Effect of water pH on spring onion growth

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Abstract

Since spring onion (*Allium fistulosum)* is one of the most commonly cultivated vegetables around the world (Food and Drug Administration, n.d.), it is important to understand optimal growth conditions for this crop. Despite its culinary and economic importance, there is no consensus on how the pH value of water affects spring onion growth. So, the objective of our study is to determine the effect of water pH on spring onion growth. We watered spring onion roots with water mixed with vinegar to make four different pH treatments (pH = 4, 5, 6, 7), and measured stem height over time as a proxy for growth. We hypothesized that there would be significant differences in growth heights between different pH groups, and specifically that onions in the slightly acidic treatment group pH = 6 would have optimal growth, since other crops were shown to prefer slightly acidic pH solutions, but suffer root damage at more acidic pH's (Islam et al. 1980). After 13 days of observation, all 4 groups of spring onions grew and appeared healthy. A two-way ANOVA, with days after planting and pH as the two factors, was performed and showed there was a significant effect of time on onion growth, but no significant effect of pH on spring onion group means. This result differs from our hypothesis, but could be because other factors such as mineral content in soil, or sunlight, had a larger effect on spring onion growth and so the effect of pH was negligible in comparison to other factors.

Introduction

The spring onion (*Allium fistulosum*), also known as scallion, Welsh onion or green onion, is the one of the most widely cultivated species of its genus (WCSP, n.d.). Although the species is native to China, agriculture sites for the welsh onion can be located all around the globe (WCSP, n.d.). Spring onions are popular culinary additions to many dishes, and are good sources of dietary fiber and vitamin C (Chilukoti, 2015).

As such an important crop, much research has been done regarding how spring onion growth is affected by factors such as sowing rate (Zurawik et. al., 2013), shading (Kadowaki et. al., 2012), or even wavelength of light (Gao et. al., 2020). However, the effects of pH, specifically soil and water pH on spring onion growth, remains largely uninvestigated. While some websites provide their various recommended pH values for onion growth, their values vary greatly. For instance, the Explore Cornell gardening site states an optimal pH of 6.2-6.8, while an article in Farmnote prescribes an optimal pH of 6.2-6.8 (Burt, n.d.; Cornell University, 2006). Moreover, these sites do not provide or cite research-based evidence for their stated optimal pH values.

For other crops, studies have shown that some crops such as maize and wheat, pH had an effect on crop growth (Islam et al. 1980). Specifically, Islam et al. found that ginger, cassava, maize, wheat, french bean, and tomato all achieved maximum or near-maximum grouwh in nutrient solutions of pH 5.5-6.5, and that at lower pH values of 3.3-4.0, growth was inhibited for many species due to hydrogen iron injury of plant roots (Islam et al. 1980). Indeed, this presents a challenge for farmers and gardeners trying to achieve optimal spring onions growth: there is evidence that many crops prefer a slightly acidic pH, but pH that is too low acidic can hinder plant growth by inhibiting soil life and causing an excess buildup of manganese, aluminum and iron which damages roots (*pH acidity: what it does to your plants,* n.d.).

Given the importance of spring onions, and the demonstrated importance of pH on crop growth, a key knowledge gap is how water pH affects spring onion growth. So, the purpose of this study is to bridge this gap by investigating if watering spring onions with acidic and neutral pH solutions (pH 4, 5, 6, 7) affects their growth, and if so, what pH levels lead to optimal growth. Our null hypothesis is that the water used to irrigate our spring onions plants at varying pH levels (pH 4, 5, 6, and 7) would have no effect on relative stem growth. Our alternative hypothesis is that spring onions watered with slightly acidic solutions (pH 6) will grow faster compared to those watered with neutral solutions (pH 7) or more acidic solutions (pH 4, 5). This prediction is based on previous research suggesting that a slightly acidic pH result in greatest relative plant growth for other plants, but very acidic pH levels can also hinder plant growth (Islam et al. 1980).

