemical	Quantity	Uncertainty
IAA solution 0.010/0.060/0.100/0.600/1.000 g cm^{-3}	100ml each	0.001g cm^{-1}
Apparatus	**Quantity**	**Uncertainty**
Petri dish	18	
Calliper	1	±0.01cm
String	10cm	
Pipette	1	±0.01cm^3
Filter paper	18	

Table 2, Materials - chemicals and apparatus used in the investigation.

Method[11]

1. A filter paper was put in a petri dish
2. The filter paper was soaked with 4 ml of water in the petri dish
3. 5 seeds are placed in the middle top area on the filter paper, in a line
4. Repeat 1-3 with concentrations of IAA 0.01, 0.06, 0.1, 0.6, 1.0 g cm^{-1}
5. Cut the top half of a 1L coke bottle open
6. The petri dishes are placed in the coke bottle with the seeds on top
7. The petri dishes and coke bottle are then placed in a non-transparent box
8. The root growth is measured using a string
9. The string was then straightened and measured with a calliper in centimetres
10. Using a pipette, 1 millilitre of the same concentration of IAA is added to the petri dishes after the measurement
11. Rinse the pipette after every use
12. The root growth is measured every day, about 24 hours apart from each measurement

Measurement: After the seed germinates, it is hard to determine where the root and the stem meet. However, besides of the primary root, root hair is grown as well. Therefore, where the root hair starts is where the root starts to be measured[12]. Setup: See appendix 6.

Safety, Ethical and Environmental Considerations

- As this investigation involves a chemical that may possess a potential risk, a risk assessment was carried out on indole-3-acetic acid (IAA) solution by producing a statement by MSDS. IAA is classified as potential hazards with low toxicity.
- The Global Harmonised System of Classification and Labelling of Chemicals (GHS) states that IAA may cause skin irritation, serious eye irritation and may cause respiratory irritation (National Center for Biotechnology Information, 2019)[13].
- Any ingestion of any substance in the investigation should be avoided, general laboratory safety should be taken, gloves, lab coats and safety goggles should be worn to avoid any contact with skin and eyes.
- Care should be taken when handling glassware and the calliper as it may cause potential damage when broken.

[11] Method derived from (Science&Plants for Schools, 2019)

[12] See Appendix 3: Measurement of the root, for how to measure

[13] National Center for Biotechnology Information. (2019, April 30). *PubChem Database*. Retrieved from Indole-3-acetic acid: https://pubchem.ncbi.nlm.nih.gov/compound/Indole-3-acetic-acid

- Although IAA is not classified as environmentally hazardous, care should be taken when disposing, and local regulations should be followed.

Data collection

Quantitative: See Appendix 4: Data collection of root growth

Qualitative: Before the seeds germinated, there is a thin layer of water surrounding the seeds (See Appendix 5). Ideally the roots should grow downwards as the petri dishes are fixed in the coke bottle (See Appendix 6). However, the roots didn't always grow downwards, they grew sideways; they grew up first and then downwards.

The first day, barely any seeds germinated, except for 0.06 concentration. The second day, most of the seeds germinated, except for 0.6 and 1.0 concentration. Only one of the five seeds germinated in the 0.6 concentration and none germinated in the 1.0 concentration. After first two days, most of the roots were clearly visible. 0.6 concentration still only had one seed germinated and seeds in 1.0 concentration didn't germinate at all. Additionally, one seed from 0.01 concentration and one seed from control group didn't germinate.

Data Analysis

Control

Day5	2.73	1.65	0.680	2.66	0.00	Group 1
	1.37	2.00	0.390	1.20	1.78	Group 2
	0.560	0.320	1.67	1.10	2.02	Group 3

Table 3, Raw data example[14]

Because there are too many raw data, an example collection of data is shown above. This is an example from the control, Day5. The numbers in the table are the measured root length. 'Control' is the independent variable, the concentration, 'Day5' is the day when these lengths are measured, 'Group1' is the 1st group as there are three total groups with fives seeds in each group to minimize the uncertainty of utilizing plants.

ANOVA[15]

Analysis of Variance (ANOVA) is a collection of statistical models. The most important one is the P-value. ANOVA can be done with excel, when inputting data, the alpha value was set to 0.05, this means if the P-value is smaller than 0.05, then with 95% confidence, there is a significant difference between at least one of the factors. The P-value is higher than 0.05, then there is no significant difference between the factors and the null hypothesis is supported (Millar, 2001)[16]. ANOVA is chosen for this investigation because there are two factors, time and concentration. Therefore, two factor ANOVA with replication is used. The generated summary table is shown below:

[14] Full raw data table, see Appendix 4.
[15] See Appendix 8: Full Analysis of Variance
[16] Millar, N. (2001). Biology statistics made simple using Excel. *School Science Review*, 23-34.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Concentration (Rows)	170.7829	17	10.04605	26.56967	2.47E-53	1.65121
Days (Columns)	143.2194	4	35.80485	94.6962	5.9E-55	2.396743
Interaction	188.0479	68	2.765411	7.313922	1.53E-37	1.337092
Within	136.1168	360	0.378102			
Total	638.1671	449				

Table 5, ANOVA

Uncertainties

The standard deviation of each day and concentration is calculated. This first, can allow the standard deviation error bars to be drawn; second, to allow the amount of variation and dispersion of the data to be quantified. Following the equation:

$$\sigma = \sqrt{\frac{\sum (x-\bar{x})^2}{n}}$$

Where σ is standard deviation, n is the number of seeds in each Group, x is the length of the seed, $\bar{x}$ is the mean of the Group. For example, Control, Group 3 Day 5:

$$\sqrt{\frac{(0.56-1.3375)^2+(2.02-1.3375)^2+(1.67-1.3375)^2+(1.1-1.3375)^2+(0.32-1.3375)^2}{5}} = 0.640$$

