Kidney Bean Germination In 0.1%, 1%, 2%, and 5% Vinegar

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Abstract

As a result of pollutants in the air, acid rain is a worldwide environmental problem that has severe consequences in plants and germination in seeds (Manisalidis, et al. 2020). Emulating the “acid rain” effect at home, different concentrations of vinegar solutions will be used to observe the germination rate of kidney beans. Kidney beans are one of many species that may be impacted by increasing acidity levels. In this study, we investigate whether incremental increases in vinegar such as 0.1%, 1%, 2% and 5% would affect the time it takes for kidney beans to germinate. The kidney beans were pressed against a damp paper towel soaked with 4 different vinegar dilutions – 0.1%, 1%, 2%, and 5% over the course of 14 days. After 24 hours of overall incubation, 20 samples were observed daily. It was discovered that there was a significant difference between the 0.1% treatment group and the 1%, 2% and 5% treatment groups. Our results indicate that the lower vinegar concentrations are an effective environment for kidney bean germination, while higher vinegar concentration conditions are not.

Introduction

The rapid development of technology and societal changes throughout the world result in undesirable consequences in environments such as pollution. The pollution caused by burning fossil fuels, greenhouse effects, carbon dioxide generation, etc. has impacted soil, water and air in different magnitudes. (Manisalidis, et al. 2020) These anthropogenic activities produce nitric acids, sulfuric acids and other harmful substances. The acids later combine with water and water vapour in the atmosphere and precipitate in the form of rain. Due to acid rain, the pH levels of soil decrease. The change in the soil pH influences microbial growth in soil, nutrient availability, as well as plant metabolism. Thus, plant productivity and seed germination are susceptible to these impacts. (Lal 2016). Lal (2016)

also stated that seed germination of three test species did not take place at the pH level of 2.0. However, the germination occurred at pH levels between 3.5 and 5.0.

The objectives of this study were to simulate and to examine the effects of acid on bean germination at home. Different concentrations of vinegar (0.1%, 1%, 2% and 5%) were prepared to observe the germination of kidney beans and how long it takes to germinate at each treatment. The number of germinated kidney beans in each day was collected for two weeks. According to Gonzales (2015), there was no seed germination at 10% vinegar solution, as a high concentration of acetic acid in vinegar had a detrimental effect on eggplant seeds. Thus, it was hypothesized that as the acidity of treatments increases, the rate at which kidney beans germinate will decrease. The more and faster kidney bean germination would be observed from 0.1% acetic acid solution after 2 weeks than other treatments (1%, 2% and 5%).

Methods

1. Bean Materials

Kidney beans were purchased from local stores. Beans in uniform size were selected for this experiment.

2. Experimental Treatment and Design

Vinegar, containing approximately 5% of acetic acid, was used in this study with the following concentration as a treatment:

T1	Control (tap water)
T2	0.1% vinegar
T3	1% vinegar
T4	2% vinegar

T5	5% vinegar

Table 1. Treatments with different concentration of acetic acid in vinegar

3. Vinegar Solution Preparation

Five clean, empty cups were prepared and labelled as control or with its corresponding vinegar concentration. As a control, the first cup, T1, was filled with 50 mL of tap water. After that, 49 mL of tap water was added to the second cup, T2, with 1 mL of vinegar. Likewise, 40 mL of tap water was transferred to the third cup, T3, with 10 mL of vinegar. 30 mL of tap water and 20 mL of vinegar were added to the fourth cup, T4. Lastly, 50 mL of vinegar was only transferred to the fifth cup, T5. A piece of paper towel was soaked in T1 and placed in a clean, empty Ziplock after gently wring it. Five dry kidney beans were placed and evenly distributed on the wet paper towel. This process was repeated four more times for the remaining vinegar treatments (0.1%, 1%, 2% and 5%). Finally, the five Ziplock bags with kidney beans were put aside in a cool and dry location for two weeks. During this period, the number of kidney beans germinated and any other changes on beans were recorded on a daily basis.