Methods

The spring onions were sourced from local supermarkets in Vancouver, B.C. They were cut 2cm above the roots, and each root was then placed in a separate small pot with potting soil barely covering the top. The pots were cylinder-shaped, 10 cm high with an 8cm diameter round opening, and had drainage holes to prevent root rot. The pots were all kept indoors, at room temperature (around 20-22C), and placed close to windows so plants had sufficient access to natural light. Experimental groups with watering pHs of 4, 5, 6, and a control group with a watering pH of 7 were set up. Each of the three experimenters grew two onions per treatment, for a total of 6 replicates per treatment. Figure 2 shows the set-ups of two of the experimenters. Evidently, they differed slightly from person to person, though we attempted to keep many environmental variables (listed above) constant.

Fig 1. Spring onion set-ups on the first day of planting by Y. Zhou (left) and A. Dhillon (right).

The spring onions were then grown for two weeks in May 2021. For the first three days of the experimental period, they were watered daily with a quarter cup (60mL) of solution each. Then, we noticed that the soil was already soaked on the second and third day, and our watering solution would immediately leak out of the bottom of the pots. So, from the fourth day onwards we only watered the spring onions every other day with 60mL of solution. Since this change in watering amount is consistent across treatment groups, and since the soil still looked damp each time we watered, this change should not affect our results regarding the effect of water pH on growth for different treatments. For the acidic treatments (pH = 4, 5, 6), vinegar was added to the watering solution. For the neutral pH treatment (pH = 7), only water was used. The amount of vinegar to add to the acidic treatments was determined using pH strips manufactured by Haobase. On each watering, and for each pH level, we added vinegar drop by drop to a container of tap water, continuing until the pH strip turned the appropriate color for the desired pH. Then, 60mL of this solution was used to water each spring onion in the corresponding pH treatment.

To quantitatively measure spring onion growth, stem height (in cm), from where the onion was cut to the tip of the stem, was used as a proxy for growth. In cases where there were multiple stems on a single plant (all growing out of the same cut root), we measured the height of the tallest stem. This measurement was taken on the first day after the onions were planted, then every other day onwards for 13 days. So, data was collected at six time points, over a growth period of 13 days. After the experimental period, data from all three people conducting this study were compiled and analyzed together.

The data was statistically analyzed in R with a two-way ANOVA, where one factor was pH and another was time (number of days since planting). First, outliers were detected but not removed. Then, a Shapiro-Wilk test of normality and Levene's test for homogeneity of variances were performed to check whether our data meets ANOVA assumptions. The data was log-transformed to fit ANOVA assumptions, and finally a two-way ANOVA was performed to assess whether the difference between mean spring onion height in different pH treatments over time is significant.

Results

All spring onions grew and appeared healthy, with a green stem or stems thinner than the original cut spring onion sprouting from the roots. Figure 2 is a boxplot showing stem height at each time of recording and for each pH group. The colored boxes indicate interquartile ranges (ranges between the 25th and 75th percentiles), and the median for each group is represented by the black line through the middle of each box. See the figure legend for an in-depth box-plot description. Visually, there was a clear effect of time on stem growth, as the mean heights increased over time for every pH group, with the highest spring onion reaching a height of 23.6cm by the final day (day 13). However, there is no obvious trend in terms of pH levels. The relative growth of spring onions in different pH groups changed over time, and also differed for the mean and median measures of growth. For instance, on the final day, pH 7 onions had a lower mean than pH 6 onions, but a higher median.

For our statistical analysis, an alpha level of 0.05 was used, since it is the most commonly accepted level for biological research. Starting with outlier detection, several outliers were found, but not removed from the dataset. Specifically, there is one outlier in the pH 5 treatment on day 5, and one outlier in each of the pH 7 treatments on days 5, 7, and 9. For the raw data, Levene's test found a p-value of 0.0074, indicating the variances are not equal. In addition, the Shapiro-Wilk test of normality found a p-value of 5.66×10^{-5}, indicating the data is not normal. So, a log transformation was performed on the raw data. After the log transformation, Levene's test found a p-value of $0.1613 > 0.05$, so we can assume equal variances. However, Shapiro-Wilk test produced a p-value of 0.01256, so the log-transformed data is still not normal.