Concentration (g cm^{-1}) /Day (24h)	Group 1[17]				
	1	2	3	4	5
Control	0.00	0.120	0.130	0.480	0.620
0.01	0.0500	0.250	0.770	0.490	0.590
0.06	0.180	0.300	0.350	1.02	1.74
0.1	0.0600	0.0500	0.380	0.920	1.33
0.6	0.00	0.100	0.200	0.300	0.400

Table 4 continued

Concentration (g cm-1) /Day (24h)	Group 2				
	1	2	3	4	5
Control	0.100	0.0600	0.360	0.240	0.600
0.01	0.110	0.0800	0.350	0.450	0.710
0.06	0.00	0.0600	0.0900	1.04	1.70
0.1	0.0400	0.0500	0.610	0.610	1.21

Concentration (g cm-1)	Group 3				
	1	2	3	4	5

[17] This experiment consists of three different groups of seeds, split into three different petri dishes for each concentration. Five seeds in each group.

/Day (24h)					
Control	0.0500	0.0800	0.220	0.370	0.640
0.01	0.120	0.250	0.320	0.390	0.310
0.06	0.280	0.530	0.560	0.710	0.910
0.1	0.0400	0.0300	0.280	0.170	0.270

Table 4, Standard Deviation

Standard error of each day and concentration are calculated. This allows the dispersion of the sample mean around the population mean to be quantified. Therefore, the errors can be discussed later. Following the equation:

$$SE = \frac{\sigma}{\sqrt{n}}$$

Where SE is Standard Error, σ is standard deviation, n is the number of seeds in the Group. For example: Control, Group 3, day 5:

$$\frac{0.64}{\sqrt{5}} = 0.29$$

Concentration (g cm-1) /Day (24h)	Group 1				
	1	2	3	4	5
Control	0.00	0.0540	0.0580	0.210	0.280
0.01	0.0220	0.110	0.340	0.220	0.260
0.06	0.0800	0.130	0.160	0.460	0.780
0.1	0.0270	0.0220	0.170	0.410	0.590
0.6	0.00	0.0450	0.0890	0.130	0.180

Concentration (g cm-1) /Day (24h)	Group 2				
	1	2	3	4	5
Control	0.0450	0.0270	0.160	0.110	0.270
0.01	0.0490	0.0360	0.160	0.200	0.320
0.06	0.00	0.0270	0.0400	0.470	0.760
0.1	0.0180	0.0220	0.270	0.270	0.540

Table 5 continued

Concentration (g cm-1) /Day (24h)	Group 3				
	1	2	3	4	5
Control	0.0220	0.0360	0.0980	0.170	0.290
0.01	0.0540	0.110	0.140	0.170	0.140
0.06	0.130	0.240	0.250	0.320	2.03
0.1	0.0800	0.060	0.630	0.380	0.600

Table 5: Standard Error

Interpretation

The graphs in appendix 7 show the relationship between the concentration of IAA and the length of root growth. To find the rate of growth, the generated trendline equations are differentiated. Following the equation: $\frac{dy}{dx}f(x)$

Where $\frac{dy}{dx}$ is the difference in y (length of root) divided by the difference in x (days), $f(x)$ is the function of the trendline.

Then, the mean of the three groups were found to show the overall trend of the effect of IAA concentration on the rate of root growth

For example, Control:

$$\frac{dy}{dx}0.2924x - 0.3524 = 0.2924$$

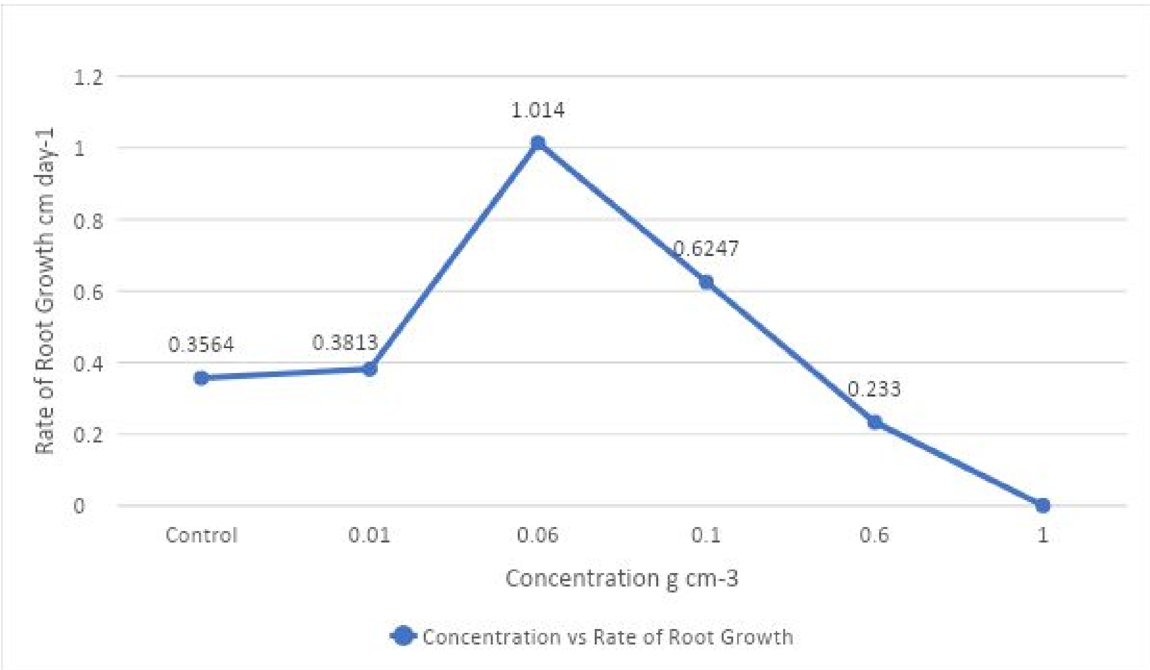
$$\frac{dy}{dx}0.4623x - 0.7293 = 0.4623$$

$$\frac{dy}{dx}0.3144x - 0.4116 = 0.3144$$

$$\frac{0.2924+0.4623+0.3144}{3} = 0.3564\ cm\ day^{-1}$$

Concentration g cm^{-3}	Control	0.01	0.06	0.1	0.6	1.0
Rate of growth cm day^{-1}	0.3564	0.3813	1.014	0.6247	0.2330	0.00

Table 6, Concentration vs Growth Rate



Graph 1: Rate of Growth.

