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The Effect of the Sodium Bicarbonate Buffer on the Acidity of Hydroponically Grown Kale

Original Article

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Abstract: The purpose of this experiment was to test the effectiveness of the sodium bicarbonate buffer in decreasing the pH of carbonated water when used to grow kale hydroponically. The results of our previous experiment showed that 75% carbonated water was most effective for kale growth. This might have been because of the acidity of the carbonated water, which is why a buffer was used to stabilize the pH and further enhance the growth of kale. Previous research shows that bicarbonate plays an important role in pH and stabilization of plants. Sodium bicarbonate also promotes the intake of carbon dioxide and other carbons, which is beneficial to plants. In this experiment, there were 6 groups, 3 of which received the buffer with 0%, 75%, and 100% concentration carbonated water and the other 3 that received no buffer with 0%, 75%, and 100% concentration carbonated water. Plant height, root length, dry mass, wet mass, number of leaves, and days the plants took to sprout were all recorded. Bar graphs and line graphs were made, and ANOVA and Tukey HSD tests were conducted to determine statistical significance. It was hypothesized that the buffer combined with the 75% and 100% carbonated water would enhance kale growth by stabilizing the pH. Results showed that the group given 75% carbonated water with no buffer, 100% carbonated water with no buffer, and deionized water with no buffer solution grew most efficiently and the groups given the buffer solution did not grow as efficiently, not supporting our hypothesis.

Keywords: sodium bicarbonate buffer • buffer • acidity • hydroponics • curly kale • carbonated water

1. Introduction

Hydroponics

Humans will have a population of 9 billion by 2050 [1]; therefore, humans are in desperate need of a stable way to increase food production. Conventional farming harms the surrounding environment [2] which was known for a very long time; therefore, the use of hydroponic systems would be beneficial to our society. Hydroponically grown plants use no soil; seeds are planted in water with a base such as Rockwool and given a nutrient solution.

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Various plants are grown in hydroponic systems, such as tomatoes, cucumbers, peppers, eggplants, strawberries, etc. Leafy vegetables also work well when grown hydroponically, such as lettuce, kale, basil, cilantro, and mint [2]. Hydroponic systems are efficient because they are not affected by the weather [3]. These plants are grown indoors where the temperature is controlled. Most farms rely on precipitation for irrigation; therefore, yields are not consistent—growing plants with hydroponics use much less land than soil-grown plants [4]. Additionally, hydroponics systems use much less water than conventional farming. When farming using soil, water that is not quickly absorbed by the roots is lost to percolation [2].

In conventional planting, diseases are passed on through the touching of healthy and diseased stems; however, this is not a problem in a hydroponics system because the plant's roots are distanced so that diseases do not jump from one plant to another. Also, there is no soil through which diseases can be spread; therefore, hydroponically grown plants cannot spread diseases to each other. Throughout the world, about 40-50 percent of all conventionally grown crops are lost to diseases. In the United States, this number is 20-30 percent [5].

Additionally, plants grown hydroponically are not exposed to pests. Pests led to a 26% primary yield loss of crops in the United States and a secondary yield loss of 38% of crops [6]. As you can see, there is a significant loss of crops and money when using conventional farming techniques; however, with hydroponics, pests and diseases will not harm the plants. Because hydroponically grown plants are not as affected by pests and diseases as soil-grown plants, pesticides are not needed [7]. These pesticides affect many aspects of nature, including the surrounding air, lakes, rivers, and food sources for animals [8]. This dramatically impacts the biodiversity of the ecosystems exposed to pesticides. Pesticides kill beneficial microorganisms, fungi and contaminate nutrients. This causes the soil to degrade much quicker; therefore, it can no longer be used for farming. According to a study conducted by US Geological Survey, 90% of water and fish samples from streams in the United States contained at least one chemical from a pesticide in them [9]. Pesticides can also cause serious health problems in humans if exposed. Farmers who apply these pesticides are at high risk of cancer because of the chemicals found in these solutions. The worldwide deaths and diseases due to pesticide poisoning are about 1 million per year [8]. The use of hydroponics systems may significantly reduce these pesticide-induced problems.

Hydroponically grown plants grow much faster in comparison to conventionally grown plants. When using a hydroponic system, many nutrients that would typically be scarce in soil are abundant in the nutrient solution given to them. Furthermore, hydroponic plants grow 25-30% faster than traditionally grown plants [10]. Additionally, the perfect amount and type of nutrients are directly given to the plant; therefore, the plant does not waste any energy for its roots to find viable food. When it comes to leafier plants like kale, lettuce, and spinach, the growth rate is even faster. According to Hydroponics.net, The growth rate for a leafy green hydroponic plant is 30-50% faster than within soil, grown under the same conditions [11]. Due to the growing human population, this would be highly beneficial because farmers would increase the food supply and feed more people across the globe.