4. Measurement of Germination

Kidney beans were considered germinated when there was a visible sprout coming out from the sample.

5. Data Gathered

Each group member conducted this experiment independently for two weeks which gave a total sample number of 20 for each treatment group.

6. Data Analysis

Once all data was collected after two weeks, a one-way ANOVA test and the Tukey-Kramer post-hoc test were performed on the data.

Results

The number of germinated kidney beans was collected for one control group and four different treatment groups: 0.1% vinegar solution, 1% vinegar solution, 2% vinegar solution, and 5% vinegar solution. An alpha value of 0.05 was used for all statistical tests.

The Shapiro-Wilk test was performed for normality (p-value = 1.26e-05) and Levene's test was performed for homogeneity of variances (p-value = 0.52). The results were found to be non-normal but had equal variances. Although the normality assumption for ANOVA tests was violated, the ANOVA test is robust enough to handle this violation with only a small effect on the Type I error rate. Thus, a one-way ANOVA test was performed on the data.

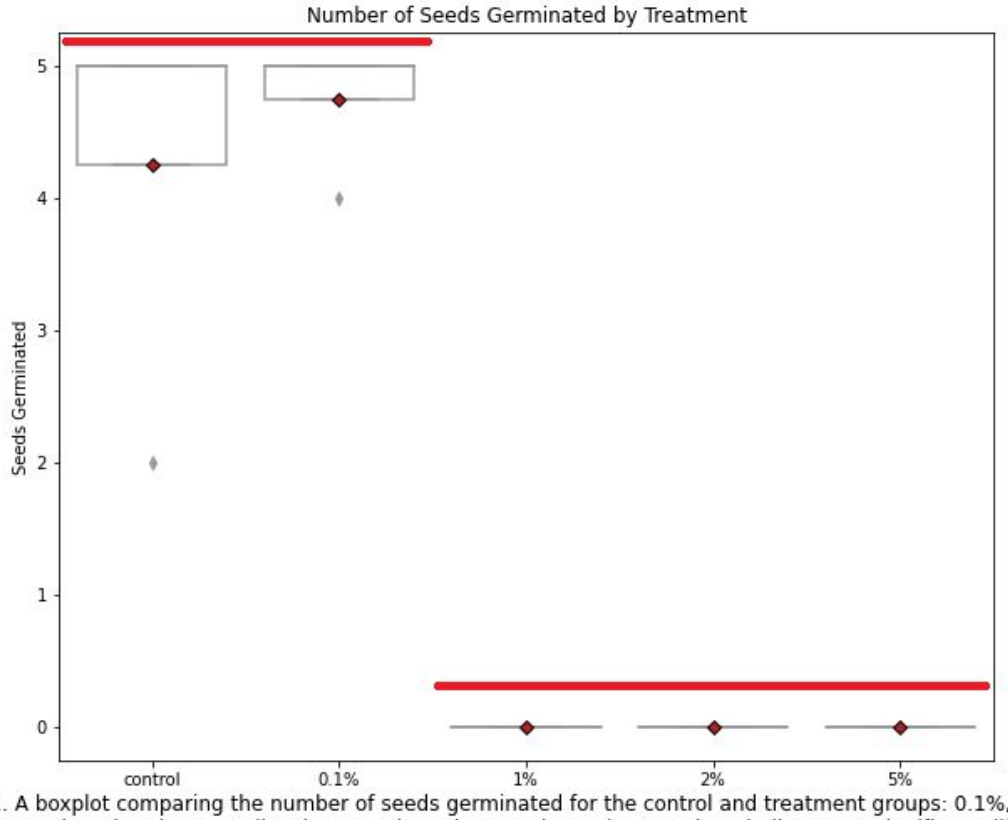

Figure 1. A boxplot comparing the number of seeds germinated for the control and treatment groups: 0.1%, 1%, 2%, 5%. Box = 25th and 75th percentiles; bars = min and max values. Line over bars indicates no significant differences.