Schmider et al. showed that the ANOVA test is robust to violations of normality (Schmider et al. 2010), so despite the violation of normality we proceeded with a two-way ANOVA on the log-transformed data. The ANOVA found a p-value of 0.419 for the effect of pH on group means, and a p-value of 1.59×10^{-14} for the effect of day on group means. Thus, both visually and statistically, there is a significant effect of days since planting, but no clear effect of pH level, on spring onion growth.

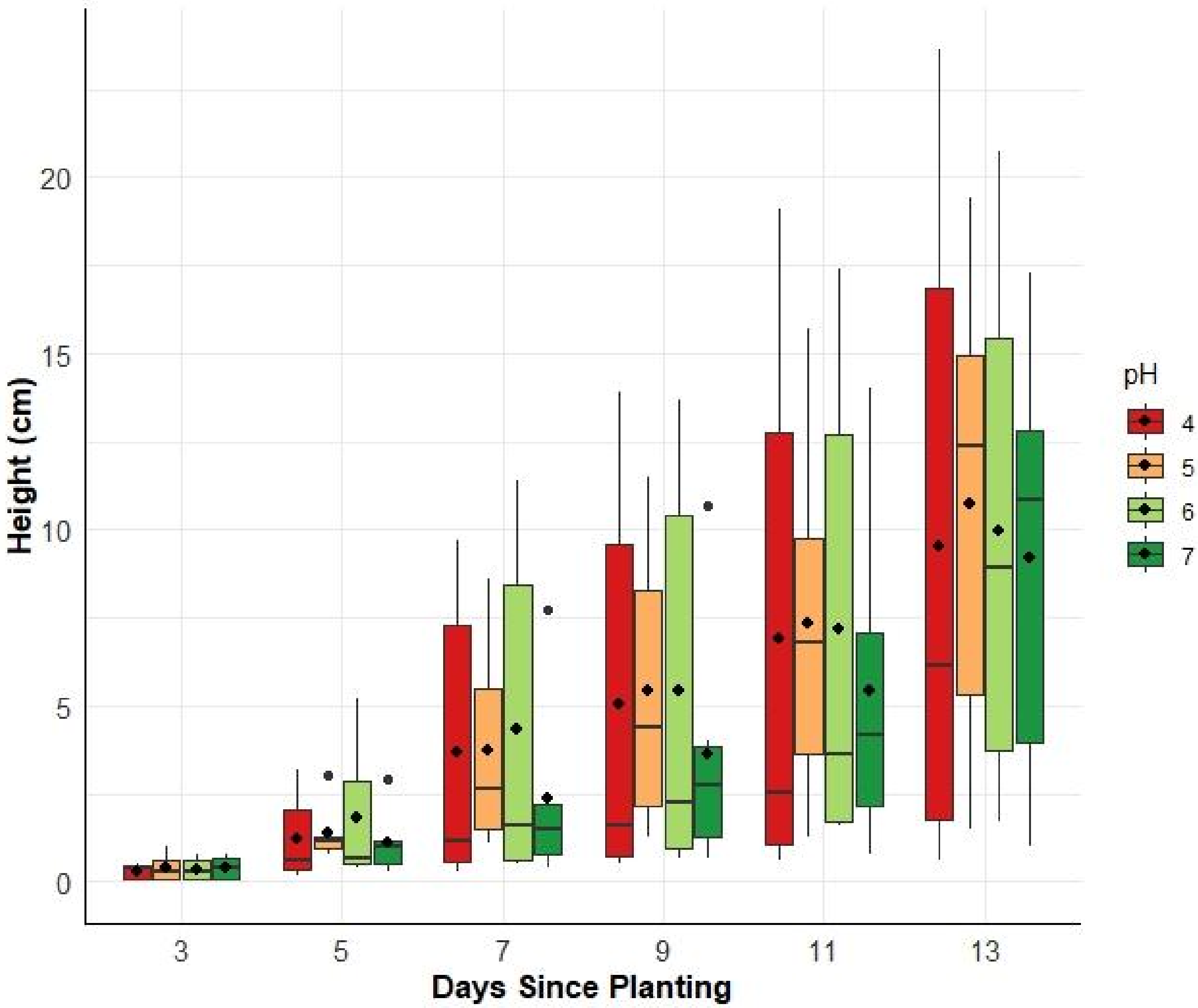

Fig 2. Box-plot showing the stem heights (cm) of spring onions in different treatment groups (n = 6 for each group) on different days since planting. The colored boxes indicate the interquartile range (between the 25th and 75th percentiles). The black line within each box marks the median; the black diamond within each box marks the mean. Whiskers above and below each box indicate the 10th and 90th percentiles. Black dots above and below the whiskers indicate outliers, For the group means, a two-way anova found $p = 0.419$ (not significant for $\alpha = 0.05$).