One of the final benefits of hydroponics is that you can control the pH levels exceptionally easily. The

Figure 1. **Photosynthesis reaction**

normal pH range for hydroponically grown plants should be maintained around 5.5-6.5 pH. When growing plants conventionally, you must find soil with a suitable pH that can be scarce. With hydroponics, you can add any premixed solution such as pH UP or pH DOWN. The plant is also not hurt by the acidity or basicity of the solution. Plants are usually harmed when there is a pH lower than four or higher than 8. This immediately causes the roots of the plant to be damaged. In turn, the plant starts absorbing harmful heavy metals such as magnesium and zinc [12]. pH levels such as five or seven will not have immediate consequences and only affect the plant's size; therefore, there is more time to change the pH to minimize the effects [13].

Carbonated Water

Carbonated water (CW) is water that has dissolved carbon dioxide gas bubbled into it. This process is called carbonation, which causes the water to have small gas bubbles throughout. Joseph Priestley invented CW in 1767 when he accidentally put a bowl of water above a beer vat in England. He found that this was a method to infuse CW with carbon dioxide; however, this method is very different from today's method. CW is created when water is infused with carbon dioxide gas under pressure, causing small bubbles to form. Furthermore, CW has very minuscule effects on humans and greatly promotes plant growth.

Past research suggests that CW helps soil-grown plants in various ways. According to one experiment that involved applying CW to soil-grown tomato plants soil, nutrient availability for certain nutrients could have been increased by the lower soil pH produced by CW application [14]. As you can see, the plant was able to intake more nutrients when the CW was added. Furthermore, they found that root growth increased greatly due to an increase in carbon dioxide throughout the plant. Carbon dioxide is crucial for the plant to partake in photosynthesis.

The photosynthesis equation in figure 1 1 shows carbon dioxide is important for the plant to make glucose as well as oxygen. Glucose is the food the plant makes on its own to power its other functions. Therefore because of the increase in carbon dioxide from the CW, the plant will be able to make more glucose and be healthier. When more glucose is made, the molecules are combined to make cellulose, which is used to make the cell walls of the plant much stronger. In turn, the plant is more durable, and its leaves will be larger. CW also has other benefits when used with plants. According to an experiment done at Colorado University Boulder, CW makes plants grow much faster and makes plants grow greener than soil plants [15]. Due to the increased greenery of the plant, it would most likely stay fresh for a longer time and because it is growing faster you could grow more plants than usual during a season.

In an experiment in which CW was applied to a soil-grown plant, the yield of the plant being grown increased as CW was added to the soil. This would make the harvest the farmers are growing even more profitable than

usual when using CW. Specifically, marketable fruit yields increased by 7.8% when CW was applied to the soil-grown plant [14]. Furthermore, fruit yields had increases in fresh-market and total fruit yields that averaged 16.4% and 15.996%, respectively [14]. Clearly, CW has very profitable and beneficial effects on the yields of soil-grown plants.

In our previous research experiment, different concentrations of CW were tested on hydroponically grown kale and 75% CW (1000mL of water in total with 750mL of CW and 250mL of distilled water) was the most efficient at growing kale in a hydroponic setting. It was hypothesized that the CW might have been too acidic for the plants as the 100% concentration of CW had lower efficiency than the 75% concentration. This acidity resulted in less efficient plant growth. A low pH within a hydroponic plant's water/nutrient solution has been observed to cause the plants to be smaller due to nutrient deficiencies, possibly lowering the rate of photosynthesis within the plant. This was what inspired this experiment, to combat the adverse effects of the low pH in the CW.

Buffers

A buffer is a solution that is used to stabilize the pH of the nutrient system at a preferred level. A buffer consists of a weak conjugate acid-base pair [16], explaining its slight pH changing abilities. Buffer solutions are used in many previous experiments to alter the pH of the solution due to an additive. The pH could be monitored using a pH meter. In one experiment based on the effect of buffers on the stability of plants' phenolic compounds, the pH was unstable, affecting the outcomes of the experiment. However, when introducing a buffer solution composed of chlorogenic and ferulic acid, the stability of the plant's growth was directly connected to the pH, which made adding a buffer to this planting system beneficial [17]. A buffer resists any major pH changes.