Overall from the graph, it is shown that initially, as the concentration of IAA solution increases, the rate of growth increases. This shows that IAA has the effect of increasing the rate of root growth in lower concentrations. 0.06 g cm^{-3} concentration has the fastest growth out of all concentration, any higher results in a decrease of growth rate. As IAA concentration increases, the inhibition effect of IAA in more significant. As the concentration keeps increasing, IAA has inhibited the growth rate and even germination. For 0.6 g cm^{-3}, only one group has germinated, the other two groups didn't, for the 1.0 g cm^{-3} concentration, none of the seeds germinated. In average, it can be identified that in lower concentrations, IAA has the effect of enhancing root growth, with the 0.06 g cm^{-3} concentration of IAA showing the best enhancement to the root growth speed of *Raphanus sativus*.

More precisely, for all the concentrations, the roots grew exponentially, the plants all had similar rate of root growth at first, as time goes, the rate of root growth increases. However, the difference in the rate increases as time goes, 0.01 g cm^{-3} concentration is higher than control group and 0.06 g cm^{-3} concentration is higher than 0.01 g cm^{-3} concentration group. There's a decrease in the difference in rate for the 0.1 g cm^{-3} concentration but it's still higher than the control group and the 0.01 g cm^{-3} concentration group. For the 0.6 g cm^{-3} concentration there is a large decrease in the rate of root growth. For the 1.0 g cm^{-3} concentration none germinated so it's not applicable here.

Moreover, from the ANOVA test, it is shown that all p-values are significantly smaller than 0.05, which means with 95% confidence, both time and IAA concentration have significant effect on the growth of root. Additionally, all the F value is significantly higher than 1 and are higher than the F critical values, so, the null hypothesis can be rejected. However, judging by the standard deviation, it could be seen that as time goes, the variance of the data increases. Also gives a higher standard error as time goes. Which explains the large error bars in the relationship graphs[18]. Therefore, it could be claimed that either or both root growth and IAA plant growth hormone has high uncertainty, some of the seeds didn't grow at all.

Conclusion

The research question of this investigation is 'how does indole acetic acid (IAA) concentration affect the rate of root growth of *Raphanus sativus*.' The hypothesis made is 'If the concentration of IAA increases, then the root will grow at a lower rate compared to without IAA. Because IAA is a plant growth hormone responsible for the regulation of growth. Higher concentration of IAA solution decreases the growth rate.' However, the hypothesis is substantiated to some extent. Because in the lower concentrations, as the concentration of IAA solution increases, the rate of growth increases, after a confirmed concentration: 0.06 g cm^{-3} concentration, the rate of growth decreases as IAA inhibits the root growth. Additionally, this can be seen from the trendlines on the growth models[19], the larger the gradient, the faster the growth rate[20], the smaller the gradient, the slower the growth rate. From the statistical analysis, it can be concluded that IAA concentration has a significant effect on the rate of root growth, therefore the null hypothesis 'All concentrations have equal mean root growth' can be rejected by at least 95% confidence.

[18] See Appendix 7
[19] See Appendix 7
[20] Growth rate calculated in Table 6

Strength and Limitation

Strength	Explanation
Demonstrates the effectiveness of the concentrations and the optimum concentration on the macro level	Different than common studies such as (Shinkle & Briggs, 1984)[21], this investigation has included a wide range of IAA concentrations. This shows the effectiveness of each concentration at a macro level, which we can use to determine the optimum concentration of IAA. Additionally, it shows the maximum concentration that the plant can handle.
High range of Groups	Five seeds per Group, three Groups per concentration is used in this investigation. The major advantage of this high range of Groups is to cover the uncertainty of seed germination. Because some didn't germinate, some have slow germination, high range of Groups can cover the uncertainty to some degree. If the Group size is small, there would be an even higher uncertainty.
Daily measurements	The measurements in this investigation is done daily. This gives the clear Day (24h) by day trend of the root growth. Both the effect of time and IAA on the rate of root growth can be seen.

Limitations	Explanation	Suggested Improvements
The range of variable is too high	This experiment is focused on the macro scale of the effect of IAA. A large scale of concentrations with large gaps between is used in this experiment. Although it could be seen that 0.06 g cm^{-3} concentration produced the highest growth rate, more accurate concentrations and experiments can be made to discover exactly which concentration produces the best results.	Ideally, a second experiment focused on the range around 0.06 g cm^{-3} concentration can be made with more time. Therefore, the more accurate trend and effect can be found.
Uncontrollable germination and growth speed	Firstly, seed germination time highly depends on the seed. For this experiment, the range is from1 to 4 days. Second, root growth is not the same for every seed. There are multiple factors involved in germination (Koger, Reddy, & Poston, 2004)[22]. Some of the roots grew extremely slow while others grew extremely fast regardless of the IAA concentration. Fortunately, these still follow the trend and have effects of the IAA solution. Therefore, the uncertainty, including standard deviation, standard error is high in this experiment.	Planting in a greenhouse can control the temperature, humidity and carbon dioxide concentration. Although it is impossible to fully eliminate the uncertainty, it can greatly reduce the uncertainty.

