Effects of pH on Growth of Green Onion, *Allium fistulosum,* placed in soaking solutions made of Vinegar, Bleach and Tap Water

Gurleen Gill, Dilraj Josan, Eleanor Liu, Ella Shen

Abstract

The use of soil culture for vegetable growth has negatively impacted the environment. Pesticides and fertilizers used in traditional soil culture methods are proving to have detrimental effects on surrounding biodiversity (Mahmood et al., 2016). Hydroponics, growing vegetables in solution rather than soil, is an environmentally friendly method and a solution to these problems (Jensen, 1999). Although growth of most vegetables has been studied through hydroponic methods, there is a lack of research on green onion growth. Our study soaked green onion bulbs, *Allium fistulosum,* in solutions of pH 3.00, 6.06, 7.07, 8.00, and 11.03 made from vinegar, bleach, and tap water. We measured the total stalk length of green onions after each week. We hypothesized a slightly acidic environment to be the optimal pH for green onion growth. The results showed that pH 6.06, 7.07, and 8.00 had no statistically significant difference in green onion growth. However, the tests also tell us that soaking solutions of pH 6.06, 7.07, and 8.00 treatment groups did have statistically different results from the pH 3.00 and 11.00 treatments. While we could not identify the specific optimal pH, we found that generally neutral solutions with a range of pH 6.06-8.00 provided optimal growth for green onions compared to highly acidic or basic solutions.

Introduction

Over the years, soil culture has been a source of increasing environmental costs. Most soils used daily by home gardeners and agriculture companies have a negative effect on the environment (Mahmood et al., 2016). The use of pesticides and fertilizers, which are toxic chemical substances, in agriculture is benefitting the growth of crops but at the cost of biodiversity (Mahmood et al., 2016). Pesticides are used to kill insects, pests, and other unwanted organisms that attack and cause harm to crops (Mahmood et al., 2016). According to research done by Majewski and Capel (1995), around 90% of pesticides become volatile within a few days of application resulting in severe damage to non-target organisms. The chemicals are able to contaminate surrounding bodies of air, water, and soil, affecting biodiversity present in those areas. (Mahmood et al., 2016). Furthermore, the ingredients used in many potting soils are made for use on a wide variety of plants, so that gardeners don't have to go through the trouble of purchasing many different soils (Resh, 2015). However, this comes with many drawbacks as most plants have their own specific nutritional needs and these are not met with basic potting soils (Resh, 2015). These problems, however, can be solved by the use of hydroponics.

In North America, hydroponics is becoming an increasingly popular and environmentally friendly method of growing vegetables (Jensen, 1999). Hydroponics is an advanced agricultural technique that allows for vegetables to be grown in a solution rather than soil (Jensen, 1999). This technique allows for the nutrients in the solution to be controlled and formulated specifically for the plant type and eliminates the need for pesticides because plants are grown in a pest free environment (Resh, 2015). There has been significant research conducted on many different vegetables grown with hydroponics however there seems to be a lack of research on growth of green onion, *Allium fistulosum*, stalk.

In order to determine the optimal conditions of green onion growth through hydroponics, we will be examining how a wide variety of pH soaking solutions made from vinegar and bleach, which are cheap and readily available ingredients, affects green onion stalk growth. pH testing is extremely important in the field of hydroponics because it determines whether plants are being able to uptake the essential nutrients they need for growth (Kane et al., 2006). Thus, we based our project around pH to determine the optimal environment for growth of green onions. Our study will place store bought green onion bulbs of similar and standard size into pH solutions of 3,6,7,8, and 11 to see the effects of acidity and basicity on growth. Although previous experiments conducted before have analyzed onion growth by examining the biomass (Kane et al., 2006), we will be examining changes in green stalk length of green onion bulbs. We chose stalk length as an accurate measurement of green onion growth because it is a sign of primary growth in plants (Baucher et al., 2007). Primary growth is cell division in the shoots and roots that allows the plant to seek more water and sunlight (Baucher et al., 2007). We will not be analyzing root length in this study because most homeowners and companies are concerned with the growth of the edible stalk; the growth of stalk length will promote the switch from soil culture to hydroponics. Ultimately, this study will allow common households and massive agricultural companies to grow fresh green onions with increased efficiency and enhanced results without harming the environment.

