**I. EXPERIMENT OVERVIEW**

Initially, we used vinegar to answer the investigation question: To what extent do different concentrations of vinegar (%) affect the growth of lentil seeds over 9 days as measured by the length of the roots (mm)? Our hypothesis was that higher concentrations of vinegar have a negative correlation with the growth of lentil seeds over 9 days as measured by the length of the roots (mm). Below are the materials, procedure used to experiment, deviations from the procedure, and observations while the procedure was being executed:

*Materials*:

1. 200 mL tap (distilled) water - distributed 50 mL, 45 mL, 40 mL, 30 mL (35 mL extra to flush out pipette)
2. 35 mL white distilled vinegar - distributed 0 mL, 5 mL, 10 mL, 20 mL
3. 4 transparent plastic cups
4. 1 graduated cylinder, 100 mL capacity
5. 1 pipette
6. 4 resealable plastic bags
7. 4 pieces of paper towel
8. 40 lentil seeds
9. Millimeter ruler
10. A short piece of string

*Procedure*:

1. Gather all materials
2. Measure out needed amounts of water/vinegar with the graduated cylinder as per condition (see "experimental set up/how to prepare") and pour into the cup (repeat 4x for all conditions) - label cups with name, solution details
3. Use the pipette to soak a paper towel with the corresponding solution such that it is fully wet but not dripping
4. Put the paper towel back in a plastic bag so that it is flat and placed in the lower 1/3 of the bag
5. Carefully place 10 lentils on top of the paper towel, neatly spaced apart
6. Seal bag closed - Keep flat to allow for ease of measuring and observation - label bag with table, period, concentration, date
7. Repeat steps 3 to 6 three more times until bags are ready for all conditions - make sure to flush out the pipette with spare tap water (35 mL) after each repetition to prevent contamination
8. Pour out excess solution and clean up the workspace
9. Gather data (length of roots in mm) after ~~5~~ *9* days using the millimeter ruler and the short piece of string to measure curved roots

*Deviations/Observations during the procedure*:

1. Got 3 (instead of just 1) graduated cylinders, 100 mL capacity each, to measure & store 200 mL water and to flush out the pipette
2. Used distilled water as needed to flush out the pipette instead of just 35 mL
3. Used an unspecified amount of vinegar as needed from a cup instead of just 35 mL
4. Dipped a paper towel in the 0% solution instead of using the pipette since it was faster and was just water - ended up squeezing some of the water out so that the paper towel would be "fully wet but not dripping"
5. Poured 20 mL of the 40% solution from a graduated cylinder onto the paper towel instead of using the pipette since it would be much faster than using the pipette 8 times (we found that it was best to take 2.5 mL solution into the pipette in one time since it would be the most efficient)
6. Had to make the 10% solution twice because the paper towel was not saturated enough, and we had already dumped out the excess solution while cleaning up
7. Found that it was easy to mix up the different solutions because they were all clear - we should have labeled them first but ended up labeling after we started making solutions. However, the cup of vinegar was easy to distinguish from the rest of the solutions because of its strong odor.
8. Gathered data after 9 days instead of 5 days due to little growth in the class

After 9 days, there were only 6 data points, all in the control condition (0% vinegar: solely distilled water), with root lengths of 22, 31, 12, 23, 15, and 35 mm. In this way, the data was insufficient, although it became clear that the presence of vinegar in a lentil-growing solution inhibits lentil growth.

**II. EXPLANATION FOR NO GROWTH FOR LENTILS PLACED IN VINEGAR SOLUTIONS**

Exposure to vinegar has a negative correlation with the growth of lentils. Our lentils did not grow because the pH of the vinegar solution is far from the ideal pH for lentil growth (7+). [1,2] Our group exposed vinegar solutions of variable concentrations to the lentil seeds in our experiment. The white distilled vinegar used in our experiment has a pH of 2.5.[3] In this way, the pH levels that the lentils were exposed to are far too low for any growth, even though the vinegar was diluted with water. (As a sidenote, this dilution would not increase the pH by much since the pH scale is logarithmic, multiplying by powers of 10 for each unit. In that sense, a solution with a pH of 7.5 is 105 times more basic than a solution with a pH of 2.5.) Strongly acidic conditions such as those created by the presence of vinegar mainly inhibit growth through the direct toxicity of the H+ ion in these solutions, causing root growth of mung beans to become nearly completely inhibited.[4,5] Furthermore, it has been suggested that “excess H+ competes with other cations for root absorption sites, interfering with ion transport and uptake, and causes root membranes to become leaky,” thus leading to a change in root system architecture and consequently reducing a plants ability to absorb water and nutrients.[6,7]

**III. C****OFFEE EXPERIMENT**

After understanding this, we used coffee to answer a modified investigation question: To what extent do different concentrations of coffee (%) affect the growth of lentil seeds over 5 days as measured by the length of the roots (mm)? Sample data was used to answer this question instead of experimenting ourselves, thus, the investigation question specifies 5 days and not 9. **FIGURE 1** shows the processed data presented in the form of a box-and-whisker plot.