[21] Shinkle, J. R., & Briggs, R. W. (1984). Auxin Concentration/Growth Relationship for Avena Coleoptile Sections from Seedlings Grown in Complete Darkness. *Plant Physiology*, 335-339.

[22] Koger, C. H., Reddy, K. N., & Poston, D. H. (2004). Factors affecting seed germination, seedling emergence, and survival of texasweed (Caperonia palustris). *Weed Science 52(6)*, 989-995.

Too much exposure to light	IAA requires darkness to fully express its functions (Monteuuis & Bon, 2000)[23]. This is because auxins tend to cumulate in the dark to elongate the cells, either for roots to grow deeper or for leaves to bend towards the light. Therefore, the plants are normally kept in a non-transparent box. However, by measuring the root growth every day, the plants needed to be taken out. This may result in higher uncertainty as it makes the effect of IAA less accurate.	Take measurements during the night. As there are not sunlight during the night, the light exposure can be limited greatly. Therefore, the roots will be able to grow more consistently with decreased uncertainty caused by light intensity. Additionally, using night vision goggles can greatly eliminated the uncertainty.
Difficult to differentiate between radicle and cotyledon	Because after germination of a while, the seed coat falls off, the radicle and cotyledon grow out. Therefore, it's hard to identify which part is the radicle and which part is the cotyledon. The method I have used it to measure from the first turning point above the root hair. However, the mid-point of the turning point is also hard to identify. Therefore, there are errors and uncertainties associated with the measurement.	It is worth a try to mark the point where the cotyledon starts to grow before the seed coat falls off. Between the cotyledon and the seed coat. This might highly improve the accuracy of the measurement as the identification between the cotyledon and the radicle is clear. Theoretically, IAA only affects the tip of the root (Overvoorde, Fukaki, & Beeckman, 2010)[24]. However, it is unclear whether the cotyledon would elongate where the mark is at.
Unable to fully explain the micro level of the effect of IAA	This experiment is focused on the macro level of the effect of IAA, however, it is hard to explain how IAA effects on the root cells without qualitative data.	By using a microscope, if microscopic observations are made and qualitative data are obtained, the effect of IAA and the different concentrations can be explained better. The inhibition effect, possibly dehydration can be seen on a microscopic level and explained clearly.
Inconsistent temperature	Temperature is also an important factor in seed germination and initial root growth. Each plant has their own favourable temperature. However, low temperatures generally would cause dormancy or generally slow germination speed (Koger, Reddy, & Poston, 2004) (Vassilevska-Ivanova & Tcekova, 2002). It's hard to keep a consistent	Planting in a greenhouse would eliminate the error associated with temperature, resulting in less uncertainty.

[23] Monteuuis, O., & Bon, M.-C. (2000). Influence of auxins and darkness on in vitro rooting of micropropagated shoots from mature and juvenile Acacia mangium. *Plant Cell, Tissue and Organ Culture*, 173-177.
[24] Overvoorde, P., Fukaki, H., & Beeckman, T. (2010). *Auxin Control of Root Development.* New York: Cold Spring Harbour Laboratory Press.

	temperature in the school while the temperature in Melbourne varies a lot. Also, the temperature in Melbourne tends have sudden changes which makes the controlling of temperature in schools hard. Therefore, this could cause some seeds to fail germination, or have varied growth speed.	

Extension

1. How does IAA, IBA and NAA concentration (0.050, 0.052, 0.054, 0.056, 0.058, 0.060, 0.062, 0.064, 0.066, 0.068, 0.070 g cm^{-3}) effect the rate of root growth of *Raphanus sativus.*

   This research question would compare and contrast the effect of different auxins on the rate of root growth of *Raphanus sativus.* As every auxin has a different effect on plant growth. Additionally, the concentrations are more accurate and more focused on the range where the maximum effect of IAA is shown. Therefore, the accurate maximum effect of auxins can be discovered.

2. The effect of abscisic acid concentration on the dormancy time of *Raphanus sativus.*

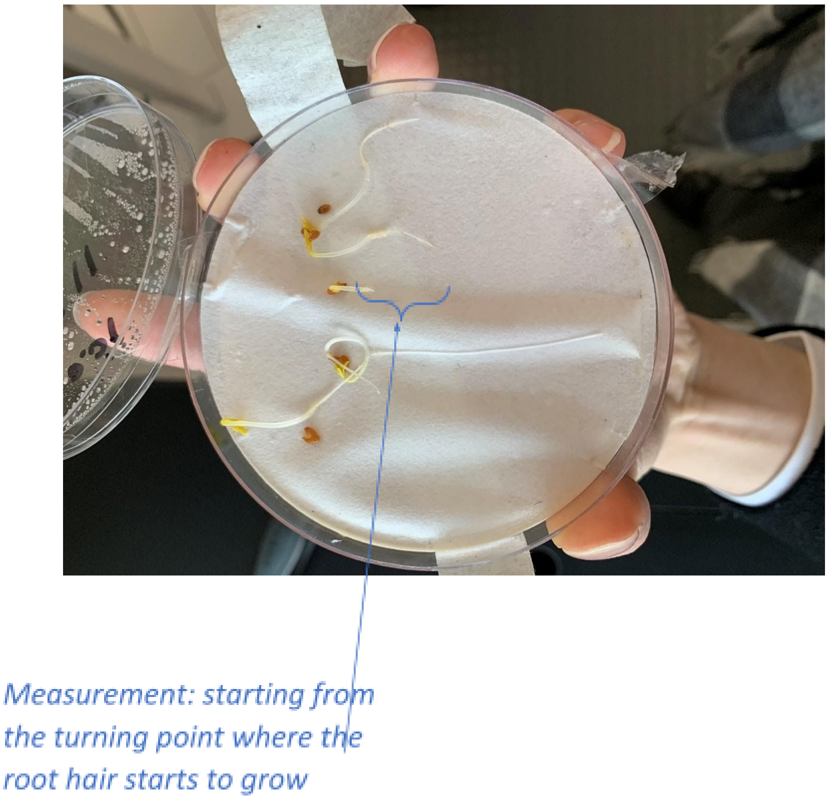
   This research would focus on the effect of plant hormone on seed germination. Different concentrations of abscisic acid may have different effects of the dormancy time of certain seeds before germination. This investigation could explain the hormonal control of dormancy by abscisic acid and whether artificial procedures can forcefully decrease dormancy time.

