Optimal Detergent Concentration in Greywater for Plant Growth using a Mung Bean Model

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Abstract

Greywater is lightly used non-toilet water (e.g. bathwater) that can be reused to irrigate plants. The reuse of greywater is receiving increasing global attention as many cities are trying to conserve freshwater resources amid changing climates and extended dry seasons. Here, we aim to understand how greywater affects plant growth using a mung bean model. We hypothesize that mung bean sprout length will be affected by greywater irrigation. We grew mung beans using different solutions of greywater (1% , 0.1%, 0.01%, 0.001%, 0.0001%) and recorded the sprout length after five days. We conducted a one-way ANOVA analysis and a post-hoc Tukey's test for our data analysis. The data analysis demonstrates that the mean sprout length was significantly different between the 1% and 0.0001% treatments, as well as between the 1% and 0.001% treatment groups, with a p-value of 0.00186. We conclude that greywater affects plant growth, where 0.0001% and 0.001% greywater solutions yielded the longest bean sprouts, whereas 1% yielded the shortest bean sprouts. This difference in plant growth can be attributed to surfactant concentrations, where low concentrations nourish plants but high concentrations of surfactant inhibit plant growth.

Introduction

Freshwater is a globally scarce resource. Water reservoirs and other freshwater sources are susceptible to water shortages, especially during dry seasons (Pfister et al., 2020). This phenomenon is exacerbated by global climate change, which elongates and intensifies dry seasons and subsequently threatens the stability of local watersheds and reservoirs (Pinto et al., 2009). In response, municipalities have implemented restrictions on using potable water for irrigation (Water Restrictions Explained, n.d.). Although this strategy is effective, it is often inconvenient for homeowners. Many countries promote greywater reuse to preserve freshwater resources and to reduce water restrictions (Travis et al., 2010).

Greywater is gently used non-toilet wastewater from sinks, showers, bathtubs and washing machines from households (Pinto et al., 2009). In addition to preserving freshwater, irrigating plants with greywater also alleviates the stress on municipal wastewater treatment plants as it reduces the run-off of detergents and surfactants into local waterways. Additionally, research has shown that greywater can act as a fertilizer to increase agricultural yield, as plants irrigated with greywater yield higher biomass than those irrigated with tap water (Misra et al., 2010). The improved plant growth could be attributed to the various nutrients in greywater that promote plant growth, such as phosphate salts, minerals, surfactants and organic matter (Misra et al., 2010). However, it has also been shown that an overly high concentration of surfactant in greywater can inhibit plant growth (Misra et al., 2010).

In our research, we aim to evaluate the effect of greywater on plant growth using a mung bean model to determine the optimal detergent concentration in greywater. We chose a mung bean model to quantify greywater's effect on plant biomass because mung beans have fast germination and growth rates, which is ideal given the short timeframe provided to carry out the experiment. We created serial dilutions of dishwasher detergent as a model for greywater, although real greywater solutions often contain other organic components as well.

Our null hypothesis is that greywater will not have an effect on mung bean sprout length. Our alternative hypothesis is that mung beans treated with greywater will have longer sprout lengths than ones treated with tap water because the detergent in greywater provides nutrients to the growing sprouts. We predict that 0.001% is the optimal greywater concentration for promoting mung bean growth, and that mung beans irrigated with 0.001% greywater will have the highest mean sprout length compared to the other greywater concentrations (1%, 0.1%, 0.01%, 0.001%, 0.0001%, and 0%). Our prediction is based on Misra et al.'s findings that tomato plants irrigated with 15.5 mg/L (0.00155%) concentration of surfactant yielded the highest plant biomass among treatments with different concentrations of greywater.