Discussion

Using a two-way ANOVA, different pH levels for the treatment groups and days since planting (independent variables) were compared with stem height (dependent variable) for the spring onion plants grown. We found a p-value of 0.419 for the effect of pH on group means, and since this is above the alpha value of 0.05, we cannot reject the null hypothesis of pH levels having no effect on spring onion growth (functionally defined as stem height). We also found a p-value of 1.59×10^{-4} for the effect of days since planting on stem height (far below 0.05) which indicates that the relationship of spring onions growing taller over time is, unsurprisingly, significant. The null hypothesis not being rejected indicates that there is a > 5% chance that varying mean heights between the treatment and control groups can be explained by chance alone, suggesting there is no clear relationship between pH levels and stem height.

Some plant heights in the same treatment group were much higher than others, resulting in high in-group variability. This is evident in the large ranges for the IQRs (some ranging from close to 0cm all the way to 20cm) from our data, which suggests there may be other confounding variables affecting plant growth. As each group member conducted the experiment with two replicates from each pH group, it is probable that the spring onions in different locations could have experienced different environmental conditions. Although we tried to keep many environmental variables (such as temperature and pot size) constant, we could not fully control for other variables such as the amount of sunlight in different locations. Various gardening guides suggest that cultivating spring in a high sun environment is optimal for growth, which could also explain our large variations in stem height (Cornell University, 2006). In addition, there could be slight differences in soil composition, specifically in mineral content, from the potting soils that were sourced from by each group member in their local stores. Previous research by Abbey et. al. indicates significant effects of soil type, genotype of the spring onion plant, and soil sulphur content on spring onion growth/dry-matter production which demonstrates some variables that could explain the vast differences in variation between/within pH groups (2002).

From our experiment, we can now discern the reason behind wide inconsistencies in optimal pH values listed on internet websites, with some sources citing ranges such as 5.3-5.8 or 6.2-6.8 (Burt, n.d.; Cornell University, 2006). The lack of relationship between various acidic pH levels and plant growth observed can reveal the difficulties in determining an exact optimal pH range for spring onions. The varying ranges that other sources observed could be due to the other confounding variables mentioned above which could affect plant growth more.

Also, it is interesting that for many treatment groups, the mean and median values differed on different days. For instance, on day 13, the mean height for spring onions in pH 4 group was around 10cm, while the median height was around 5cm, so the mean was noticeably higher. In contrast, on the same day, the pH 7 group had a higher median (around 11cm) compared to the mean (around 9cm). This suggests that for some groups, the distribution of data is skewed right (median less than mean), while other groups have a distribution skewed left (mean less than median). This lack of normality could have affected the results of our analysis, as normality is one assumption for a two-way ANOVA.

Changes to the experimental design for other studies that control for confounding variables from environmental conditions would greatly benefit future studies on spring onion growth, and would further provide insight on effects of watering pH in particular. Other factors for determining plant success and growth should also be considered like leaf greenness, number of green stems, bulb diameter, total soluble solids content , and percentage dry matter, all of which have been measured in other studies (Abbey et. al., 2002). Finally, we recommend testing spring onion growth in a greenhouse setting for all replicates, to best regulate and standardize environmental conditions such as lighting and humidity.