Appendices

Appendix 1: Preliminary practical work failed experiment 1

Appendix 2: Preliminary practical work fail experiment 2

Appendix 3: Measurement of the root



Appendix 4: Data collection of root growth

0.01 Concentration

	Root length cm					
Day 1	0	0.1	0	0	0	Group 1
	0.19	0.23	0.25	0	0	Group 2
	0.23	0	0	0	0	Group 3
Day2	0.2	0.3	0.22	0	0	Group 1
	0.4	0.31	0.42	0.12	0.26	Group 2
	0.72	0.13	0.29	0.31	0	Group 3
Day3	1.8	0.45	0.39	0.49	0	Group 1
	0.19	0.3	0.3	1.19	0	Group 2
	0.84	0.21	0.2	0.19	0	Group 3
Day4	1.18	0.39	1.7	2.16	0	Group 1
	0.72	1.35	0.25	0.62	1.2	Group 2
	1.1	0.62	1.51	0.5	1.33	Group 3
Day5	2.11	2.13	0.45	1.1	2.12	Group 1
	3.71	2.3	0.52	3.1	0	Group 2
	0.88	0.26	1.49	1.22	2.17	Group 3

0.06 concentration

	Root length cm					
Day 1	0.12	0.42	0.14	0.37	0	Group 1
	0	0	0	0	0	Group 2
	0.71	0.12	0.15	0.33	0	Group 3
Day2	0.52	0.98	0.41	0.61	0	Group 1
	0.2	0.19	0.1	0	0	Group 2
	1.41	0.11	0.41	0.21	0.79	Group 3
Day3	1.69	1.06	1.11	1.62	1.95	Group 1
	0.59	1.82	1.69	3.72	1.45	Group 2
	0.2	0.9	0.19	1.3	0	Group 3
Day4	0.38	0.4	2.14	0.41	2.22	Group 1
	2.74	4.28	5.42	4.2	2.22	Group 2
	1.52	1.9	3.41	1.9	2.29	Group 3
Day5	4.1	2.45	0.61	0.21	0.63	Group 1
	6.55	5.19	2.06	3.18	3.82	Group 2
	8.06	4.52	9.21	8	2.93	Group 3

0.1 concentration

				Root length cm		
Day 1	0.14	0	0	0	0	Group 1
	0.1	0	0	0	0	Group 2
	0.19	0	0	0	0	Group 3
Day2	0.18	0.18	0.2	0.2	0.31	Group 1
	0.22	0.12	0.12	0.18	0.21	Group 2
	0.32	0.28	0.21	0.29	0.19	Group 3
Day3	1.72	1.99	1.98	1.42	1.1	Group 1
	0.29	1.52	1.26	0.2	1.22	Group 2
	0.7	0.4	0.95	1.81	0.2	Group 3
Day4	0.24	2.14	1.02	0.53	2.26	Group 1
	0.3	2.33	2.21	1.2	0.89	Group 2
	1.79	2	2.03	2.79	2.18	Group 3
Day5	2.94	1.99	3.65	0.29	3.2	Group 1
	3.93	1.49	3.05	3	1	Group 2
	3.26	2.89	1.71	2.24	2.29	Group 3

0.6 concentration

				Root length cm		
Day 1	0	0	0	0	0	Group 1
	0	0	0	0	0	Group 2
	0	0	0	0	0	Group 3
Day2	0.22	0	0	0	0	Group 1
	0	0	0	0	0	Group 2
	0	0	0	0	0	Group 3
Day3	0.45	0	0	0	0	Group 1
	0	0	0	0	0	Group 2
	0	0	0	0	0	Group 3
Day4	0.67	0	0	0	0	Group 1
	0	0	0	0	0	Group 2
	0	0	0	0	0	Group 3
Day5	0.89	0	0	0	0	Group 1
	0	0	0	0	0	Group 2
	0	0	0	0	0	Group 3

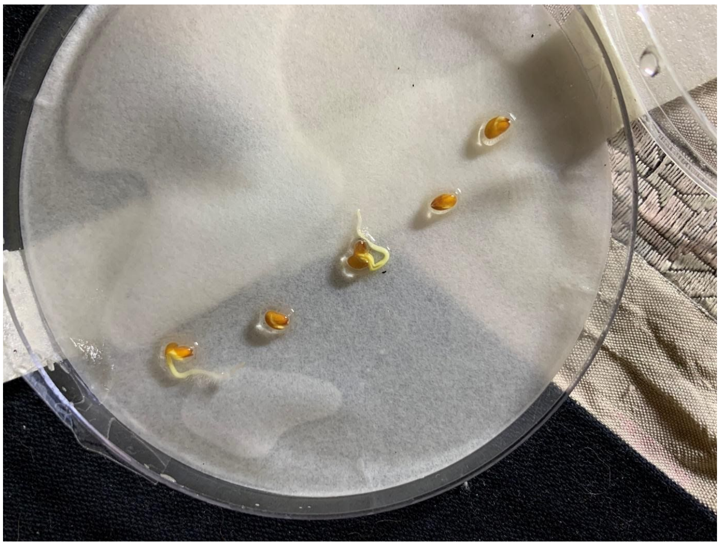
1.0 concentration

	Root length cm					
Day 1	0	0	0	0	0	Group 1
	0	0	0	0	0	Group 2
	0	0	0	0	0	Group 3
Day2	0	0	0	0	0	Group 1
	0	0	0	0	0	Group 2
	0	0	0	0	0	Group 3
Day3	0	0	0	0	0	Group 1
	0	0	0	0	0	Group 2
	0	0	0	0	0	Group 3
Day4	0	0	0	0	0	Group 1
	0	0	0	0	0	Group 2
	0	0	0	0	0	Group 3
Day5	0	0	0	0	0	Group 1
	0	0	0	0	0	Group 2
	0	0	0	0	0	Group 3