Methods

Mung Bean Treatments

Within our research team, a total of four trials were conducted. For each trial, we soaked 30 mung beans in room temperature water for around 12 hours. Using serial dilution methods, we prepared 6, 1 L solutions of Dawn Original Dish Dishwashing Liquid diluted in tap water (hereinafter referred to as greywater) at 1%, 0.1%, 0.01%, 0.001%, 0.0001%, and 0% (control treatment of tap water) detergent by volume using measuring cups and spoons. The calculations for the serial dilutions for each of the greywater solutions are outlined in Appendix 1. After 12 hours, we moistened six pieces of kitchen paper towels with each of the greywater solutions. We then placed five mung beans atop each of the paper towels, folded the paper towels to cover the beans, and placed each of the folded paper towels in a clear cup labelled with its corresponding greywater solution concentration. We placed the cups in a warm area with moderate amounts of sunlight and let the beans grow for 5 days (Figure 1). During this time, we added 1 tbsp of the respective greywater solution every 8 hours.

Figure 1: *A picture of our experimental setup for 5 treatments and a control. Each clear cup contains 5 mung beans wrapped in a damp paper towel.*

Data collection and analysis

Once the experiment was complete, we measured each mung bean’s length and compiled the results from the four trials. The 0.01% detergent treatment for one of our trials was excluded from the analysis as the sprouts were damaged by mold growth. Since the bean sprouts were not straight, we used a string to trace the shape of the bean sprout and measured the string to obtain an accurate length of each mung bean.

We then analysed the compiled data using R (version 4.0.2) and Jupyterlab. A table of the compiled raw data is in Appendix 2. The analysis consisted of a square root transformation, a one-way ANOVA, and a post-hoc Tukey Test. First, we created a histogram to view the distribution of our data. Since our original data was left-skewed (Appendix 3), we used a square root transformation to correct the data and fulfil the ANOVA normality assumptions (Appendix 4). After meeting the required assumptions for ANOVA, we ran a one-way ANOVA on the transformed data (Appendix 5). Consequently, we conducted a post-hoc Tukey test to see which groups were different from each other (Appendix 6). Finally, we created a Tukey test plot (Appendix 7) and a box plot of the square root transformed data to display the results as shown in Figure 2.

Results

The 0.001% treatment yielded the longest sprouts with a mean sprout length of 6.32 cm, whereas the 1% treatment yielded the shortest sprouts at 1.71 cm (Figure 2). Broadly, our results resemble a bell-curve distribution, although the mean for the 0.01% greywater treatment appears to be lower.

The ranges of values for the different trials are represented by the 95% interval whiskers (Figure 2). Based on those we can see that the 0.001% treatment has the highest variability of data (longest whiskers) while 0.01% has the least variable data. A one-way ANOVA analysis returned a p-value of 0.00186, which is less than our alpha value of 0.05. We chose an alpha level of 0.05 as it is the most commonly accepted level in the scientific community. The p-value indicates that the differences in mung bean sprout lengths grown in different greywater concentrations were statistically significant. Since the results were significant, a post-hoc Tukey test was conducted and revealed 2 pairs of treatments with statistically different means (1% and 0.0001%, 1% and 0.001%).