The average number of kidney beans germinated across all treatment groups was measured to be 1.8 (SD = 2.35); the 0.1% treatment had an average number of germinated seeds of 4.75 (SD = 0.50), and each of the 1%, 2%, and 5% treatments had an average

number of germinated seeds of 0 (SD = 0). The control group had an average number of germinated seeds of 4.25 (SD = 1.50). The ANOVA test reports there is a statistical difference between the four means and the Tukey-Kramer post-hoc test was performed (p = 1.99e-08, F(4, 15) = 48.85).

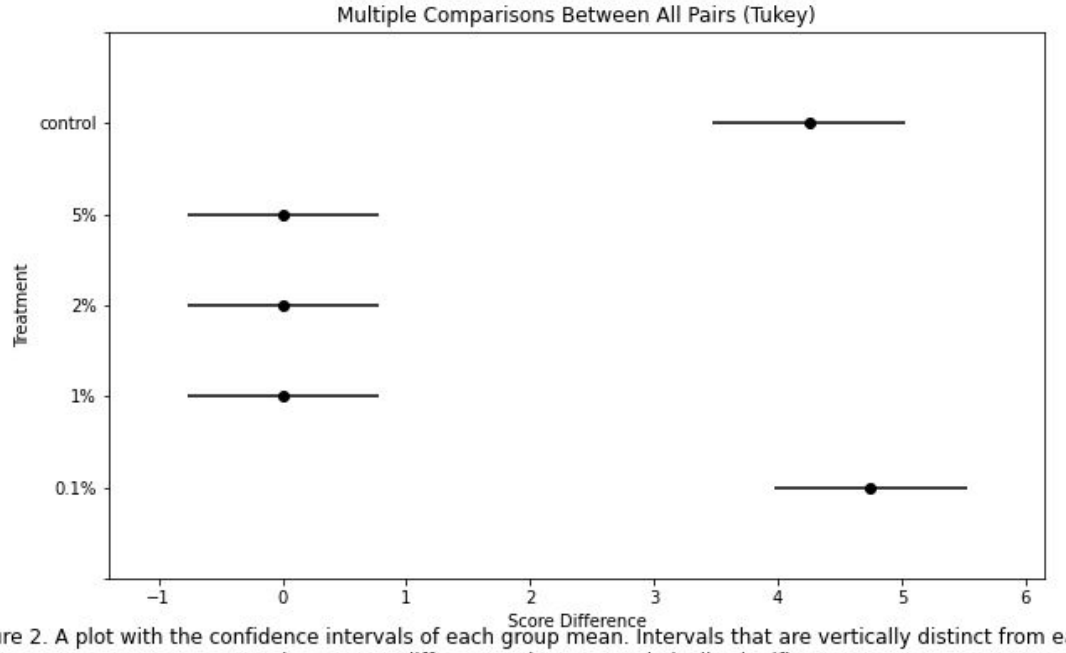

Figure 2. A plot with the confidence intervals of each group mean. Intervals that are vertically distinct from each other have mean differences that are statistically significant.

From Figure 2, there is a statistically significant difference in the number of germinated kidney beans between the 0.1% treatment and the 1%, 2%, and 5% treatments and between the control group and the 1%, 2%, and 5% treatments. There was no significant difference between the 1%, 2%, and 5% treatment groups and between the control and 0.1% treatment group.

Discussion

The Turkey-Kramer and the ANOVA test were used to determine the significance of the results. In this study we based the hypothesis on a study done by Gonzales (2015). The increase in acidity or concentration of acetic acid would have a negative effect on the rate at which the kidney beans germinated. Thus, we would observe more and faster kidney beans germination when wrapped within the paper towel wetted with the lowest acetic acid

concentration (0.1%). The p-value (1.99e-8) calculated from the ANOVA test was less than 0.05, implying that the null hypothesis is to be rejected and there is a statistical difference between the means of the four treatment groups. The Turkey-Kramer post-hoc test was used afterwards and showed that there was a statistical difference only between the 0.1% treatment group and the other groups. Through both statistical tests and observations, using lower concentrations of vinegar solution was better at germinating kidney beans than higher concentrations.