Sodium Bicarbonate Buffer

Sodium Bicarbonate (NaHCO3) is a salt of a weak acid containing sodium, hydrogen, carbon, and oxygen molecules with a pH of about 8.3 when higher than 0.01 M [18]. This makes a bicarbonate buffer, which is commonly used in biochemistry as a method of neutralizing acidity. Adding sodium bicarbonate to the CW would stabilize the pH of the CW and allow for the plant to be able to thrive. Specifically, this buffer works best to stabilize alkaline solutions, making it a good buffer for this experiment due to the need for the solution to become basic. Using sodium bicarbonate as a buffer can also make it harder for fungus to infect the plants due to keeping the plant at a healthy pH. According to the National Center for Biotechnology Information, sodium bicarbonate is a white, crystalline powder that is not only a buffer but an electrolyte replenisher, systemic alkalizer, and a cleansing solution [18]. Bicarbonate in general plays a fundamental role in the pH status and stabilization of many organism cells. Sodium bicarbonate also causes plants to intake carbon dioxide and other carbons more efficiently [19]. The bicarbonate helps contribute to carbon concentration mechanisms [19].

According to another previous experiment, when a pH 9.5 carbonate/bicarbonate buffer solution, similar to a sodium bicarbonate buffer was used for the soaking of bacalao (salted cod), the yield increased, and the cod presented better protein functional quality [20]. A Sodium bicarbonate buffer can stimulate the growth of plants

when deficient in nutrients like iron with the desired pH of 7.3 as seen in a previous experiment [21]. According to a previous study, sodium bicarbonate combined with Metschnikowia fructicola and ethanol also significantly reduce the number of decayed berries [22]. According to another experiment, adding sodium bicarbonate to non-heated fruit significantly reduced the decay severity caused by P. expansum [23]. Sodium bicarbonate reduces decay rates in plants. This outlines several benefits of this buffer in addition to its ability to neutralize the pH of substances that are added to the buffer.

Kale

In this experiment, kale was used for a variety of reasons. Specifically, curly kale was used, also known as Brassica oleracea. An image of curly kale is shown in figure 2. Kale originated in northern Turkey and Greece. The curly kale that was used grows up to 2-3 feet, can withstand cold temperatures, and has rough curly leaves [24]. Kale belongs to the mustard family. Plants like broccoli, cabbage, cauliflower, and arugula are also in the mustard family [25]. The mustard family is defined by the word "Brassica" which is shown in their scientific names. The mustard family is one of the largest plant families as it contains around 3,000 species of plants [25]. They are found on all continents except Antarctica. The plants of the mustard family grow in different climates. Some grow in temperate and hot climates while others like kale grow in colder climates. The ideal soil pH for growing kale is 6.0 to 7.5 [26]. Many members of the mustard family are of extreme economic importance to different countries. For example, cabbage, canola, radish, kale, and mustard make up a large part of the agricultural trade [17]. While kale does share many similarities with the other plants in the mustard family, it also has some differences. Kale is one of the only mustard plants that do not form a compact head like lettuce. Instead, its leaves are thin and spread out. Furthermore, kale's flavor and color differ when it is grown in a warmer or cooler temperature unlike other plants found in the mustard family.

Figure 2. **Curly Kale Plant.** [Source]

Kale is rich in nutrients and vitamins. Specifically, it is extremely rich in Vitamin K, Vitamin C, Vitamin A, and Vitamin B. It also has a large amount of fiber, which maintains stomach and bowel health [16]. Kale,

as well as other leafy green vegetables, could also benefit people with certain health problems. Kale has many antioxidants, which help protect the body's cells from radiation and other toxins. Antioxidants can also prevent heart disease. According to the USDA, "green leafy vegetables may be one of the best cancer-preventing foods. Studies have shown that eating 2 to 3 servings of green leafy vegetables per week may lower the risk of stomach, breast, and skin cancer" [5]. Due to their low-calorie count and high nutrient count, they are very good for maintaining a healthy body weight [27]. Additionally, people with type-2 diabetes can benefit from kale's large number of dietary fibers.

Kale has recently grown in popularity due to its relatively cheap price and the nutrition gained from consuming it. Kale is now served in many popular restaurants in salads and smoothies due to its versatility. Kale is often a choice for many different types of athletes. This spike in popularity is due to its abundance in the colder regions of the United States; however, kale has always been relatively popular throughout the Middle East. It is used in a variety of different cultural foods. In the north of Turkey, it is especially consumed as a major food during the winter and spring months.

