# Analysis of Green Onion (genus *Allium*) Root and Stem Growth in Water at Different pH Levels

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#### Abstract

The mild flavour of green onions compared to white onions allows it to be used in various dishes in a raw or cooked form while also providing an abundance of nutrients to those that consume it (Gallary). This has prompted the question of what pH level is optimal for green onions to grow so that green onion farmers and gardeners will adjust their gardens/crops to maximize their green onion yield? Therefore, this study was created to see if the pH level influences green onion growth in water in a way that causes the stems and roots to grow more in certain pH levels compared to others. The members conducting this study grew twelve green onions at varying treatment pH levels of 6.30, 6.46, 6.72, and the control of ~7.5; each sample's root and stem growth was then monitored over the next 20 days. Overall, with the one-way ANOVA test, it showed that there was no significant difference between the length of roots or stems in green onions. This study did not show any significant differences in growing green onions in the tested pH conditions.

#### Introduction

Green onions provide essential nutrients, including vitamin A and vitamin K, with virtually no calories (Tremblay). One cup of green onions accounts for less than 1% of a person's daily calorie limit while covering nearly all daily vitamin A and vitamin K needs for both men and women (Tremblay). Receiving such a large amount of nutrients from such a small amount of green onions is impressive, considering that vitamin A plays a vital role in proper vision and creating new white blood cells, while vitamin K is an essential factor in blood clotting and in cartilage growth (Tremblay). To ensure that green onions remain a staple in our diet and abundant in our refrigerators, producers of green onions must grow green onions as optimally and efficiently as they can. Then green onions producers can maximize their yield and profit, which will, in turn, benefit the food industry as a whole due to this ingredient's widely applicable use in ethnic cuisines (Bahram-Parvar & Lim).

Some of the essential factors that affect green onion growth include: nutrient availability, oxygen concentration, temperature and pH (Hong & Kim; Kane et al.). During the planting and growth processes, if just one of these factors is out of optimal range, it may prevent the green onion yield from being maximized (Kane et al.). Furthermore, pH is a factor of interest for our study as nutrient availability to facilitate green onion growth depends heavily on the pH level (Kane et al.). The optimal pH for green onion growth has been previously determined to be 6.5 (Kane et al.). It is important to ensure the pH is at an optimal level as several nutrients (elements), including: magnesium, calcium, potassium, phosphorus, copper, manganese and iron, can be utilized best under optimal pH conditions (Kane et al.). Therefore, our experiment will test different pH levels that are near 6.5 to see which pH level has the largest root and stem growth (in centimetres). If the pH level of the water in which the green onions are placed is the only variable between the green onions, then the roots and stems of green onions grown closest to a pH level of 6.5 will show more significant growth (in centimetres) than green onions grown outside of this optimal pH level. To test the hypothesis, we each grew green onions in water with pH adjusted by lime juice - and monitored the green onion root growth at each pH treatment level over the next 20 days to see what level yields the highest growth.

#### **Materials & Methods**

This experiment was conducted in each of the study members' houses. The list of materials required per household was as follows: 1) tap water -  $\frac{1}{3}$  cup per plant, 2) ruler to measure the plants, 3) 12 clear cups, 4) 12 green onion bunches, 5) a sharpie pen to label each of the cups holding the plants, and 6) lime juice to alter the pH of the solution submerging the green onion.

In an effort to minimize uncertainty, all households purchased green onion bunches from a local Save On Foods. The rationale behind this decision was an assumption that as the households are all in the Lower Mainland area, the same distributor will supply the region's Save On Foods branches. Furthermore, all households used ReaLime Lime Juice, a product produced by a company called ReaLemon.

The following methodology subsection describes the processes taken in one household.

#### 1. Preparation for plant growth phase:

Each of the 12 cups was labelled according to the treatment group (e.g. control group; sample 1). Then, the relevant solutions that the green onions will be submerged in were created. The first type of solution was for the control group. The control samples' cups were filled with <sup>1</sup>/<sub>3</sub> cup of tap water. The pH for the control equates to tap water pH in Metro Vancouver, which is ~7.5 (YourWaterMatters). The second group of cups had its solution sourced from a 2L container of water with one drop of lime juice. The solution's pH level was approximately 6.72 (see below for calculation details). The third group had its solution sourced from a 2L container of water with three drops of lime juice. The first group's calculated pH levels were 7.5, 6.72 for the second group, 6.46 for the third group, and finally, 6.30 for the last group. Then, the green onion bunches were cut such that only the white coloured part of the stem and the roots remained. For each of the green onions, the stem's length - from the start of the root to the spot where the green onion was cut - was measured in cm and recorded. Then, the root's length was measured from the

start of the root to the end of the longest root. The onions were then placed into the relevantly labelled cups (refer to Figure 1).