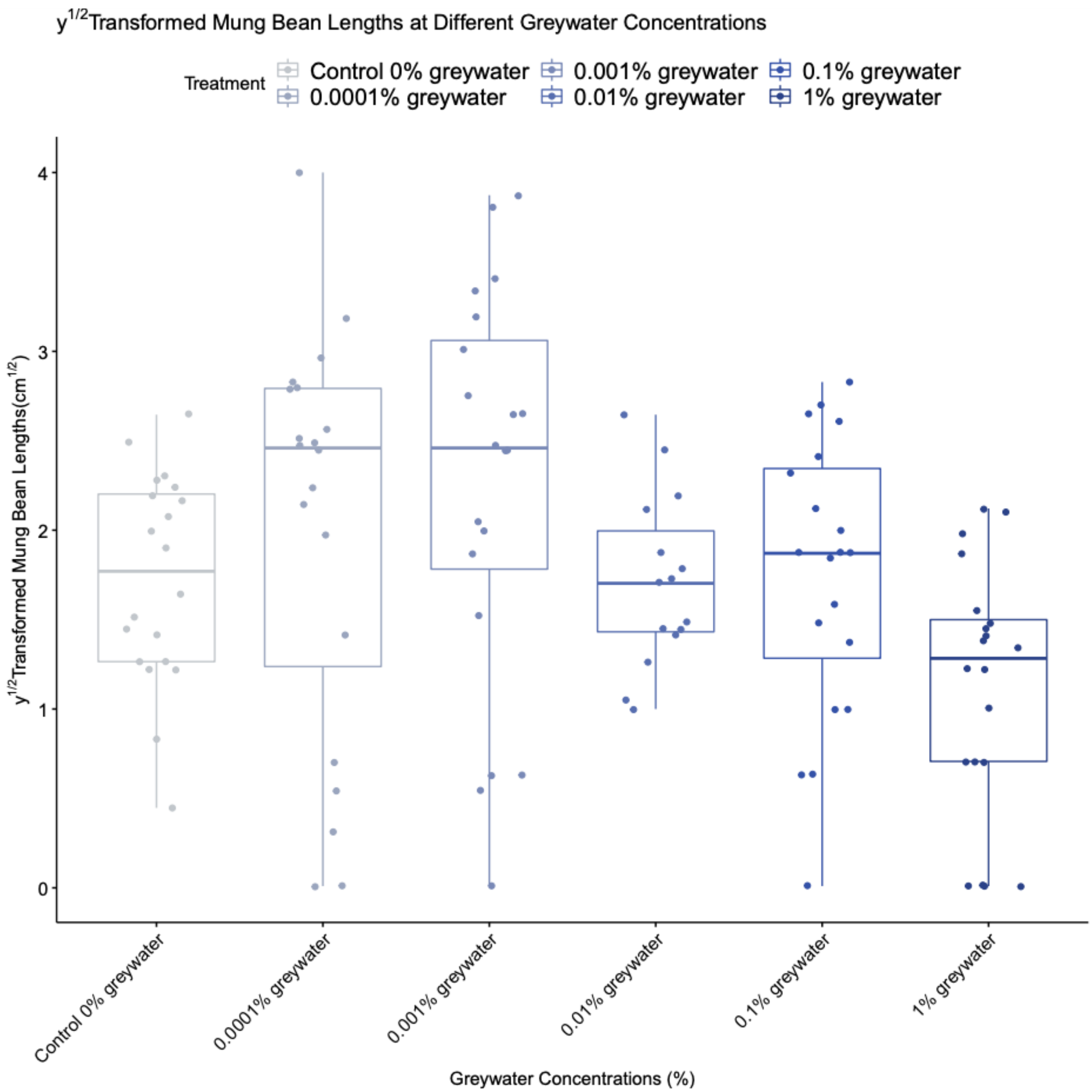

Figure 2: *A box plot showing the square root ($y^{1/2}$) transformed mean mung bean lengths at different greywater concentrations. The dots represent individual data points, the boxes show the interquartile range, and the whiskers indicate the 95% interval. Sample sizes for all treatments were 20, except 0.01% greywater where n=15. A one-way ANOVA produced a p-value of 0.00186, indicating there are significant differences among the greywater concentrations.*

Discussion

We conducted this experiment to determine if greywater has an effect on sprout growth and if sprout growth is affected differently when irrigated with different concentrations of greywater using a mung bean model. Our results show that the 0.001% greywater solution yielded the highest growth, while the 1% greywater solution yielded the lowest growth. Our ANOVA analysis shows that there is a significant difference in plant growth among treatment groups, and our Tukey's test further establishes that sprout growth between the 1% treatment and the 0.001% treatment are statistically different, as are the 1% treatment and the 0.0001% treatment. Therefore, we reject the null hypothesis that greywater does not have an effect on mung bean sprout length and instead support our alternative hypothesis that greywater does contribute to mung bean sprout growth. The data from the 0.01% treatment does not seem to follow the overall bell curve trend. However, this discrepancy can be attributed to the fact that one of our trials for the 0.01% detergent treatment was excluded from the analysis as the sprouts were damaged by mold growth.