Control

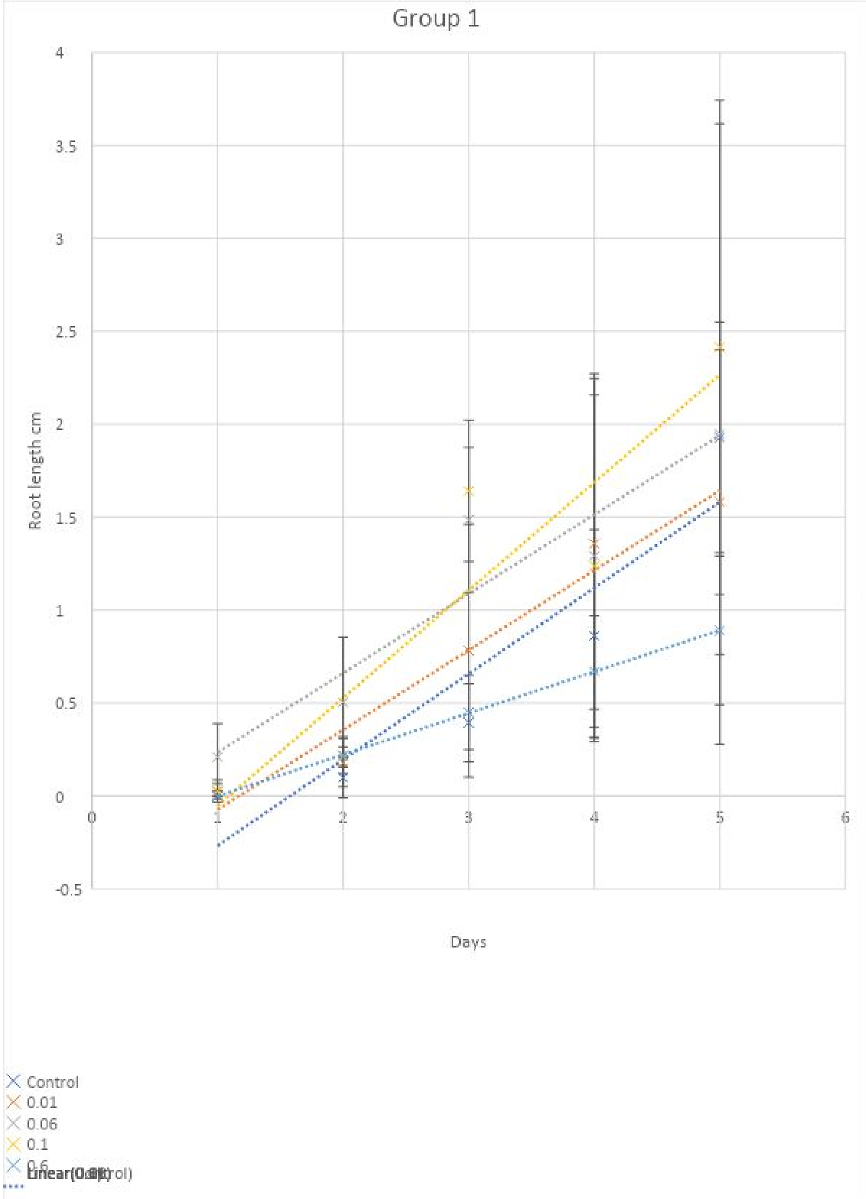
	Root length cm					
Day 1	0	0	0	0	0	Group 1
	0.21	0.12	0	0	0	Group 2
	0.11	0	0	0	0	Group 3
Day2	0.22	0.18	0	0	0	Group 1
	0.21	0.31	0.11	0	0	Group 2
	0.14	0.32	0.1	0	0	Group 3
Day3	0.33	0.45	0.25	0.55	0	Group 1
	0.41	0.21	0.13	0.69	0	Group 2
	0.35	0.21	0.7	0.2	0	Group 3
Day4	1.12	0.78	0.23	1.32	0	Group 1
	0.68	1.11	0.3	0.45	1.3	Group 2
	1.2	0.34	0.77	0.85	1.26	Group 3
Day5	2.73	1.65	0.68	2.66	0	Group 1
	1.37	2	0.39	1.2	1.78	Group 2
	0.56	0.32	1.67	1.1	2.02	Group 3