Figure 1. Finished set-up for sample 1 within the control group.

### 2. <u>During the plant growth phase:</u>

Every five days, the solutions of the cups were replaced. Each replacement occurred at 10 pm PST. For each time the solutions were replaced, the same methodology in creating the relevant solutions was used. The replacement was to minimize pH level discrepancies. Every five days, the length of the stem and the root for each of the green onions were measured and recorded. The measurements also occurred at 10 pm PST. The plants were grown for 20 days (refer to Figure 2) - providing five data points if the initial measurement is included.



Figure 2. Samples 20 days into experiment.

Additional actions, on top of the aforementioned standardization of materials used for this study, were taken to minimize any other alterations in variables that may taint this study's results. First, all the plants were placed by windows facing south. This decision was taken in an attempt to standardize the amount of sunlight the green onions received. Also, just before the time and date the solutions needed to be replaced or onions needed to be measured, all members of this study corresponded through online communications to prevent a scenario where one or more members forgot to carry out the necessary task(s). The thermostats in the rooms that housed the plants were all set to 25°C. Finally, some plants died before the study's conclusion; in such cases, their last measurements before death were used for the one-way ANOVA analysis.

#### Calculations

pH calculations:

#### <u>Setup:</u>

Equations used:  $pH = -log[H^+]$  and  $M_1V_1 = M_2V_2$ 

2L = 2000 mL of tap water used for each treatment, pH of tap water = 7.5 (YourWaterMatters)

pH of lime juice = 2.2 (Alcademics)

 $pH = log[H^+]$ 

 $10^{-2.2} = [H^+] = 0.00631M$  for lime juice

 $10^{-7.5} = [H^+] = 3.16 * 10^{-8} M$  for tap water

*One drop pH:* 

 $M_1V_1 + M_2V_2 = M_3V_3$ 

 $0.00631(0.05) + 3.16*10^{-8}(2000) = M_3(2000.05)$ 

 $M_3=1.893*10^{-7}M$  so one drop pH =  $-\log(1.893*10^{-7}) = 6.72$ 

One drop pH = 6.72

*Two drops pH:* 

 $M_1V_1 + M_2V_2 = M_3V_3$ 

 $0.00631(0.10) + 3.16*10^{-8}(2000) = M_3(2000.10)$ 

 $M_3=3.47*10^{-7}M$  so two drops  $pH = -log(3.47*10^{-7}) = 6.46$ 

Two drops pH = 6.46

*Three drops pH:* 

 $M_1V_1 + M_2V_2 = M_3V_3$ 

 $0.00631(0.15) + 3.16*10^{-8}(2000) = M_3(2000.15)$ 

 $M_3=5.048*10^{-7}M$  so three drops  $pH = -log(5.048*10^{-7}) = 6.30$ 

<u>Three drops pH = 6.30</u>

#### Results

The results appeared to be quite inconclusive. The root growth did not vary greatly in any of the treatments (refer to Figure 3). All the growth appears to have been between the range of 3.5-4cm on average and then the error bars appear to overlap greatly as they are quite large (~3.5-4cm on average). Below is the sample calculation of root growth:

Final Root Measurement - Initial Root Measurement = 8.5cm - 0.8cm = 7.7cm



Figure 3. Graph of Root Growth for all the different treatments and control (pH levels). There were three samples for each group. p-value = 0.8003. Error bars represent 95% Confidence Intervals. Generated on GraphPad Prism.

Furthermore, the one-way ANOVA test revealed that the p-value for the root growth was p=0.8003, which is greater than 0.05. Therefore, the difference between the root growth is not statistically significant, and no post-hoc test is needed. The stem growth was obtained using the following calculation for each treatment (e.g. Control 1):

Final stem Measurement - Initial stem Measurement = 36cm - 10.5cm = 25.5cm The stem growth varied greatly (~10cm on average) in some treatments, such as two drops versus three drops (refer to Figure 4). Even in these cases, the error bars still overlap and are quite large (~15cm on average).



Treatment (pH Level)

Figure 4. Graph of stem Growth for all the different treatments and control (pH levels). There were three samples for each group. p-value = 0.1906. Error bars represent 95% Confidence Intervals. Generated on GraphPad Prism.