One research study examined the effects of environment pH levels on onions and found that soil acidity played a huge role in the development of the onions (Kane et al., 2006). Islam et al. (1980) discuss how plants grown in soil culture generally show optimal development in an environment with pH range of 5.5-6.5. This is due to the unavailability of nutrients for uptake by roots when the pH levels are highly acidic or basic. Thus, if a slightly acidic (pH 6) soaking solution is optimal for stimulating green onion growth, then the green onions that are soaked in a slightly acidic solution will have the longest total stalk length compared to others because of higher nutrient availability.

Methods

Data Collection

In order to determine the effect of pH on green onion growth, we made four soaking solutions at pH of 3, 6, 8 and 11. To make a soaking solution at pH 3, we mixed 0.30 mL of concentrated vinegar, 10% acetic acid (CH_3COOH), and tap water to make a 500 mL acidic solution. Then, we took one mL of this acidic solution and performed serial dilution to dilute it 1000x in order to make a solution with a pH of 6. Alternatively, to make a soaking solution at pH 11, we mixed 1.24 mL of bleach, 3% sodium hypochlorite (NaOCl), with tap water to create a 500 mL basic solution. Similarly, we took one mL of the basic solution and performed serial dilutions to dilute it 1000x to make a pH 8 soaking solution. Furthermore, 500 mL of tap water was used as a neutral soaking solution. The pH of each soaking solution was tested using a calibrated pH meter and recorded. The exact pH levels of the soaking solutions were determined to be 3, 6.02, 8.00, 11.03, and 7.07 for the neutral solution. Subsequently, we poured 20 mL of each solution into three clear 50 mL plastic cups, totaling 15 clear plastic cups.

Later, we rinsed 15 green onions, cut the roots to approximately 0.20 cm, and cut the overall length (Figure 1) to 7.62 cm. This was done to ensure all green onions are equal in length before being placed in soaking solution to allow for an accurate measurement of growth. Green onions were then randomly placed in the 15 cups filled with soaking solutions.

The green onions were soaked in solution for a total of two week. At the end of each week, the green onions were removed from the solution and placed on a counter surface. The stalk length and overall length were measured using (Figure 1). The total stalk length was defined as the overall length minus the initial length of 7.62 cm and the addition of any additional stalks if they grew. In between measurement of green onions, the counter surface and ruler were cleaned and dried thoroughly to avoid contamination.

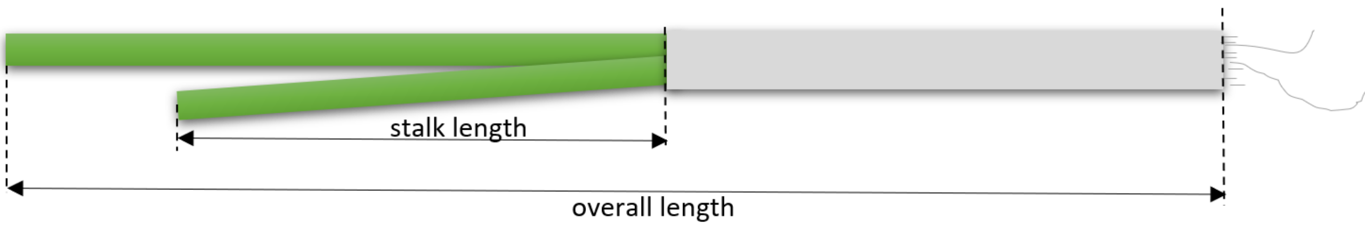


Figure 1. **Graphical representation of the green onion stalk data that was collected.** Green onions soaked in different pH levels (N=5) were measured after week 1 and week 2. All parts of the green onion were straightened out when measured. Overall length is from the tip of the longest green stalk to the end of the white stalk. Stalk length indicated the length of any additional stalk, if any. The total stalk length is calculated to be the sum of the overall length and any other unincluded stalk length, then minus the initial length of green onions (7.62cm).