The purpose of this experiment is to use the combination of a buffer and CW to effectively grow kale in a hydroponics system. The novelty of this experiment is that kale is a very beneficial plant and if grown faster and more efficiently, can benefit humans in many ways. This method can also be used to effectively grow other plants as well.

2. Methods

Preparation

To prepare testing for this experiment a variety of different materials and systems were used. To grow the plants in the hydroponic system, rock wool/mineral wool squares were used. These pieces of molten rock and minerals act as a sponge to hold in water for the seed to grow in [29]. Throughout each rock wool square, there was a hole to place each seed. The roots of the plant attach around the square. The rock wool almost acts as soil because the roots of the plant absorb the water and nutrient solution from there.

The trays the plants were grown in were simple takeout trays. Each tray could fit ten mineral wool squares that held ten kale seeds. These trays were not changed throughout the experiment. After the growing cycle was completed, the trays were washed and reused for other planting experiments. To supply the plants with the correct amount and type of nutrients, an already mixed solution called MaxiGro was used. This solution is not harmful or considered hazardous, therefore, only gloves and goggles were worn when handling it. According to the MaxiGro manufacturer website, MaxiGro contains Ammonium Nitrate, Ammonium Molybdate, Calcium Sulfate, Cobalt Sulfate, Copper Sulfate, Iron, Manganese, Magnesium Sulfate, Potassium Borate, Potassium Nitrate, Potassium Phosphate, and Zinc Sulfate (Alternative Garden, 2013). All these compounds and minerals account for the plant's nutrients that promote growth. Without these nutrients, the plant would not grow as fast or as healthy.

Nutrient solutions are crucial to the hydroponic system because this is the only way the plant can get the correct amount of nutrients.

The buffer solution used was simply added to the plant's nutrient solution before being placed into the trays with the plants. As stated, it was composed of sodium bicarbonate (NaHCO3) with a molarity of 0.05M. These were both obtained in powder form as both chemicals are soluble in water. As seen in Table 1, Groups 1, 3, and 5 were given the buffer solution while groups 2, 4 and 6 were not. The buffer solution was changed along with the nutrient solution twice a week with measurements in proportion to the amount of water supposed to be added to the plants.

The CW that was used contained no other minerals and nutrients because this would interfere with our experiment. Instead, a brand of CW, specifically from Trader Joe's, which only contained dissolved carbon bubbles was used. Due to the data obtained from the previous experiment, the groups were given a 75% concentration of CW within the nutrient solution as seen in table 1, because the plants exposed to the concentration had the most efficient growth. A 100% concentration was also used because it contains the most CW even though it did not grow as efficiently as the 75% due to a change in the pH from the CW. A 0% concentration was given to monitor the full effects of the buffer on the growth of the plants. Therefore, if the pH is stabilized, it may be even more beneficial than the 75% concentrations. The water as well as the rest of the nutrient solution was changed once a week. The amount of water, however, was changed throughout the experiment.

Planting and Maintenance

Goggles, gloves, and an apron were worn as safety measures due to the chemicals being worked with. The steps shown depict how to make 2L of nutrient solution for a 75% CW concentration and a 100% CW concentration. Firstly, a 2 L solution of 75% CW was prepared by measuring out 1.5L of pure CW with 0.5L of deionized water in a graduated cylinder and combining. Proceeding that, 8.4 grams of sodium bicarbonate were weighed using an electronic balance. The sodium bicarbonate was added into the beaker with the 2L CW solution. To prepare 2 L of 100% CW solution simply measure out 2 L of CW with a graduated cylinder and combine with the same amount of the buffer as the 75% concentration. The final pH was 8.3 with the buffer. Depending on the growing period of the plants, the necessary quantity of nutrients was added to the solution as well.

Twenty Rockwool squares were wet using deionized water and placed on paper towels and into two trays. A kale seed was placed in each of the ten holes in the rockwool mineral squares. The plants were kept in a cart with a light to mimic the natural sunlight given to the plants. As seen in Table 1, a 75% concentration of CW was used for Group 2 and the 75% concentration of CW with the buffer was used for Group 1. The solutions were added until the level was 1cm and this water level was maintained until the plant sprouted. Furthermore, a 100% concentration was given to groups 3 and 4, with the buffer being given to group 3 but not group 4. Lastly, a 0% concentration was given to groups 5 and 6. The buffer was given to group 5 and not group 6. The water solutions were changed once a week. When the plant sprouted, the water level was adjusted so that the Rockwool was 1/3

submerged. When there was no access to the plants, extra water was added, or the container was covered with saran wrap to account for evaporation.