Conclusion

Through our experiment investigating the effect of acidic and neutral pH levels (pH 4, 5, 6, and 7) on spring onion growth which was functionally defined as stem height (in cm), we could not reject the null hypothesis as the p-value > 0.05 ($p = 0.419$). This provides support for the null hypothesis that different acidic to neutral pH levels for water used to irrigate spring onions have no effect on plant growth. There was high in-group variability in our data suggesting confounding variables creating unequal variances between replicates. This indicates a strong effect of external variables between the different environmental conditions where this experiment was conducted. Future research into optimizing spring onion growth from alterations in environmental factors should work to restrict confounding variables as much as possible and further investigate the role of other factors like soil mineral content in growth levels.

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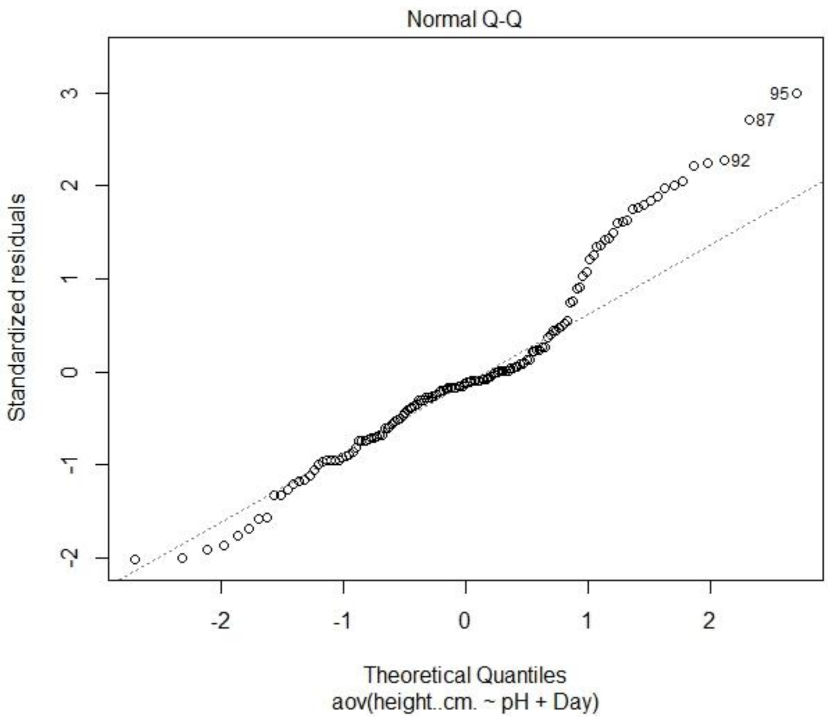
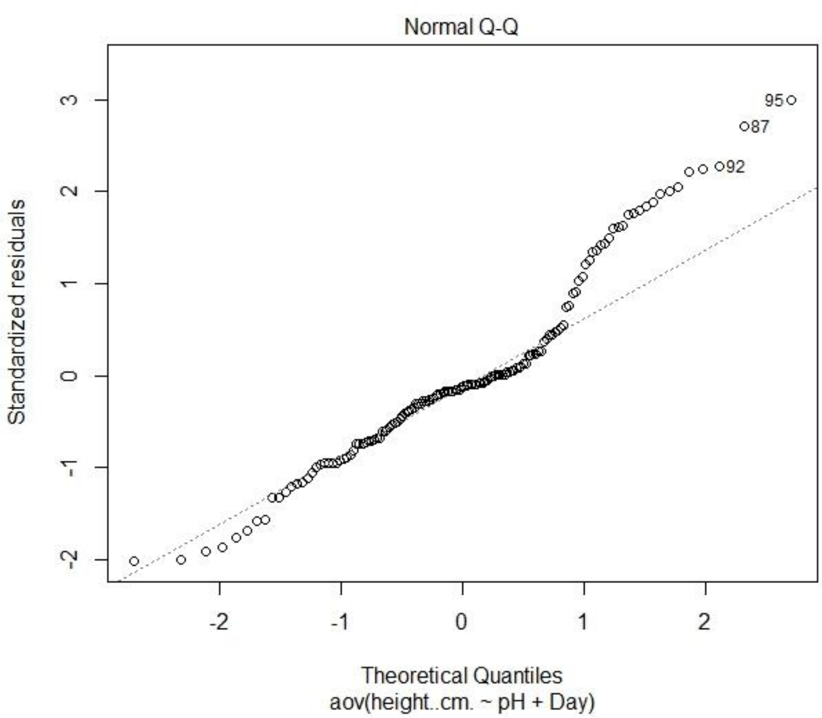
Appendix A