Appendix 5: Qualitative data of the seeds



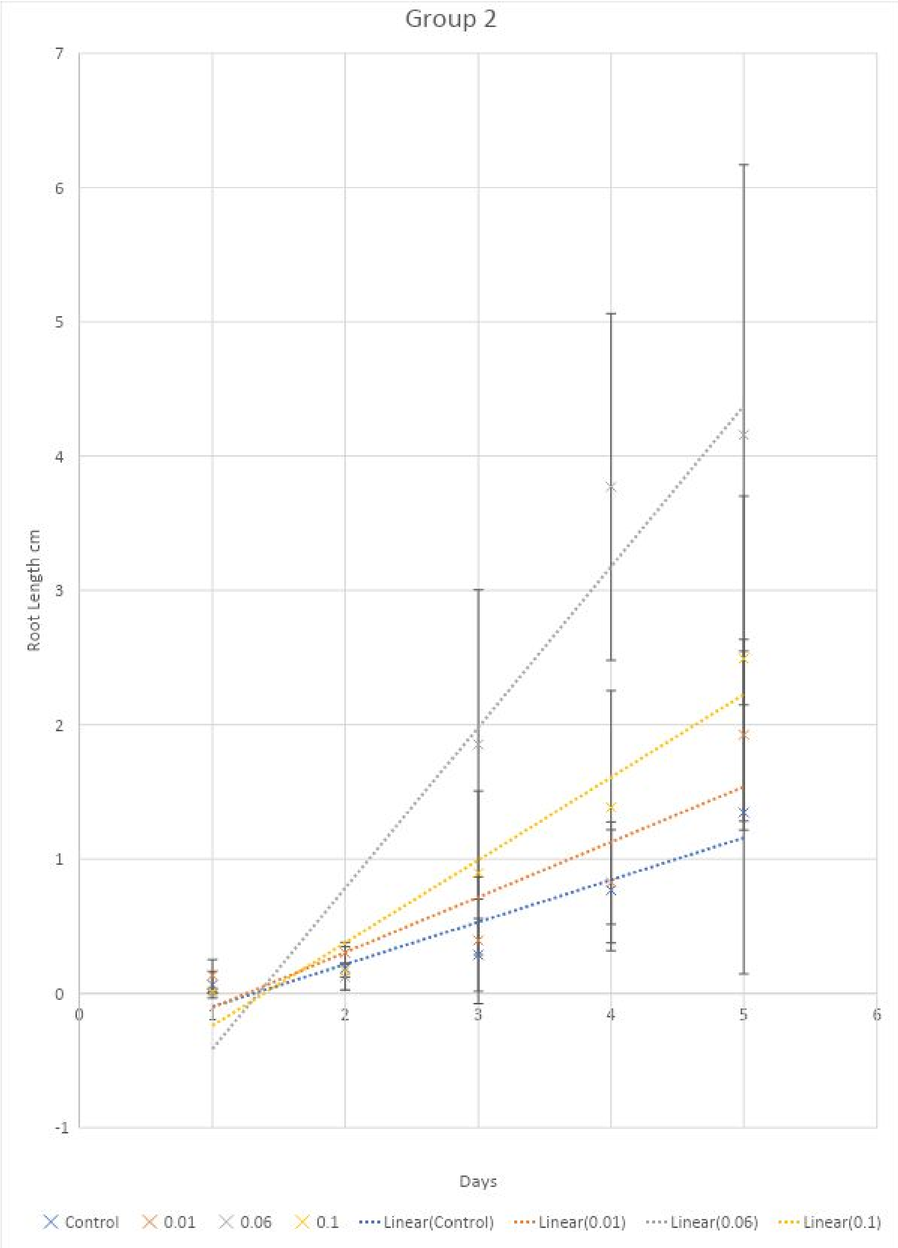
Appendix 6: Set up

Appendix 7: Relationship between concentration and root growth diagram (3 Groups)



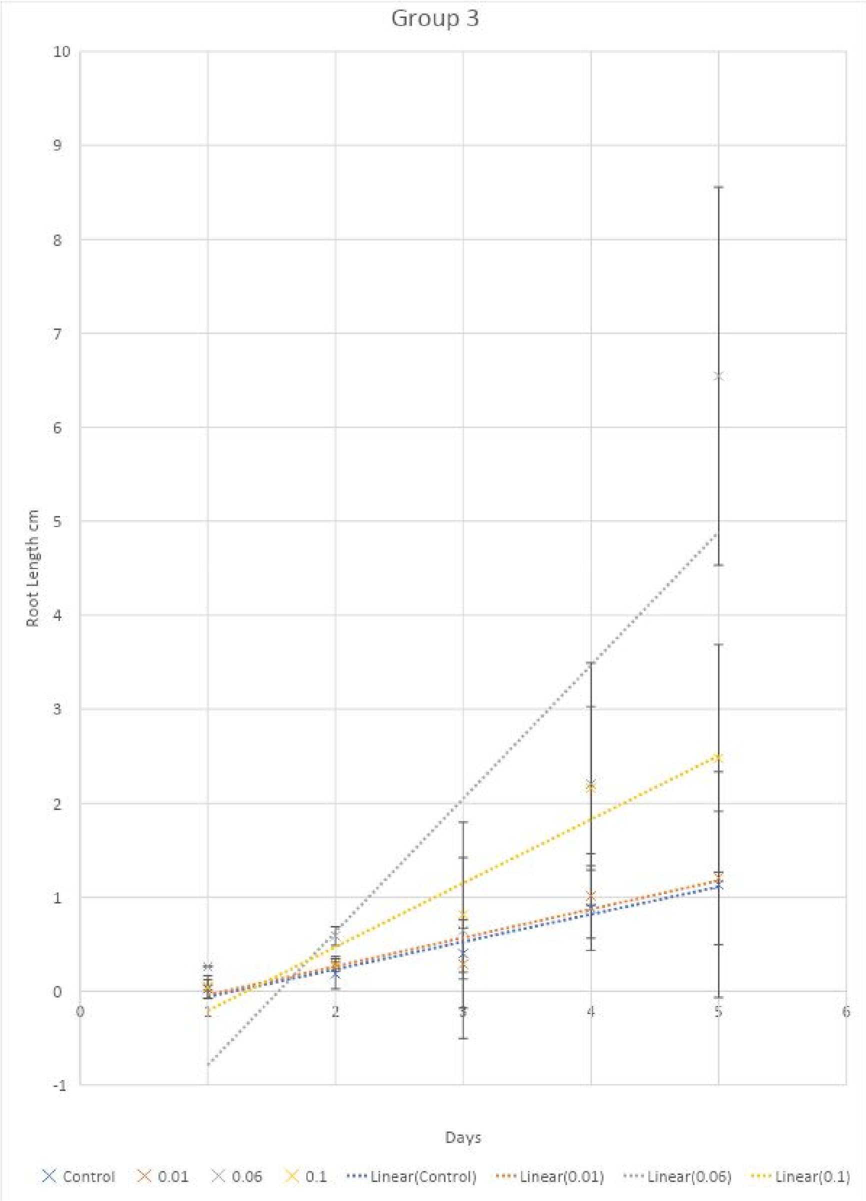
[25]