Indole acetic acid (IAA) is an auxin that is found in various plant species and is one of plant hormones required for plant development including seed germination (Zhao, 2010). In this study, vinegar was used as a source of acetic acid due to its availability. It was found that 1% or higher vinegar solution had an inhibitory effect on bean germination. An inhibition feedback loop could have resulted from the exposure to higher vinegar solutions, and the endogenous pathway for germination is affected. This is reflected in Zhao and Zhong's 2013 study, where the exogenous IAA at 10^{-4} M resulted in lower IAA concentrations but higher gibberellin (GA), and a high germination rate was observed.

It is noted that through observations, all of the beans subjected to the treatments and control solutions were wrinkly after two weeks of being doused in vinegar-soaked paper towels. However, on the sixth and eighth day of the study mold was growing on the beans and paper towels soaked in control (tap water) and 0.1% solution respectively. Whereas no mold was observed on the beans or paper towels soaked in higher concentrations. Our observations agree with studies from Gonzales (2015) and Othmen et al (2018), where it is possible that vinegar can be used as an alternative germinating solution while having microbial-inhibiting properties. In this and in Gonzales' (2015) study, the optimal vinegar concentration that produces a decently high germination rate that also inhibits microbial growth is quite low. However, our study shows that, at most, the optimum vinegar solution is 0.1%.

In our study, the lowest vinegar concentration tested was 0.1% (approximately 0.0166 M). Due to certain circumstances, we were unable to use accurate measuring instruments to produce the treatment solutions, therefore errors in measurements could have occurred. The errors in turn can also change the statistical significance of our results and the study must be repeated with highly accurate instruments in the future. It was found that 0.1% vinegar solution had the highest germination rate, and an inhibitory effect with others. Our study was also not able to explain the relationship between IAA and other plant growth hormones or the inhibition mechanism that potentially occurred. To understand the relationship, molecular biological techniques must be performed in future studies.

Conclusion

This study aimed to determine which vinegar concentration would germinate kidney beans the quickest and the germination rate for each group was measured using the number of beans germinated. It was hypothesized and found that 0.1% vinegar solution had the highest germination rate out of all the treatment groups and was statistically different. This study was able to confirm some of Gonzales' (2015) and Othmen's (2018) work. However, limitations of the study include no molecular biological tools used to understand the relationship between acetic acid and other plant hormones or specifics about its inhibition mechanism. Measurement errors were made as there was no opportunity to use highly accurate instruments and is to be included as a limitation.

Acknowledgements

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Citations and Literature Cited

Gonzalez, L. M. R. Germination Response of Eggplant (Solanum Melongena L.) Seeds to Different Vinegar Concentration as Seed Priming Agents. Int. J. Sci. Res. Pub. 5, 1–4 (2015).

Lal, N. “Effects of Acid Rain on Plant Growth and Development”. *Researchgate*, vol. 11, pp. 85-101, 2016, https://www.researchgate.net/publication/310954525_Effects_of_Acid_Rain_on_Plant_Growth_and_Development. Accessed 25 November 2020.

Manisalidis, Ioannis et al. “Environmental and Health Impacts of Air Pollution: A Review.” Frontiers in public health vol. 8 14. 20 Feb. 2020, doi:10.3389/fpubh.2020.00014

Zhao, Guangwu, and Tailin Zhong. "Influence of Exogenous IAA and GA on Seed Germination, Vigor and their Endogenous Levels in Cunninghamia Lanceolata." *Scandinavian Journal of Forest Research*, vol. 28, no. 6, 2013, pp. 511-517.

Zhao, Yunde. "Auxin Biosynthesis and its Role in Plant Development." Annual Review of Plant Biology, vol. 61, no. 1, 2010, pp. 49-64