Moreover, our results support the prediction that the 0.001% greywater will yield the highest level of sprouting activity. Our results demonstrate that 0.0001% greywater also promotes sprout growth since both treatments yielded sprout lengths that are significantly higher than those treated with 1% greywater. Results from both Misra et al. (2010) and our experiment can be explained by research that shows that surfactants can promote water uptake by improving hydraulic conductivity. Similarly, results from Rodda et al. (2011) demonstrated that swiss chard and carrot crops had higher growth and yield when irrigated with greywater compared to tap water. This is also reflected in Shankhwar & Srivastava's (2014) research, which demonstrated higher biomass for Eucalyptus hybrid plants irrigated with greywater. On the other hand, mung beans grown in 1% greywater have significantly lower mean sprout lengths than those grown in 0.001% and 0.0001% greywater. This observation is congruent with the findings of Misra et al. (2010), whose research showed that plants irrigated with a high concentration of detergent yielded lower biomass than those irrigated with tap water or greywater with lower concentrations of detergent. Further, a high concentration of surfactants is toxic to plants as they can disrupt membrane permeability and subsequently lead to dehydration and degradation of plant tissues (Garland et al., 2004).

Many sources of variation may have contributed to the discrepancies in mung bean sprout length among each trial conducted by our four researchers. The volume of water used as well as the time elapsed between watering the sprouts differed between experimenters since our methods dictated watering only when the paper towel was becoming dry. Also, the size of paper towels used to wrap the mung beans and the orientation, looseness or tightness of the wrapped towel may have allowed or restricted the bean's ability to grow sprouts. This could have contributed to variation among trials or even within trials. Another source of error is the uncertainty associated with the volume measurements for our serial dilutions, as we used 10 mL measuring cups and did not have graduated cylinders to measure 990 mL of a solution. Moreover, room temperature and humidity differed among the trials. The ambient temperature of our experimental setups ranged from approximately 18°C to 26°C (Hanif et al., 2019). The temperature of the water used to soak and germinate the beans also varied among trials. In one trial, mung beans were soaked in 15°C water, whereas in the other three trials, mung beans were soaked in 25°C water. According to Hanif et al. (2019), the optimal temperature for mung bean sprouting is between 30°C, so the lower temperature could have stunted the bean sprout growth. This trend was also seen in our data as the trial performed in the lowest room temperature (18°C) yielded the lowest overall growth, and the trial performed in the highest room temperature (26°C) yielded the highest overall growth. Also, light intensity varied among our trials, as different researchers used cups of varying opacity and exposed the mung beans to different amounts of sunlight. Research has demonstrated that sprouts grown in darkness had a higher germination rate than those grown in sunlight (Jamhari, 2018).

For future experimentation, we would limit uncertainties by conducting trials in the same environment and using mung beans from the same source, as well as performing measurements for the serial dilutions using a graduated cylinder rather than a measuring cup for more precision. We would also measure the biomass or volume of the sprouts rather than their lengths because sprouts vary in thickness and there were secondary roots emerging from some of the sprouts which were not accounted for in this study. To further expand on our findings, we could also conduct a toxicology assay to investigate the effect of individual ingredients in detergent on mung bean sprout growth.

Conclusion

Our experiment reveals that greywater has an effect on plant growth. Irrigation with 0.001% and 0.0001% greywater yield the highest mung bean growth whereas 1% greywater yields the lowest growth out of the six treatments. Thus, if the concentration of greywater is monitored, the reuse of greywater can simultaneously alleviate freshwater scarcity and promote plant growth to increase agricultural yield.