Statistical Analyses

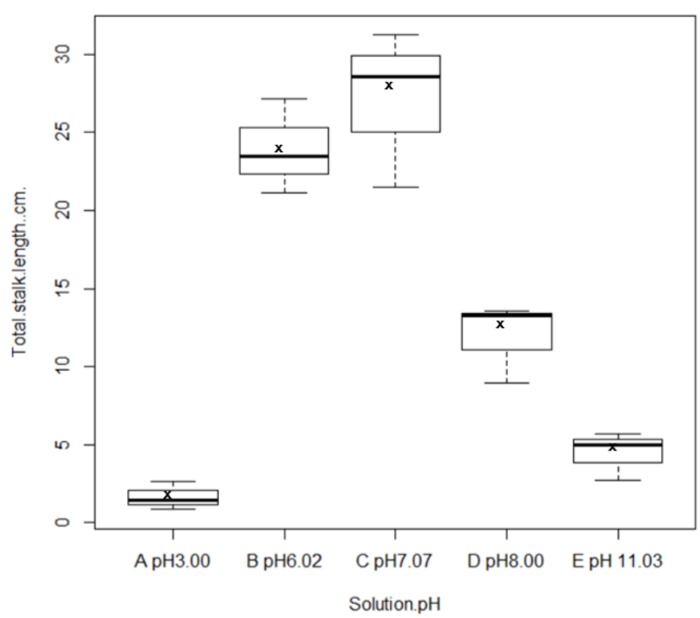
Statistical analyses of data were done using R Commander. We analyzed the data collected after week 1 of soaking and week 2 of soaking separately. We used an ANOVA test to compare the mean total stalk length of green onions soaked in solution with different pH. After obtaining the ANOVA analysis, Tukey's test was performed to determine the significant differences, if any, between the different pH treatment groups.

Result

The ANOVA tests showed that an overall statistically significant difference existed among all groups of green onions that were soaked in different pH levels after both 7 days (p-value = 0.00000274) and 14 days (p-value = 0.00000175). The green onions soaked in pH 7 observed the greatest mean (±95% CI) total stalk length of 27.09±2.77 cm after week 1 and 46.65±4.39cm after week 2. For both weeks, the green onion soaked in pH 7.07 and pH 6.02

solution grew significantly longer total stalk length (Figure 2). The slightly basic pH (pH 8.00) solution showed slow stalk growth in the first week but showed significant stalk growth after the second week (Figure 2, Table 1). The total stalk length of the green onions soaked in pH 3.00 and pH 11.03 solution were found to have significantly shorter length at the end of both weeks (Figure 2). Any difference in stalk length between the green onions soaked in pH 6.02 and 7.07 solutions, pH 6.02 and 8.00 solutions, and pH 3.00 and 11.03 solutions are suggested due to random chance by Tukey's test (Table 1). The standard deviation in both stalk lengths increased as the pH of the solution was closer to 7.00. The green onion soaked in acidic solution generally had smaller standard deviation in stalk length than the ones soaked in basic solutions (Figure 2).