**IV. COFFEE DATA INTERPRETATION (CER)**

Based on the data gathered, exposure to coffee at higher

concentrations cause larger variations in root growth

while not significantly influencing the average, and

exposure to coffee at low concentrations has a negative correlation with lentil growth.

The former is shown by the fact that the standard deviation is much higher in 20% coffee than in 0% coffee, as indicated by the tick marks on the box-and-whisker plot. Meanwhile, averages for the control (0%) and 20% coffee solution were almost the same (26.9 and 27 mm, respectively), showing that higher concentrations of coffee do not have a negative correlation with root growth like the lower concentrations do, nor a positive correlation—the minimum and maximum values of root growth in the 20% concentration trial had a far higher deviation from the average than the minimum and maximum in the control.

Contrastingly, lentils grown in solutions of water with low coffee concentrations (1% and 5%) had a lower average root length than lentils grown in 100% distilled water (0% coffee), at 10.9 and 21.4 mm compared to 26.9 mm, respectively, which shows how lower concentrations have a negative correlation with root length. Furthermore, the minimum and maximum values for 1% and 5% coffee concentrations are strictly less than the average for the control condition, showing that this correlation is universal across all data points.

Upon further research, it is revealed that coffee contains 2% nitrogen, which is highly beneficial to fulfilling the nutrient requisites for plant growth through the process of nitrogen fixation.[8] Nitrogen is mainly involved in photosynthesis in plants as it is an essential component of chlorophyll, a green pigment essential for photosynthesis.[9] Without nitrogen, a plant cannot grow taller because nitrogen is a critical element of proteins, chlorophyll, vitamins, enzymes, and hormones.[10] Therefore, coffee boosts the growth of all plants, including lentils. Hence, it would be expected that the average root length would be significantly higher in the 20% solution than in the 0% solution, but this is not the case. Such an outcome could be possibly attributed either to (1) errors while conducting the experiment, (2) an environment that is not controlled enough, or (3) simply that the increasing trend starts at a higher concentration (e.g., 40%).

In addition, even though the data clearly suggests that exposure to coffee at low concentrations has a correlation with lentil growth, this would be unexpected because the nitrogen present in coffee should facilitate plant growth, not inhibit it. The results of our experiment now raise more questions such as why the 20% coffee concentration did not have a higher average root growth or the low coffee concentrations inhibited lentil growth.

**V. SOURCES & CITATIONS**

Note: each hyperlink directs to the webpage scrolled to the selected text.

[1] “Lentil reaches its maximum yield potential with soil pH greater than 7” ([Soil Fertility Recommendations for Field Pea, Lentil and Chickpea in North Dakota — Publications (ndsu.edu)](https://www.ag.ndsu.edu/publications/crops/soil-fertility-recommendations-for-field-pea-lentil-and-chickpea-in-north-dakota#:~:text=Lentil%20reaches%20its%20maximum%20yield%20potential%20with%20soil%20pH%20greater%20than%207.))

[2] “A soil pH near 7.0 is best for lentil production” ([Alternative Field Crops Manual | Lentil (purdue.edu)](https://www.hort.purdue.edu/newcrop/afcm/lentil.html#:~:text=A%20soil%20pH%20near%207.0%20is%20best%20for%20lentil%20production.))

[3] “Standard 5 percent distilled white vinegar with a pH of 2.5 is [. . .] acidic enough to kill bacteria, and it probably has the most neutral smell of all the kinds of vinegar” ([pH of Vinegar: What Makes It Acidic? Plus, Strength of Vinegar (greatist.com)](https://greatist.com/health/ph-of-vinegar#:~:text=Standard%205%20percent%20distilled%20white%20vinegar%20with%20a%20pH%20of%202.5%20is%20the%20best%20around%20for%20cleaning.%20It%E2%80%99s%20acidic%20enough%20to%20kill%20bacteria%2C%20and%20it%20probably%20has%20the%20most%20neutral%20smell%20of%20all%20the%20vinegars.))