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Appendix

Greywater Concentrations	Volumes of Solutions	Calculations
1% solution	10mL soap + 990mL water	$c_1v_1 = c_2v_2$ $1\% * 1000\,mL = 100\% * v_2$ $v_2 = 10\,mL$
0.1% solution	10mL of the 1% solution + 990mL water	$c_1v_1 = c_2v_2$ $0.1\% * 1000\,mL = 1\% * v_2$ $v_2 = 10\,mL$
0.01% solution	10mL of the 0.1% solution + 990mL water	$c_1v_1 = c_2v_2$ $0.01\% * 1000\,mL = 0.1\% * v_2$ $v_2 = 10\,mL$
0.001% solution	10mL of the 0.01% solution + 990mL water	$c_1v_1 = c_2v_2$ $0.001\% * 1000\,mL = 0.01\% * v_2$ $v_2 = 10\,mL$
0.0001% solution	10mL of the 0.001% solution + 990mL water	$c_1v_1 = c_2v_2$ $0.0001\% * 1000\,mL = 0.001\% *$ $v_2 = 10\,mL$
0% solution	1000mL water	n/a

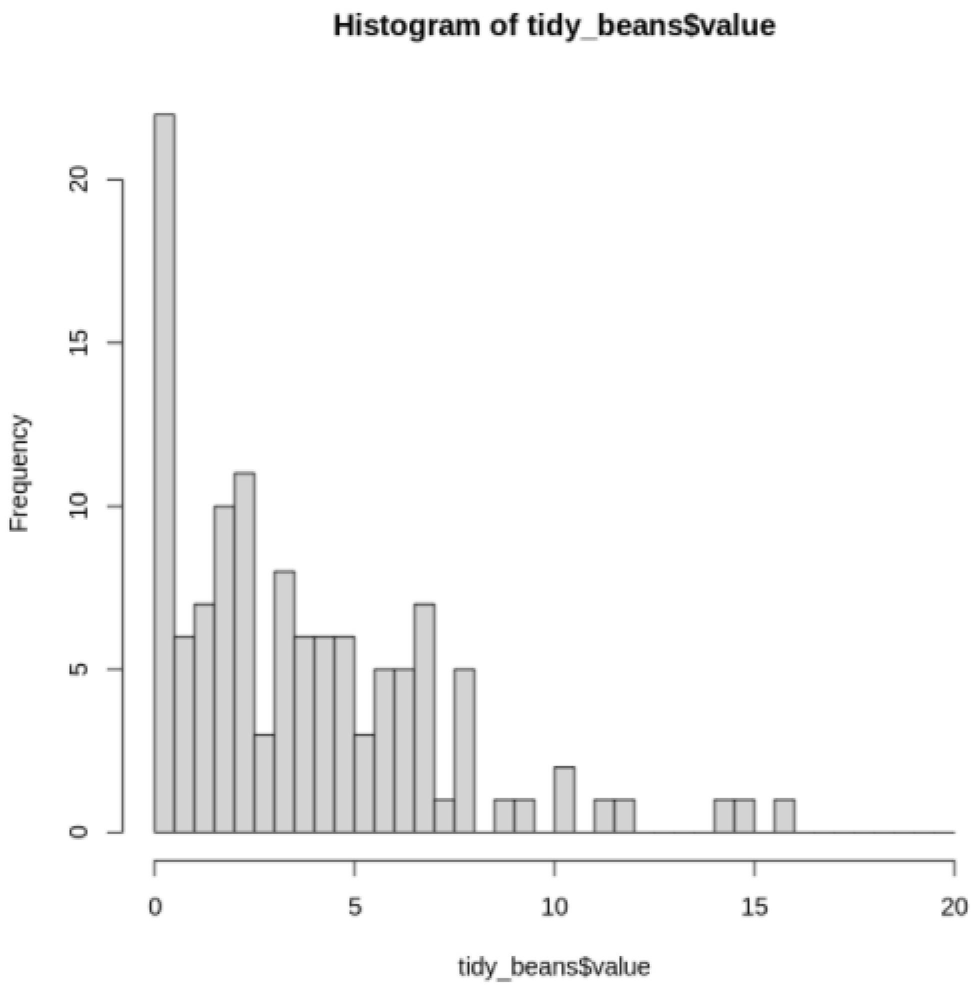
***Appendix 1**: Serial dilution volumes and calculations for our greywater solutions.*