a. Week 1 Solution pH over total stalk lengths



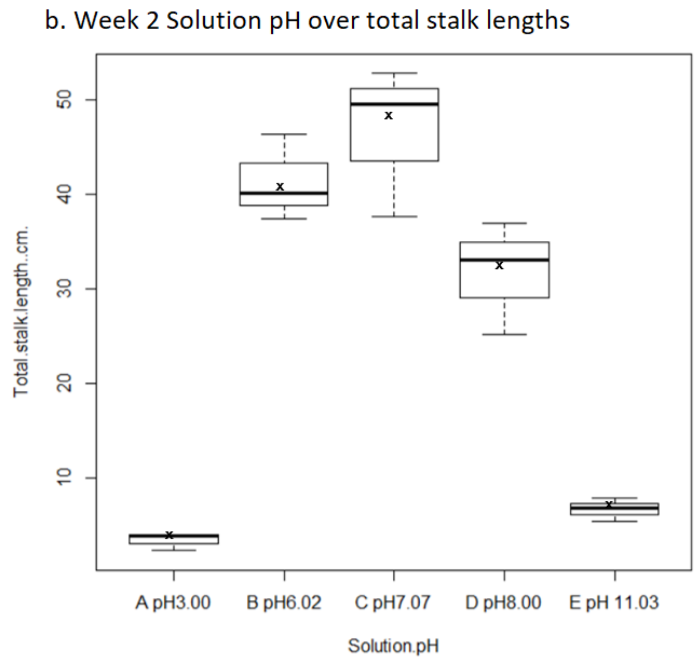


Figure 2. Box plot of the green onion total stalk length data after (a) week 1 and (b) week 2 of soaking. Green onions were soaked in different pH treatment groups (N=5) to determine the effect of pH on the total stalk length (Figure 1) of green onions. The x-axis represents the pH of the various soaking solutions green onions were soaked in and the y-axis represents the total stalk length of the green onion (Figure 1). The plot shows the mean (x), median (darken black line), 75% quartiles, and spread of green onions soaked in solutions with different pH levels. Through the ANOVA test, the p-value was determined to be 0.00000274 for week 1 and 0.00000175 for week 2. The p-value was less than alpha (0.05) in both weeks, showing statistically significant different total stalk length among the different pH treatment groups.

a. Week 1 Tukey's test total stalk length comparison between pH groups

pH 8.00	0.07207			
pH 7.07	<0.001	<0.001		
pH 6.02	<0.001	0.00426	0.69770	
pH 3.00	0.77696	0.01219	<0.001	<0.001
	pH 11.03	pH 8.00	pH 7.07	pH 6.02

b. Week 2 Tukey's test total stalk length comparison between pH groups

pH 8.00	0.0008			
pH 7.07	<0.0001	0.0274		
pH 6.02	<0.0001	0.2021	0.6896	
pH 3.00	0.9231	0.0003	<0.0001	<0.0001
	pH 11.03	pH 8.00	pH 7.07	pH 6.02

Table 1. Triangle table showing p-values analyzed by Tukey's test comparing total stalk length between pH groups after (a) week 1 and (b) week 2. Green onions were soaked in different pH treatment groups (N=5) to determine the effect of pH on the total stalk length (Figure 1) of green onions. This table compares the total stalk

length between pH groups and presents the p-value of these results. The p-values greater than 0.05 are labeled in gradient of oranges. The p-values smaller than 0.05 are labeled in gradient of green. The darker the color, the farther the p-value is away from the 0.05 critical point. Darker green colour means there is greater statistically significant difference in total stalk length.

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Discussion

Since the p-values produced from the ANOVA analysis were below alpha 0.05 at the end of both weeks, we reject the null hypothesis. The data was then further analyzed by the Tuckey's test and showed there were significant statistical differences between pH levels in the pH 6.02/7.07/8.00 group and the pH 3.00/11.03 group (Table 1). While neutral pH (pH 7) showed more growth in mean total stalk length (Figure 2) than the other treatment groups in both weeks, there was no statistical difference between it and pH 6.02. Thus, we were not able to conclude the most precise optimal growing conditions for green onions, however, we can conclude that solutions with pH 6.02-8.00 provided more optimal growth than solutions with pH 3.00 and 11.03. Kane et al. (2007) found that the most optimal soaking solution for general onion species growth should have a pH of 6.5. Our results were inconsistent with Kane's team as our results indicate that maximum plant growth should be in solutions of pH 6.02-8.00. After analyzing possible sources of error, we concluded that human error and mistakes in methods may have led to these results.

After two weeks in the soaking solution, the results showed that there was no significant difference in the mean total stalk length of green onions growing in pH of 6.02 and 7.07 solutions and pH 6.02 and 8.00 solutions (Table 1). This result could be due to contamination which could have occurred during the process of measuring the green onion stalk and root lengths. Although cleanliness was maintained, there is still a strong possibility that the bleach and vinegar transferred between solutions through the counter surface, as all the green onions were placed and measured on the same surface. This limitation could be why at first there was a significant difference between pH 6.02 and 8.00 and then at the end of the second week there was no significant difference in their total stalk length (Table 1). Since the solutions of pH 3.00 and 11.00 are more concentrated in vinegar and bleach respectively, the transfer likely didn't have an effect on the growth of green onions in these solutions.