The one-way ANOVA test revealed that the p-value for the root growth was p=0.1906, which is greater than 0.05. Therefore, the difference between the stem growth is not statistically significant, and no post-hoc tests are needed. Thirdly, the ratio of stem to root growth (Stem:Root) was obtained using the following calculation for each treatment (e.g. Control 1):

Final stem Measurement / Final Root Measurement = 25.5 / 7.7 = 3.25cm

The root growth did not vary greatly in any of the treatments (refer to Figure 5). All the growth appears to have been between the average range of 3.5-4cm, and the error bars appear to overlap greatly as they are relatively large (~2cm on average).



Treatment (pH Level)

Figure 5. Graph of stem:root for all the different treatments and control (pH levels). There were three samples for each group. p-value = 0.9267. Error bars represent 95% Confidence Intervals. Generated on GraphPad Prism.

The one-way ANOVA test revealed that the p-value for the root growth was p=0.9267, which is greater than 0.05. Therefore, the difference between the stem:root ratios are not statistically significant, and therefore no post-hoc tests are needed.



Figure 6. Graph of stem:root over time (starting at the initial planting date). There were three for each group. Generated on GraphPad Prism.

The ratio of stem to root growth was around 3.5-4cm for all of the treatments and control (see figure 5). The roots and stem were looking quite green and healthy in the first two weeks as they were growing. Near the last week of the experiment, more of the green onions (for several treatments) showed signs of stress with yellowing or browning of stems or roots. Some plants even succumbed to the stress and died; for the statistical analysis, their final measurements for root and stem length before death were used.

#### Discussion

Based on the one-way ANOVA analyses performed, the p-value for root growth at different pH levels was 0.8003, which is greater than 0.05; thus, the difference between the root and stem lengths were not statistically significant. Therefore, we fail to reject the null hypothesis and fail to support our alternative hypothesis. In other words, this experiment has found that we are unable to conclude that growing green onions in a pH closest to 6.5 will have the largest root growth compared to green onions grown at pH levels farther from 6.5.

The failure to reject the null hypothesis may have occurred because green onions could have established buffering mechanisms to cope with a change in pH conditions. There is currently no research on green onions having a buffering system to mitigate differences in pH levels - a future study that addresses this lacuna may be a great addition to scientific literature.

Numerous factors may have played a role in the results, despite our best efforts to minimize variables' differences. Firstly, the plants were grown in three different environments within Metro Vancouver. There may have been discrepancies in each location's tap water, such as pH level. Although the Metro Vancouver tap water is stated to be ~7.5, there was no accurate way to verify this in our samples as the litmus paper available to all of us was precise only up to one's place (YourWaterMatters). Moreover, pH stability is an issue as the pH of tap water may not stay constant over a five day period; degassing of chlorine in tap water increases the pH over time (Biedermann-Brem & Grob). This issue would affect the validity of the results as the true pH of tap water used may not have always been 7.5. Also, the different pH levels could be varied more in a future study as the nutrient availability might not have been significantly affected by different pH levels (Kent et al.).

Other environmental constraints include temperature and sunlight. The plants were all placed in windows facing south, and the thermostats within the households were altered to maintain room temperature (25°C). Despite these measures, factors such as the thermometers' calibration and varying sunlight conditions may have tainted the results. Temperature has been found to play an important role in green onion respiration; specifically, a temperature of 20°C has shown to have

high amounts of respiration for green onions (Hong & Kim). This high respiration promotes the degradation of green onions, a potential issue for this study as the green onions in our experiment are grown for almost four weeks (Hong & Kim). However, turning the temperature to a lower respiration rate was not feasible - doing so would provide discomfort to residents of the testing locations.

Factors such as unaccounted human errors in measuring the plants and creating relevant solutions to submerge the plants may have skewed the data. Our assumptions on Save-on-Foods in the Lower Mainland having the same green onions supplier may have been inaccurate. All these variability factors and lack of precise laboratory equipment in some combination have likely led to the discrepancies between this study and the study by Kane et al. Moreover, Kane et al. determined the optimal pH for green onion root growth was 6.5, whereas our experiment concluded that the pH levels (6.30, 6.46, 6.72, 7.5) had no statistically significant effect on green onion growth. A pH of 6.46 should have been the optimal pH for our study, but variability and lack of precise tools (sensitive litmus paper, pH meter, lab room, Etc.) likely caused the results to be different than what was expected.