Treatment		Trial # 1	Trial # 2	Trial # 3	Trial # 4
100% water (control)	Bean 1	4.8 cm	0.7 cm	1.4 cm	2.3 cm
	Bean 2	4.3 cm	2 cm	1.5 cm	1.6 cm
	Bean 3	3.6 cm	6.2 cm	4.9 cm	2.1 cm

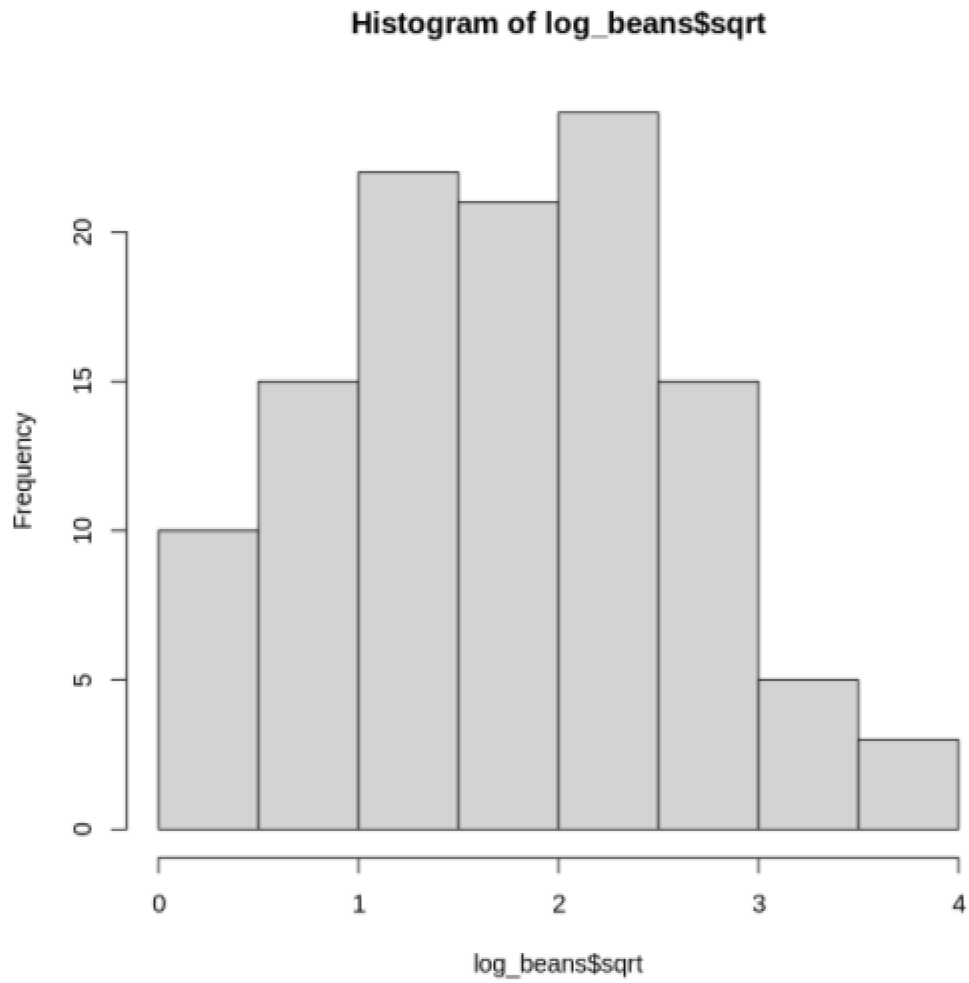
	Bean 4	2.7 cm	5.3 cm	7.0 cm	1.6 cm
	Bean 5	4.7 cm	5.2 cm	4.1 cm	0.2 cm
1% greywater	Bean 1	2.1 cm	4.4 cm	1.4 cm	0 cm
	Bean 2	1.9 cm	1.8 cm	1.5 cm	0 cm
	Bean 3	2.4 cm	4.5 cm	1.1 cm	0 cm
	Bean 4	2.2 cm	3.9 cm	0.5 cm	0.5 cm
	Bean 5	2.0 cm	3.5 cm	0.6 cm	0 cm
0.1% greywater	Bean 1	4.5 cm	7.0 cm	4.0 cm	0.4 cm
	Bean 2	6.8 cm	2.5 cm	3.5 cm	0 cm
	Bean 3	5.8 cm	3.4 cm	3.5 cm	1 cm
	Bean 4	5.4 cm	1.9 cm	1.1 cm	2.2 cm
	Bean 5	7.3 cm	8.0 cm	3.5 cm	0.4 cm
0.01% greywater	Bean 1	1.6 cm	2.1 cm	7.0 cm	0.9 cm
	Bean 2	2.9 cm	4.5 cm	3.0 cm	1.1 cm
	Bean 3	4.8 cm	1.1 cm	1.9 cm	0 cm
	Bean 4	3.2 cm	2.1 cm	1.0 cm	1.1 cm
	Bean 5	3.5 cm	2.2 cm	6.1 cm	0 cm
0.001% greywater	Bean 1	11.1 cm	7.6 cm	15.0 cm	2.3 cm
	Bean 2	11.6 cm	4.2 cm	4.0 cm	0.3 cm
	Bean 3	10.2 cm	3.5 cm	7.1 cm	0.4 cm
	Bean 4	14.5 cm	6.0 cm	7.0 cm	0 cm
	Bean 5	9.1 cm	6.1 cm	6.3 cm	0.4 cm
0.0001% greywater	Bean 1	7.8 cm	6.1 cm	8.2 cm	0.1 cm
	Bean 2	7.8 cm	6.2 cm	16.2 cm	0.5 cm
	Bean 3	8.8 cm	3.9 cm	6.7 cm	0.3 cm