When the plants were first planted, no MaxiGro was given as the seed itself contains all the needed nutrients for the plant to sprout. After the plants sprouted, however, 1 teaspoon of MaxiGro was given per refilling and the electro-conductivity (EC) was monitored daily. If the EC was lower than 1, more MaxiGro was added to keep the EC at 1. After 2 weeks, the amount of MaxiGro was increased to 2 teaspoons but the EC level was kept around 1. The plant's water was refilled more often. As mentioned, this amount should be added to the buffer solution when being made. A pH meter was used to moderate and monitor the pH. If we noticed the pH was too low or too high, the amount of nutrients given was altered. However, due to the buffer, this did not happen. Condensed methods are shown in figure 3. The methods used to find the data points are below (Tab. 1).

Table 1: Experimental Setup

Plant Group Number	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
CW Concentration	75%	75%	100%	100%	0%	0%
With or without Carbonate-Bicarbonate Buffer	Buffer was given	No buffer was used	Buffer was given	No buffer was used	Buffer is given	No buffer was used
Sodium bicarbonate Concentration (M)	0.05M	0	0.05M	0	0.05M	0
Mass of Sodium Bicarbonate Given	8.4 g per 2L of water	0	8.4 g per 2L of water	0	8.4 g per 2L of water	0

Measurement

The first data point that was found was the root length for each of the plants. This was calculated once the plant was fully-grown. To do this the plant was removed from the mineral wool base. Then the plant roots were laid out in a straight line over a paper towel and measured using a ruler. After finding this for all plants within each group, it was averaged together for each group to find the mean root length. The second data point found was the daily growth rate from the beginning of the experiment until the end. As stated, every day throughout the experiment the height was found using a ruler. The plants were measured from the top of the Rockwool to the top of the plant.

To find the number of leaves each plant had when the experiment was complete, the leaves were simply counted for each of the plants within the groups after the plant sprouted. Following this, the data was averaged for each group to find a concise mean number of leaves. The fourth data point was the leaf growth rate. As mentioned, every day, the number of leaves for each plant was counted after sprouting and separated by group number. The fifth data point was the wet mass of each of the kale plants. This was done by removing each of the plants from the Rockwool and finding their mass in grams. After this, the data was averaged together for each group. The sixth data point found was the dry mass of the kale plants. This was found by removing the plants from its Rockwool base and placing them in an incubator for 4 days at 40 °C. The mass was then found with an analytical balance.

The seventh data point found was the number of days it took for each plant to sprout. From the start of planting to when the seed sprouted, the number of days it took to sprout was recorded. After this, the number of days were averaged together for each group. The eighth piece of data found was the sprouting rate for each plant which is discussed in the data analysis below.

Data Analysis Plan

Average root length and standard deviation were calculated using Microsoft Excel. No equation was used for this data point. Only standard excel functions were used. This data was also put into a multiple bar graph showing the averages and the root length. The chart was titled Buffer Concentration vs. Root Length. The statistical method used was ANOVA followed by Tukey HSD. The application used was astatsa.com to retrieve all of our ANOVA test results.

The second data point was the growth rate for the plants. The data is simply stored in Microsoft Excel. For this data point, both mean and standard deviation were calculated in excel along with the height in centimeters for each day. To calculate the actual rate, the averages for the plants were calculated at the beginning of the growing period and the end of the growing period along with the time. The equation used was.

$$\frac{Height2 - Height1}{Time2 - Time1}$$

The statistical method used was ANOVA due to there being more than two data groups.

The third data point was the number of leaves each plant had. Like the previous data points, it was recorded in Microsoft Excel. Both the averages and standard deviation were calculated using standard functions. A bar graph was used to represent the data to clearly show the differences in leaves for the plants. The bar graph along with the table showed the leaf number over the buffer concentration of CW. ANOVA was used to compare groups.

The fourth data point was the leaf growth rate. To calculate the rate, the equation:

$$\frac{LeafNumber2 - LeafNumber1}{Time2 - Time1}$$

was used. Leaf number 1 represents the number of leaves at the beginning of the experiment. Time 2 and time 1 represent both times at the beginning and end of the experiment, respectively (day number).