Future studies may wish to consolidate the testing sites - this will eliminate concerns regarding discrepancies in sunlight, pH level, and temperature growth. Furthermore, the green onions being sourced from a singular store, which was not feasible in this study due to COVID-19 and related restrictions, will ensure some unwanted variables are not present. In addition, standardizing the

cut length to 5cm in future studies would be useful as well since the area of the cut may trigger different responses for green onion root growth.

#### Conclusion

This study did not find a significant difference in root or stem length between various pH levels of solutions green onions were submerged in. Various factors that may have affected our study are listed, and directions for potential future projects are given. Despite this study's results, gardeners and green onion farmers should remain cautious about the pH levels used when growing their green onions, as more research will need to be conducted in optimal conditions - for example, a laboratory - so that more variables can be controlled. Being able to replicate the optimal value of a pH level of 6.5 for green onion root growth derived by Kane et al. is paramount as it can provide useful information for green onion growers to help maximize their production.

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## Appendix

N/A - green onion plant died; C - control; 1 - 1 drop of lime juice in 2L of water; 2 - 2 drops of lime juice in 2L of water; 3 - 3 drops of lime juice 2L of water. Any numbers that follow the aforementioned labels denote sample numbers within the group.

STEM	GROWTH	- Tae	Young	Bae
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cm	C.1	C.2	C.3	1.4	1.5	1.6	2.7	2.8	2.9	3.10	3.11	3.12
Week 0	2.1	2.1	2.5	3.4	2.8	2.8	3.5	4.0	3.6	3.2	3.2	3.0
1	2.8	5	4.3	8.8	6.6	5.7	6	9.8	14.7	N/A	N/A	5.7
2	N/A	5	5.5	10.5	8	6.3	7	13.5	21.5	N/A	N/A	8.5
3	N/A	7	5.7	11	8	7	7.5	14	32.4	N/A	N/A	12.7
4	N/A	7.5	5.7	13.5	8.5	7	7.5	14	32.6	N/A	N/A	12.7

ROOT GROWTH - Tae Young Bae

cm	C.1	C.2	C.3	1.4	1.5	1.6	2.7	2.8	2.9	3.10	3.11	3.12
Week 0	0.5	0.5	1.3	2.1	1.5	1.7	1.3	1.0	1.3	1.7	1.7	1
1	0.8	1.5	2.2	3	3.7	3.7	2.5	3.8	5.5	N/A	N/A	4.3
2	N/A	.3	2.7	3	4	4.5	3.6	4.2	7	N/A	N/A	5.7
3	N/A	2.5	2.7	4.5	5.5	5	4.2	4.7	8.8	N/A	N/A	6.5

4	N/A	2.5	3.0	4.7	5.5	6	4.5	4.7	8.8	N/A	N/A	6.5
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## STEM GROWTH - Joven Gill

cm	C.1	C.2	C.3	1.4	1.5	1.6	2.7	2.8	2.9	3.10	3.11	3.12
Week 0	10.5	11.5	10	10	9.9	10.1	10.1	10.2	10.8	9.2	9	9.2
1	21.7	24.9	27.5	27.4	25.1	24.9	17.6	26.8	26.2	15.3	19.2	18.3
2	34.1	30	35.9	34.8	30	33.3	32.3	40.9	38.4	24.2	20.1	30.6
3	35	41.9	40.3	35	32.4	33.6	42.1	39.4	38.6	32.4	23.2	33.1
4	36	42.3	43.2	36.2	35.8	36	45	39.5	38.6	32.5	24.4	33.2

## ROOT GROWTH - Joven Gill

cm	C.1	C.2	C.3	1.4	1.5	1.6	2.7	2.8	2.9	3.10	3.11	3.12
Week 0	0.8	0.7	0.9	1.2	1	1.4	1	1.3	1.7	1	1	1.4
1	6	5.1	4.6	4.9	4.2	4.7	5.1	5.1	5	4.8	3.8	4.7
2	7	6.3	6.9	5.2	5	5.1	6	6.2	6.4	6.7	4.6	5
3	8	8.5	7	5.3	6.1	6.4	6.3	6.5	6.9	6.8	5.5	5.1
4	8.5	8.5	7.4	5.5	6.3	6.4	7	6.8	7.1	8.1	7.2	5.4