	Bean 4	10.1 cm	6.3 cm	2.5 cm	0 cm
	Bean 5	6.6 cm	4.6 cm	5.1 cm	0 cm

Appendix 2: *Raw data collected from four trials.*



Appendix 3: *Histogram of mung bean sprouts length before any transformation.*



Appendix 4: *Histogram of mung bean length after a square root transformation.*

```
            Df Sum Sq Mean Sq F value  Pr(>F)
Treatment     5  14.86   2.972    4.11 0.00186 **
Residuals   109  78.81   0.723
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

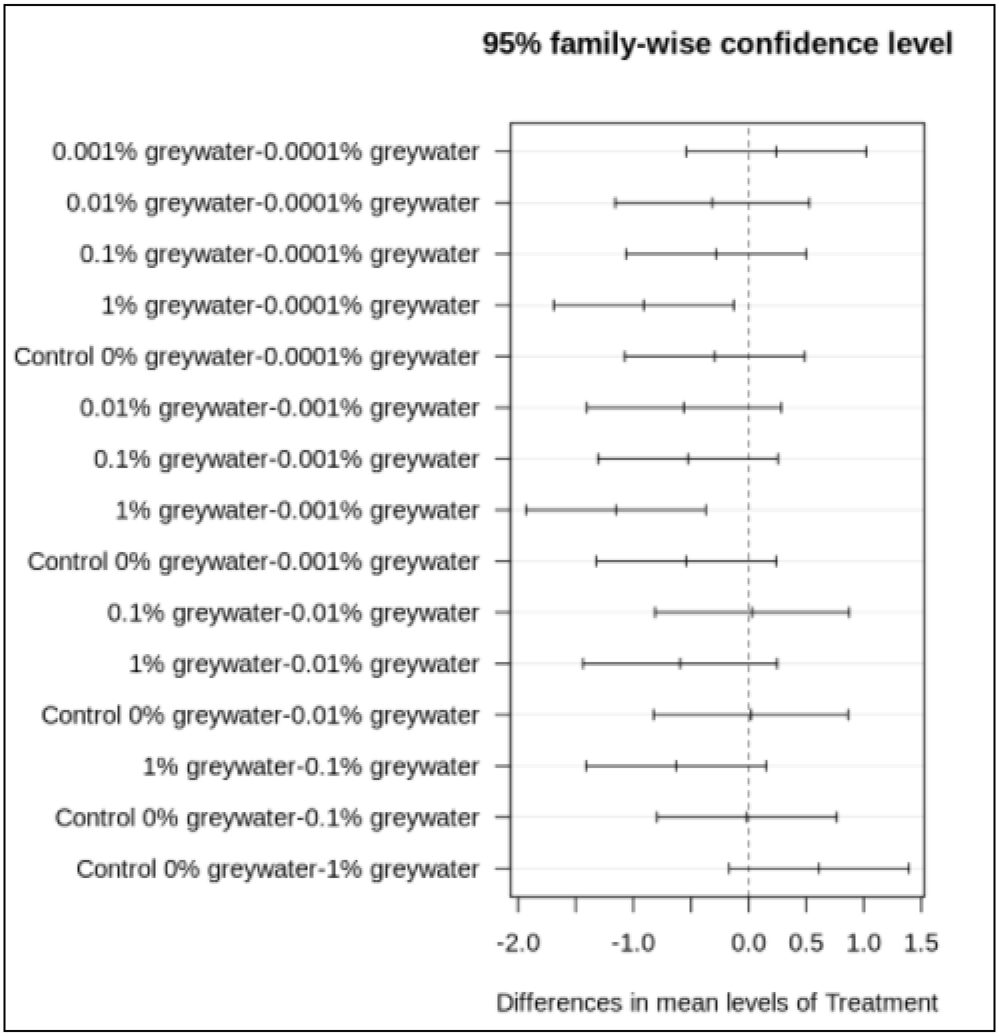
Appendix 5: *ANOVA results table*

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = sqrt ~ Treatment, data = aov_tidy_beans)

$Treatment
                                           diff        lwr        upr
0.001% greywater-0.0001% greywater      0.24487037 -0.5351994  1.0249402
0.01% greywater-0.0001% greywater      -0.31295721 -1.1555289  0.5296145
0.1% greywater-0.0001% greywater       -0.27983392 -1.0599037  0.5002359
1% greywater-0.0001% greywater         -0.90583296 -1.6859028 -0.1257632
Control 0% greywater-0.0001% greywater -0.29187280 -1.0719426  0.4881970
0.01% greywater-0.001% greywater       -0.55782758 -1.4003993  0.2847441
0.1% greywater-0.001% greywater        -0.52470429 -1.3047741  0.2553655
1% greywater-0.001% greywater          -1.15070333 -1.9307731 -0.3706335
Control 0% greywater-0.001% greywater  -0.53674317 -1.3168130  0.2433266