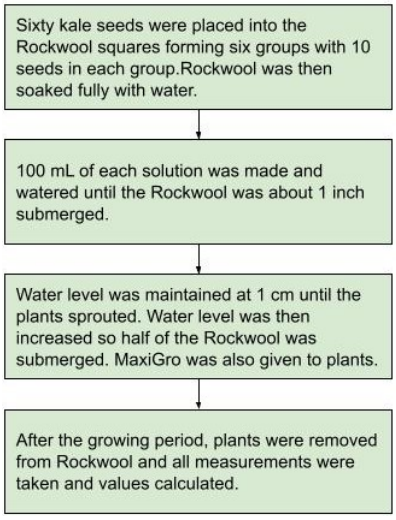

Figure 3. **Methods**

The fifth data point was the mass of the kale plants before they dried. Both the mean and standard deviation were calculated using standard functions along with the mass values in grams. The graphs included were a multiple bar graph made in excel. The statistical method used was ANOVA since there were more than 3 data points.

The sixth data point was the mass of the kale plants after drying with all moisture removed. Both the mean and standard deviation were calculated using standard excel functions. The graphs included were a multiple bar graph made in excel. The statistical method used was ANOVA since there were more than 2 data points.

The seventh piece of data that was collected was the number of days each plant took to sprout, shown in a table along with the number of days. The average was calculated using standard excel functions. The data was placed in a multiple bar graph. The statistical method used was ANOVA because there were more than 2 data points.

The eighth data point being found was the sprouting rate of each plant. Like the previous data points, it was recorded in Microsoft Excel. The equation used to find the sprouting rate was:

$$\frac{Amount\ of\ plants\ germinated}{Total\ amount\ of\ plants}$$

The top value is the total number of plants germinated within the group over the total number of plants. This was shown in a table and a multiple line graph. The line graph was the number of plants germinated vs. number of days. The table showed this as well as the rates. ANOVA was followed by Tukey HSD. The website used to find this was astasta.com.

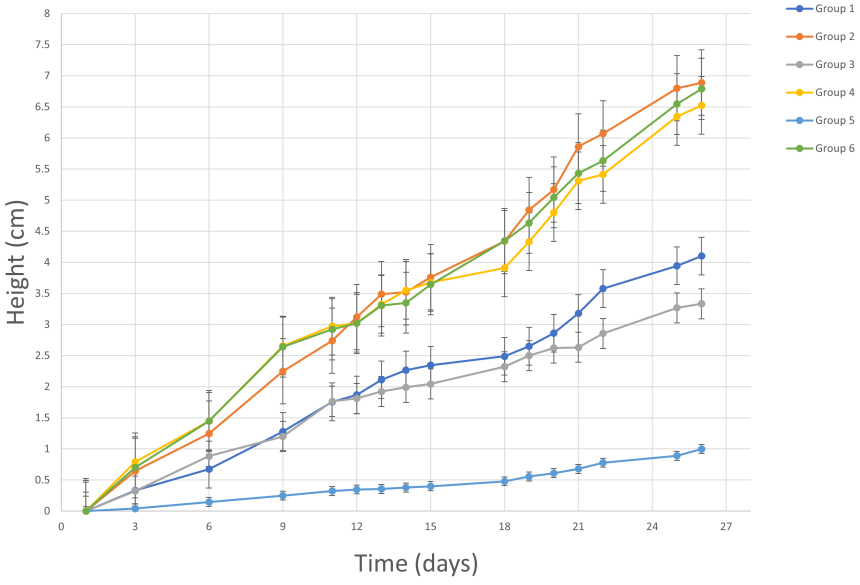

Figure 4. **Plant Height vs. Concentration.** The graph shows the mean height calculated in centimeters for all six groups of plants within the 28-day growing period. While for all groups, as time increased height also increased, Groups 2 (75% concentration of carbonated water with no buffer), 4 (100% concentration of carbonated water with no buffer), and 6 (deionized water with no buffer) all had the greatest heights over time. Groups 1 and 3 had similar growth as well but not as efficiently as the aforementioned three. Group 5 had the least growth of all the groups.

3. Results

Fig. 4 shows that all groups increased in height over time. However, Groups 2, 4, and 6 displayed the highest plant height compared to the other groups. These were the groups that received no buffer but different concentrations of carbonated water or no carbonated water at all. Groups 1 and 3, the groups that received the buffer in combination with the carbonated water, also showed similar growth but not as much as the previously mentioned groups. Finally, group 5 showed the least growth over time. This was the group given just the buffer. The average height only went up to about 1 cm while the highest groups went up to 7 cm.