During the process of growing, plants produce waste products that are released into the soaking solution. This is a process known as excretion (Ramsay & Kelley, 1998). Plants produce oxygen during photosynthesis and carbon dioxide during respiration which are excreted through the stomatal pores (Ramsay & Kelley, 1998). Colt and Orwicz (1991) state that the build-up of carbon dioxide can lead to increased dissolved carbon dioxide concentrations and thus a decrease in pH. Although they further state that the decrease in pH is dependent on the amount of carbon dioxide released, in a closed system with no carbon dioxide removal the effects of carbon dioxide on pH are prominent (Colt & Orwicz, 1991). This would explain why according to the mean, the greatest total stalk length was observed in soaking solution of pH 7. Thus, the optimal growth in green onions was actually seen in a slightly acidic soaking solution which would support our hypothesis. Moreover, Ramsay & Kelley (1998) discuss that while the majority of these excretions are gaseous, plants do excrete solid waste such as resin and latex. Since the soaking solutions were not changed for two weeks, it is likely there was an increase in solid waste in these solutions. This excessive waste would almost act like a negative feedback loop as extracellular waste could be reabsorbed by the plant, decreasing metabolic efficiency and growth (Ramsay & Kelley, 1998). We do not know if all the bulbs excreted the same or different amounts of waste and thus can not precisely identify the magnitude of the effect of metabolic waste on plant growth. Changes in pH and an increase of waste presence in soaking solution may have impacted the growth of the green onions, impacting the stalk length growth and significance of our results.

Also, the use of plastic cups made may have affected plant growth as plastic cups are manufactured with BPA (Bisphenol A). Wang et al. (2015) discussed the concentration varying effects of BPA on plants. Low amounts of BPA promoted plant growth, while higher amounts of BPA inhibited plant growth by changing the ratio of growth and stress hormones (Wang et al., 2015). BPA from our cups could have diffused into the soaking solution and gotten absorbed by green onion roots. The amount of diffusion and absorption of BPA by green roots would have different effects on stalk growth, which would impact the significance of our findings.

There are a number of possible changes that can be made for further research on this topic. A factor to consider when looking at green onion growth could be the weight of the stalks. Despite the green onions grown in pH 7 having the greatest mean in total stalk length (Figure 2), the predicted optimal pH of 6.03 could have produced the most biomass and thus had stalks with the most weight. Also, the next experiment should change their methods and conduct the experiment in BPA free cups and change the soaking solutions daily to prevent factors such as excretion of waste from impacting plant growth and pH of soaking solution.

While we were not able to deduce a specific pH value that has the most enhanced effects on green onion growth, we managed to find a rough range of pH 6.02-8.00 which showed the best development. Further studies should measure the effects of temperature, light availability, and atmospheric carbon dioxide concentrations on green onion growth in hydroponic conditions. By factoring in all variables, it will allow for the creation of the most optimal hydroponic environment for green onion growth. Our research shows that green onions were able to successfully grow in a hydroponic solution, thus this technique should be promoted and implemented in major agricultural companies. This will allow our society to step towards finding more environmentally friendly alternatives to traditional agricultural methods which would protect ecosystems from effects of pesticide and fertilizer runoffs.

Conclusion

The results of our study did not support the alternative hypothesis. We believed that a slightly acidic solution would create the most optimal environment for growth, but our results indicate that there is no statistical significance for the differences in total stalk lengths observed in pH solutions 6.02, 7.07, and 8.00. However, there was a statistically significant difference in the total stalk length between pH treatment groups of 11.03 and 3.00 compared to pH 6.02, 7.07, and 8.00, thus, we are able to reject the null hypothesis. Although we weren't able to produce a specific optimal pH, this research should narrow the range of variables, making it easier for other researchers to further study the effects of pH on plants grown in hydroponic conditions. This study can aid farmers and homeowners to step away from soil culture methods and delve into growing vegetables through an environmentally cordial method like hydroponics.