0.1% greywater-0.01% greywater          0.03312329 -0.8094484  0.8756950
1% greywater-0.01% greywater           -0.59287575 -1.4354474  0.2496959
Control 0% greywater-0.01% greywater    0.02108441 -0.8214873  0.8636561
1% greywater-0.1% greywater            -0.62599904 -1.4060688  0.1540708
Control 0% greywater-0.1% greywater    -0.01203888 -0.7921087  0.7680309
Control 0% greywater-1% greywater       0.61396016 -0.1661096  1.3940300
                                           p adj
0.001% greywater-0.0001% greywater     0.9430277
0.01% greywater-0.0001% greywater      0.8893070
0.1% greywater-0.0001% greywater       0.9030692
1% greywater-0.0001% greywater         0.0130318
Control 0% greywater-0.0001% greywater 0.8862068
0.01% greywater-0.001% greywater       0.3953061
0.1% greywater-0.001% greywater        0.3771074
1% greywater-0.001% greywater          0.0005662
Control 0% greywater-0.001% greywater  0.3512914
0.1% greywater-0.01% greywater         0.9999972
1% greywater-0.01% greywater           0.3261678
Control 0% greywater-0.01% greywater   0.9999997
1% greywater-0.1% greywater            0.1919090
Control 0% greywater-0.1% greywater    1.0000000
Control 0% greywater-1% greywater      0.2098661
```

Appendix 6: *Tukey Test Results.*



Appendix 7: *Tukey Test Plots. Lines that do not cross the dotted line indicate that the means between the pairs is significantly different.*