# STEM GROWTH - Austin Chang

cm	C.1	C.2	C.3	1.4	1.5	1.6	2.7	2.8	2.9	3.10	3.11	3.12
Week 0	6.0	4.0	5.0	6.0	6.5	5.0	5.5	6.5	6.5	4.0	4.0	4.5
1	11.0	5.0	12.0	14.0	12.0	10.0	12.5	12.0	14.5	11.0	12.0	4.5
2	12.0	5.0	12.0	25.0	18.0	16.0	21.0	20.0	24.0	12.0	16.5	4.5
3	N/A	N/A	7.0	20.0	18.0	30.0	22.0	22.0	26.0	9.0	4.0	4.0
4	N/A	N/A	N/A	6.0	6.0	30.0	21.0	22.0	20.0	1.0	N/A	4.0

ROOT GROWTH - Austin Chang

cm	C.1	C.2	C.3	1.4	1.5	1.6	2.7	2.8	2.9	3.10	3.11	3.12
Week 0	4.0	7.0	4.0	13.0	12.0	2.0	7.0	4.0	8.0	5.0	7.0	0.5
1	6.0	9.0	7.0	18.0	12.0	2.0	7.0	4.0	8.0	5/0	9.0	0.5
2	11.0	9.0	7.0	18.0	12.0	2.0	7.0	4.0	8.0	6.0	10.0	0.5
3	7.0	2.0	7.0	18.0	9.0	2.0	7.0	4.0	9.0	5.0	6.0	N/A
4	5.0	1.0	4.0	18.0	9.0	2.0	7.0	4.0	9.0	5.0	8.0	N/A

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The shirt of the	three-drops 1 (6.30)	4.8	15.3				three-drops 1 (6.30 )	6.1	ary,a	
	three-drops 216.30)	3.8	19.2			-	three-drops 2 ( 6.30)	4.6	20,1	-
	10 1000 done 2 (E.21)	U.T	182				three-drops 3 (6.30)	5	30.6	(23)
	(macyrops 3 const)	1	10.5	-+-					A TRIMINAL PROPERTY AND ADDRESS	

A A A A A A A A A A A A A A A A A A A	de ilas la sale			the l				-
Date Sample(H)	On November 12:	Pl. I with (cm)		Date	Sample (pH)	root length (cm)	stem length (cm)	-
November 12 Control I (7E)	KUUT length(cm)	Stem lengin ( um )		November 17	Control 1 (1,5)	8,5	36	-
Contract (a contract ( 105)	0	2)			Control 2(7.5)	8.5	42.3	-
Control 2(7.5)	8.5	41.9			Control 3(7.5)	74	43.2	
[introl 3 (7.5)	7	40.3	_		Une-drop 1 (6.70)	5.5	362	
One-drop 1 (6.72)	5.3	35	-		Une-drop 2 (6.70)	6.3	35.8	
One-drop 2 (6.72)	6.1	32,4	_		One-drop 2 (672)	6.4	36	
ONE-drop 3 (6.72)	6,4	33.6			Thurdhard (low)	7	UE	
Two-drops 1 (6.46)	6.3	42,1			Two-dome 21641)	6.9	39.5	
Two-drops 2 (6.46)	6.5	39,4		and the second se	Two drops of ( 16)	7.1	38.6	
Two-drops 3 (G,46)	6.9	38.6			Three-days 3 (6.46)	8.1	32.5	
Thre-dipps 1 (6.30	6.8	32.4			1 mrce-urops + 16/30)	77	24.0	
Threedom 2 (6.3)	55	13.7			Three-arops 2 (6:50)	1.2	0.1,2	
These dona 2 (6.7	0 51	27 1 22			Three - drops 3 (6,30)	5.4	35.04	37
10.55 (0.5)							STATISTICS AND	

100	una store growth 20 days after place rig	
Control 1 (7)	Total Root Growth (cm)	Total Stem Growth (cm)
Control 2 (7.5)	7.7	25.5
Control 3 (7.5)	6.5	30.8
Une-drop 1 (6.72)	4.3	26.2
Une-drop 3 (6.72)	5.3	25.9
Two-drops 1 (6,46)	6	29.2 34.9
Two-drops 2 (6,46)	5.5	37.8 29.3
1wo-drops 3 (6,46)	5.4	27.8
Three-drops 2 (6.30)	6.2	15.4
Three-drops 3 (6.30)	4	24

# One-way ANOVA for root growth:

ANOVA summary	
F	0.3347
P value	0.8003
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R squared	0.03043

# One-way ANOVA for stem growth:

ANOVA summary	
F	1.682
P value	0.1906
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R squared	0.1362

# One-way ANOVA for stem:root Ratio:

ANOVA summary	
F	0.1544
P value	0.9267
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R squared	0.002942