[25] 1.0 concentration is not included as it did not grow



[26]

26 0.6 and 1.0 concentration are not included, because they didn't grow



[27]

Appendix 8: Full Analysis of Variance

Anova: Two-Factor With Replication

SUMMARY	Day 1	Day 2	Day 3	Day 4	Day 5	Total
Control 1						
Count	5	5	5	5	5	25
Sum	0	0.4	1.58	3.45	7.72	13.15
Average	0	0.08	0.316	0.69	1.544	0.526
Variance	0	0.0122	0.04428	0.3194	1.44843	0.633825
Control 2						

[27] 0.6 and 1.0 concentration are not included, because they didn't grow

Count	5	5	5	5	5	25
Sum	0.33	0.83	1.44	3.84	6.74	13.18
Average	0.066	0.166	0.288	0.768	1.348	0.5272
Variance	0.00918	0.02463	0.07262	0.18197	0.38797	0.34857 9

Control 3

Count	5	5	5	5	5	25
Sum	0.11	0.56	1.46	4.42	5.67	12.22
Average	0.022	0.112	0.292	0.884	1.134	0.4888
Variance	0.00242	0.01732	0.06757	0.13783	0.51638	0.32589 4

0.01 1

Count	5	5	5	5	5	25
Sum	0.1	0.72	3.13	5.43	9.63	19.01
Average	0.02	0.144	0.626	1.086	1.926	0.7604
Variance	0.002	0.01868	0.46883	0.80078	2.59678	1.15010 4

0.01 2

Count	5	5	5	5	5	25
Sum	0.67	1.51	1.98	4.14	7.91	16.21
Average	0.134	0.302	0.396	0.828	1.582	0.6484
Variance	0.01543	0.01462	0.21203	0.19997	0.59557	0.45463 9

0.01 3

Count	5	5	5	5	5	25
Sum	0.23	1.45	1.44	5.06	6.02	14.2
Average	0.046	0.29	0.288	1.012	1.204	0.568
Variance	0.01058	0.07375	0.10277	0.19317	0.50283	0.36172 5

0.06 1

Count	5	5	5	5	5	25
Sum	1.05	2.52	7.43	5.55	8	24.55
Average	0.21	0.504	1.486	1.11	1.6	0.982
Variance	0.0317	0.12523	0.14943	0.955	2.7064	0.96895 8

0.06 2

Count	5	5	5	5	5	25
Sum	0	0.49	9.27	18.85	20.8	49.41
Average	0	0.098	1.854	3.77	4.16	1.9764
Variance	0	0.00952	1.31773	1.6629	3.06475	4.22469 9

0.06 3

Count	5	5	5	5	5	25
Sum	1.31	2.93	2.59	11.02	32.72	50.57
Average	0.262	0.586	0.518	2.204	6.544	2.0228
Variance	0.07667	0.27988	0.30862	0.52863	7.17083	7.207296

0.1 1

Count	5	5	5	5	5	25
Sum	0.14	1.07	8.21	6.19	12.07	27.68
Average	0.028	0.214	1.642	1.238	2.414	1.1072
Variance	0.00392	0.00298	0.14612	0.85072	1.77833	1.291454

0.1 2

Count	5	5	5	5	5	25
Sum	0.1	0.85	4.49	6.93	12.47	24.84
Average	0.02	0.17	0.898	1.386	2.494	0.9936
Variance	0.002	0.0023	0.36962	0.75753	1.46683	1.274824

0.1 3

Count	5	5	5	5	5	25
Sum	0.19	1.29	4.06	10.79	12.39	28.72
Average	0.038	0.258	0.812	2.158	2.478	1.1488
Variance	0.00722	0.00307	0.39297	0.14417	0.36577	1.178469

0.6 1

Count	5	5	5	5	5	25
Sum	0	0.22	0.45	0.67	0.89	2.23
Average	0	0.044	0.09	0.134	0.178	0.0892
Variance	0	0.00968	0.0405	0.08978	0.15842	0.053874

0.6 2

Count	5	5	5	5	5	25
Sum	0	0	0	0	0	0
Average	0	0	0	0	0	0
Variance	0	0	0	0	0	0

0.6 3

Count	5	5	5	5	5	25
Sum	0	0	0	0	0	0
Average	0	0	0	0	0	0
Variance	0	0	0	0	0	0

1.0 1

Count	5	5	5	5	5	25
Sum	0	0	0	0	0	0
Average	0	0	0	0	0	0
Variance	0	0	0	0	0	0
1.0 2						
Count	5	5	5	5	5	25
Sum	0	0	0	0	0	0
Average	0	0	0	0	0	0
Variance	0	0	0	0	0	0
1.0 3						
Count	5	5	5	5	5	25
Sum	0	0	0	0	0	0
Average	0	0	0	0	0	0
Variance	0	0	0	0	0	0
Total						
Count	90	90	90	90	90	
Sum	4.23	14.84	47.53	86.34	143.03	
Average	0.047	0.164889	0.528111	0.959333	1.589222	
Variance	0.012911	0.054969	0.501	1.239292	3.753039	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Group	170.7829	17	10.04605	26.56967	2.47E-53	1.65121
Columns	143.2194	4	35.80485	94.6962	5.9E-55	2.396743
Interaction	188.0479	68	2.765411	7.313922	1.53E-37	1.337092
Within	136.1168	360	0.378102			
Total	638.1671	449				