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Appendix

Week 1							
Solution pH	Onion #	Overall Stalk length (cm)	# of stalk	Longest green stalk (cm)	Green stalk length 1 (cm)	Green stalk length 2 (cm)	Green stalk length 3 (cm)
3	1	8.58	2	0.92	0.50	0.92	
3	2	10.27	1	1.60	1.60		
3	3	8.47	1	0.60	0.60		
6.02	1	26.06	2	18.11	18.11	2.71	
6.02	2	24.35	2	17.19	17.19	10.4	
6.02	3	25.19	2	17.26	17.26	5.90	
7.07	1	31.65	3	23.6	23.60	4..06	2.60
7.07	2	25.10	2	17.60	17.60	11.09	
7.07	3	23.26	2	15.73	15.73	5.82	
8	1	15.85	2	8.00	8.00	0.70	
8	2	14.89	2	7.72	6.26	7.72	
8	3	14.71	2	7.00	7.00	6.20	
11.03	1	12.59	1	5.45	5.45		
11.03	2	9.32	2	1.53	0.99	1.53	
11.03	3	12.95	2	5.60	5.60	0.40	

Table 2. Green onion data collected after the week 1 of soaking. Green onions were soaked in different pH treatment groups (N=5) to determine the effect of pH on the total stalk length (Figure 1) of green onions. Overall stalk length stands for the length from the tip of green stalk to the end of white stalk (right above the root). Green stalk length stands for the length from the tip of green stalk to the top of white stalk. Longest green stalk is the length of the longest green stalk.

Trial	Solution pH	Total stalk length (cm)
1	A pH3.00	1.46
2	A pH3.00	2.65
3	A pH3.00	0.85
1	B pH6.02	21.15
2	B pH6.02	27.13
3	B pH6.02	23.47
1	C pH7.07	31.23
2	C pH7.07	28.57
3	C pH7.07	21.46
1	D pH8.00	8.93
2	D pH8.00	13.53
3	D pH8.00	13.29
1	E pH 11.03	4.97
2	E pH 11.03	2.69
3	E pH 11.03	5.73

Table 3. **Green onion data calculated based on the data collected after the first week of soaking.** Green onions were soaked in different pH treatment groups (N=5) to determine the effect of pH on the total stalk length (Figure 1) of green onions. Total stalk length is the sum of overall stalk length and all the rest green stalk length except the longest green stalk and minus the initial stalk length (7.62 cm).

Week 2							
Solution pH	Onion #	Overall Stalk length (cm)	# of stalk	Total green stalk length (cm)	Green stalk length 1 (cm)	Green stalk length 2 (cm)	Green stalk length 3 (cm)
3	1	11.29	2	4.10	3.88	0.22	
3	2	11.33	2	3.42	0.32	3.10	
3	3	9.00	2	2.41	1.50	0.91	
6.02	1	32.05	3	40.54	13.89	24.82	1.83
6.02	2	33.30	3	46.47	10.71	10.01	25.75
6.02	3	30.39	2	37.74	23.13	14.61	
7.07	1	38.12	3	51.78	20.3	29.50	1.98
7.07	2	35.52	2	49.77	21.67	28.10	
7.07	3	27.72	2	37.60	17.5	20.10	
8	1	31.12	3	32.99	8.99	23.40	0.60
8	2	29.90	2	37.40	14.60	22.80	
8	3	24.76	2	25.28	7.98	17.30	
11.03	1	15.51	1	8.55	8.55		
11.03	2	11.20	2	5.42	3.62	1.80	
11.03	3	14.18	2	7.10	6.90	0.20	

Table 4. **Green onion data collected after two weeks of soaking.** Green onions were soaked in different pH treatment groups (N=5) to determine the effect of pH on the total stalk length (Figure 1) of green onions. Overall stalk length stands for the length from the tip of green stalk to the end of white stalk (right above the root). Green stalk length stands for the length from the tip of green stalk to the top of white stalk.

Trial	Solution pH	Total stalk length (cm)
1	A pH3.00	3.89
2	A pH3.00	4.03
3	A pH3.00	2.29
1	B pH6.02	40.15
2	B pH6.02	46.4
3	B pH6.02	37.38
1	C pH7.07	52.78
2	C pH7.07	49.57
3	C pH7.07	37.6
1	D pH8.00	33.09
2	D pH8.00	36.88
3	D pH8.00	25.12
1	E pH 11.03	7.89
2	E pH 11.03	5.38
3	E pH 11.03	6.76

Table 5. **Green onion data calculated based on the data collected after two weeks of soaking.** Green onions were soaked in different pH treatment groups (N=5) to determine the effect of pH on the total stalk length (Figure 1) of green onions. Total stalk length is the sum of overall stalk length and all the rest green stalk length except the longest green stalk and minus the initial stalk length (7.62 cm).