Fig. 5 shows the root length of each group. The figure depicts that Group 6 had the longest roots followed by Group 2 and Group 4. These were the groups that received 0,75, 0r 100% carbonated water with no buffer. Followed by these three groups, Groups 1, 3, and 5 had the shorter roots with Group 5 having the shortest. This group was the group that received just the buffer. It can be seen the groups given the buffer had shorter roots than the roots that were not given the buffer. In addition, the root length of Group 6 was significantly higher than all other groups. The root length of Groups 2 and 4 was only significantly higher than Groups 1, 3, and 5 but not to each other. Finally, the root length of Group 5 was significantly lower than all other groups.

Fig. 6 shows that Groups 2, 4, and 6 had the highest number of leaves but were not significant to each other. The number of leaves for these three groups was significantly different than Groups 1, 3, and 5. Groups 1 and 3 also had a similar number of leaves but were significantly higher than Group 5 which had the least number of

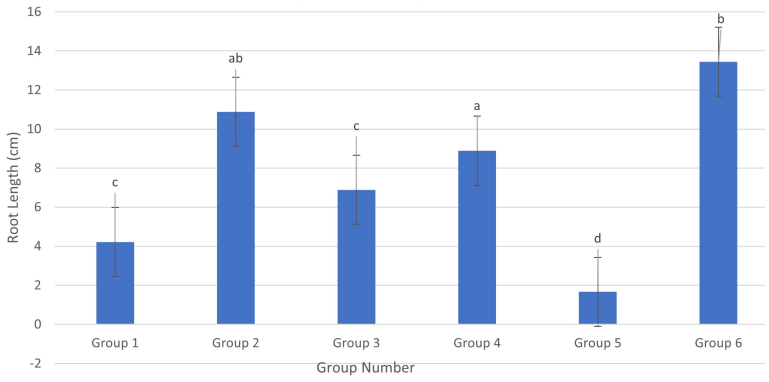

Figure 5. **Root Length vs. Concentration.** This bar graph shows the average root length in centimeters for each plant group. The error bars shown in the graph represent +/- SEM. ANOVA tests followed by Tukey HSD tests were done. Different letters on each bar represent groups that were significantly different from each other. Group 6 having the largest average root length is significantly different from the rest of the groups (p¡0.01) except group 2. Groups 2, 3 and 4 all had similar root lengths and are also statistically different from groups 1 and 3 (p¡0.05). The graph shows that when given the deionized water without a buffer, the plants had longer root lengths. Error bars represent +/- SEM.

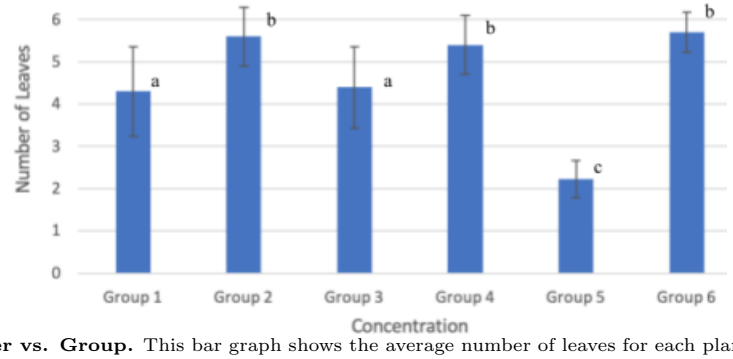

Figure 6. **Leaf Number vs. Group.** This bar graph shows the average number of leaves for each plant group. Groups 2, 4 and 6 had the largest number of leaves. The error bars shown represent +/- SEM. ANOVA as well as Tukey HSD tests were done. The groups given 75%, 100% and deionized water with no buffer (2, 4 and 6) were significantly different compared to the rest of the groups (p¡0.01). Groups 1 and 3 were significantly different from group 5 (p¡0.05), suggesting that group 5 had the least number of leaves. Different letters on each bar represent groups that were significantly different from each other.

leaves.

Fig. 7 shows that Groups 2 and 6 had the highest wet mass followed by Group 4. These three groups received just carbonated water or deionized water and were statistically similar to each other but significantly higher than Groups 1, 3, and 5. Group 5 had the lowest wet mass and was the group that received just the buffer.

Fig. 8 shows that Group 2 had the highest dry mass followed by Group 6 and Group 4. Again, these were the groups that received just carbonated or deionized water with no buffer. Group 1 and 3 had the lowest dry mass followed my Group 5.