Citations 4-7 are sourced from:

Petra S. Kidd, John Proctor, why plants grow poorly on very acid soils: are ecologists missing the obvious? *Journal of Experimental Botany*, Volume 52, Issue 357, 15 April 2001, Pages 791–799, <https://doi.org/10.1093/jexbot/52.357.791>

[4] “It has been demonstrated for races of the crops maize (*Zea mays* L.), mung bean (*Vigna radiata*L. Wilczek), and rice (*Oryza sativa* L.), that very acid soils are so toxic that their root growth is nearly completely inhibited ([Proctor, 1999](javascript:;))” ([Why plants grow poorly on very acid soils: are ecologists missing the obvious? | Journal of Experimental Botany | Oxford Academic (oup.com)](https://academic.oup.com/jxb/article/52/357/791/480277#:~:text=It%20has%20been%20demonstrated%20for%20races%20of%20the%20crops%20maize%20(Zea%20mays%20L.)%2C%20mung%20bean%20(Vigna%20radiata%20L.%20Wilczek)%2C%20and%20rice%20(Oryza%20sativa%20L.)%2C%20that%20very%20acid%20soils%20are%20so%20toxic%20that%20their%20root%20growth%20is%20nearly%20completely%20inhibited%20(Proctor%2C%201999).%20It%20appears%20that%20plants%20are%20faced%20firstly%20with%20toxic%20%5BH%2B%5D))

[5] “The direct toxicity of the H+ ion is the proximal cause of the poor growth of non‐tolerant plants on these very acid organic soils” ([Why plants grow poorly on very acid soils: are ecologists missing the obvious? | Journal of Experimental Botany | Oxford Academic (oup.com)](https://academic.oup.com/jxb/article/52/357/791/480277#:~:text=It%20was%20concluded%20that%20the%20direct%20toxicity%20of%20the%20H%2B%20ion%20is%20the%20proximal%20cause%20of%20the%20poor%20growth%20of%20non%E2%80%90tolerant%20plants%20on%20these%20very%20acid%20organic%20soils))

[6] “It has been suggested ([Foy, 1992](javascript:;)) that excess H+ competes with other cations for root absorption sites, interfering with ion transport and uptake, and causes root membranes to become leaky” ([Why plants grow poorly on very acid soils: are ecologists missing the obvious? | Journal of Experimental Botany | Oxford Academic (oup.com)](https://academic.oup.com/jxb/article/52/357/791/480277#:~:text=It%20has%20been%20suggested%20(Foy%2C%201992)%20that%20excess%20H%2B%20competes%20with%20other%20cations%20for%20root%20absorption%20sites%2C%20interfering%20with%20ion%20transport%20and%20uptake%2C%20and%20causes%20root%20membranes%20to%20become%20leaky.))

[7] “Low pH [. . .] led to a change in root system architecture which, in consequence, can reduce a plants ability to absorb water (and nutrients)” ([Why plants grow poorly on very acid soils: are ecologists missing the obvious? | Journal of Experimental Botany | Oxford Academic (oup.com)](https://academic.oup.com/jxb/article/52/357/791/480277#:~:text=Low%20pH%20not%20only%20affected%20RER%20but%20also%20led%20to%20a%20change%20in%20root%20system%20architecture%20which%2C%20in%20consequence%2C%20can%20reduce%20a%20plants%20ability%20to%20absorb%20water%20(and%20nutrients).))

[8] “Nitrogen fixation is the process whereby molecular N2 gas is converted to reactive, biologically available forms of nitrogen.” ([[Nitrogen Fixation - an overview | ScienceDirect Topics](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/nitrogen-fixation#:~:text=Nitrogen%20fixation%20is%20the%20process%20whereby%20molecular%20N2%20gas%20is%20converted%20to%20reactive%2C%20biologically%20available%20forms%20of%20nitrogen.)](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/nitrogen-fixation#:~:text=Nitrogen%20fixation%20is%20the%20process%20whereby%20molecular%20N2%20gas%20is%20converted%20to%20reactive%2C%20biologically%20available%20forms%20of%20nitrogen))

[9] “Plants need nitrogen because it helps them produce proteins that are needed for healthy growth and development like chlorophyll production, protein synthesis, seed development, fruiting/flowering” ([Why Do Plants Need Nitrogen to Get The Best Results (planteli.com)](https://planteli.com/why-do-plants-need-nitrogen/#:~:text=Plants%20need%20nitrogen%20because%20it%20helps%20them%20produce%20proteins%20that%20are%20needed%20for%20healthy%20growth%20and%20development%20like%20chlorophyll%20production%2C%20protein%20synthesis%2C%20seed%20development%2C%20fruiting/flowering.))

[10] “Nitrogen is part of the chlorophyll molecule, which gives plants their green color and is involved in creating food for the plant through photosynthesis” ([How does Nitrogen Help Plants Grow? - Phoslab Environmental Laboratories Phoslab Environmental Laboratories](https://www.phoslab.com/how-does-nitrogen-help-plants-grow/#:~:text=photosynthesis.%20Lack%20of-,nitrogen,-shows%20up%20as))