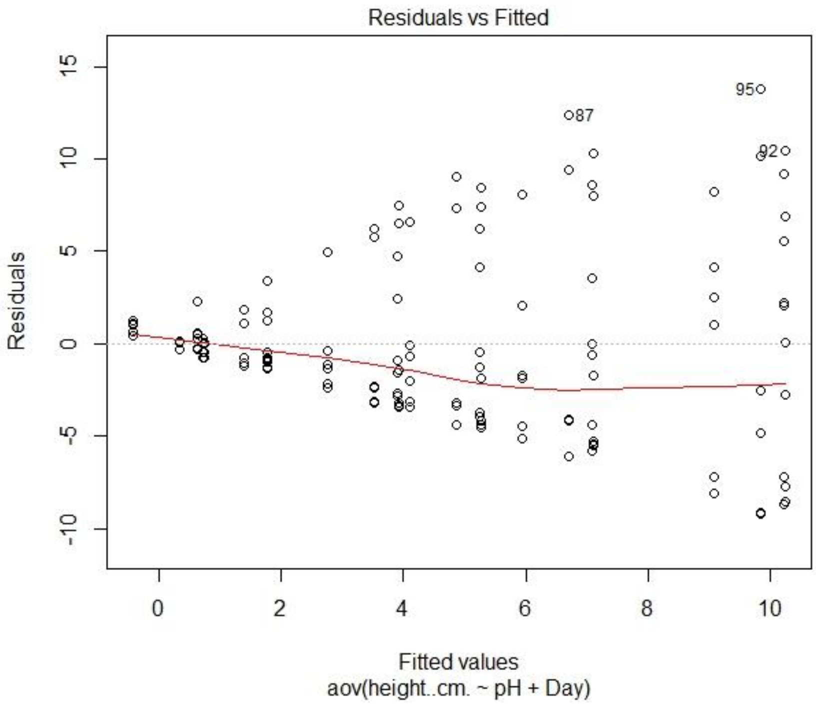


QQ Plot of data prior to log-transformation, without outlier removal.

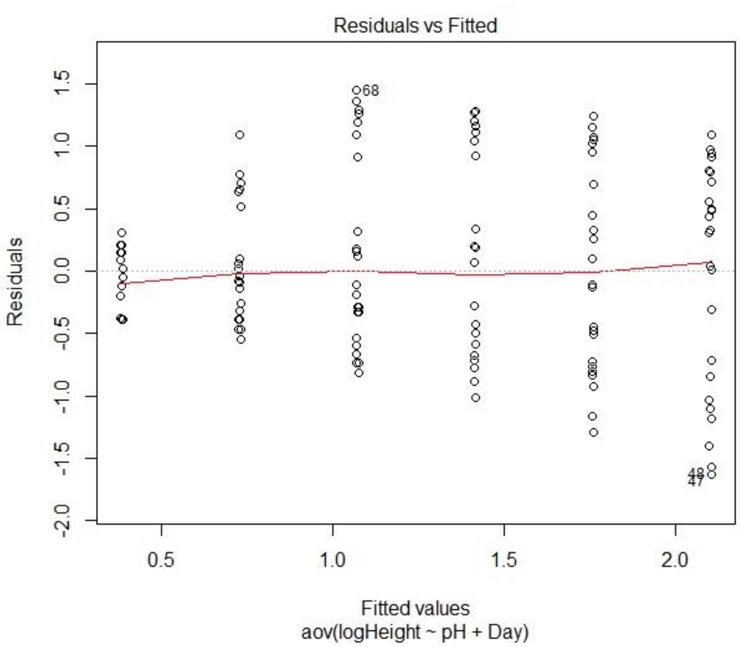


QQ plot of data after log-transformation, without outlier removal.

Appendix B



Residual plot of data prior to log-transformation, without outlier removal.



Residual plot of data after log-transformation, without outlier removal.