Fig. 9 shows that Groups 2, 4, and 6 had the highest plant height on Day 26. This was followed by Groups 1 and 3. Group 5 had the lowest plant height.

Fig. 10 shows that Group 2, 4, and 6 sprouted the fastest and Group 5 took the longest to sprout. Wile it

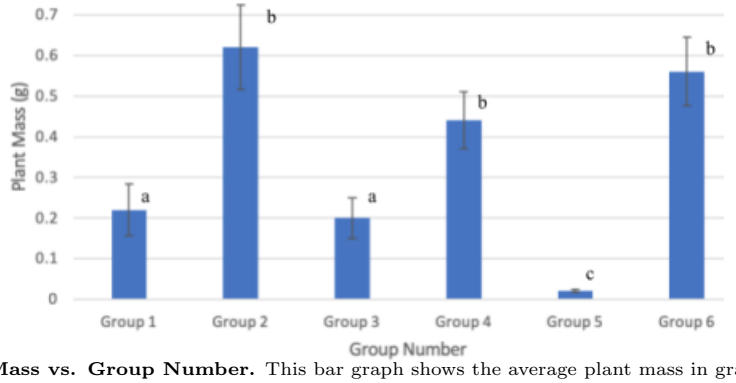

Figure 7. **Wet Plant Mass vs. Group Number.** This bar graph shows the average plant mass in grams for each plant group. The error bars shown in the graph represent +/- SEM. ANOVA as well as Tukey HSD tests were done. Different letters on each bar represent groups that were significantly different from each other. Groups 2, 4 and 6 had the largest wet mass and had data that was more significantly different from the other groups (p¡0.01). Like the previous graph, groups 1 and 2 were significantly different from group 5 (p¡0.01) showing that group 5 had the smallest average wet mass.

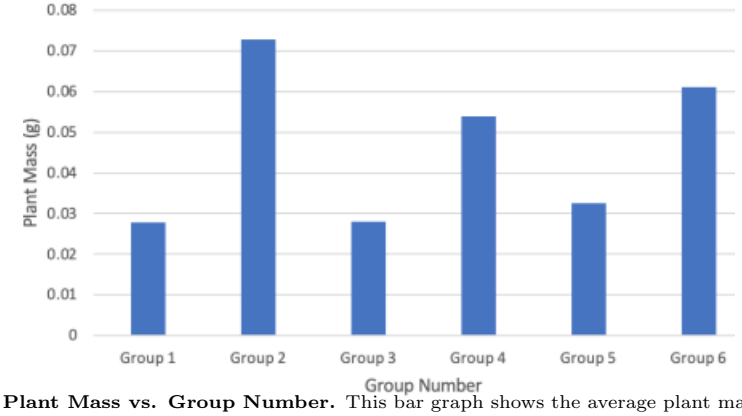

Figure 8. **Total Dry Plant Mass vs. Group Number.** This bar graph shows the average plant mass in grams for each plant group. ANOVA and other statistical tests were not able to be performed, due to the plants being stuck together when removed from the incubator after drying. However, it is clear from the graph that groups 2, 4 and 6 had the largest dry mass corresponding to the wet mass in figure 6. Meanwhile the rest of the groups all had similar masses as well.

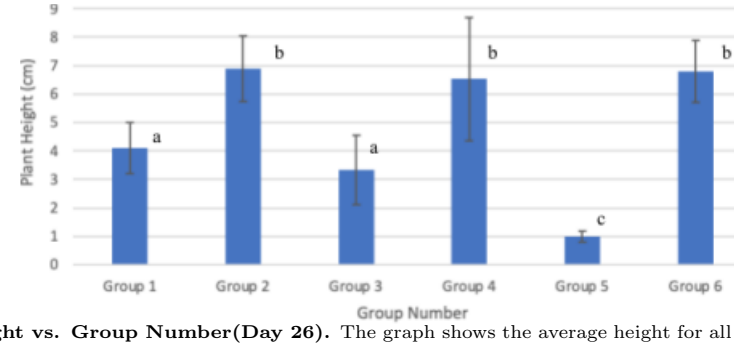

Figure 9. **Plant Height vs. Group Number(Day 26).** The graph shows the average height for all groups of plants compared on Day 26 in centimeters. This day was used because all plants grew to a tall height, and it was the final day the plants were measured. The error bars represent +/- SEM. ANOVA and Tukey HSD tests were completed. Different letters on each bar represent groups that were significantly different from each other. Group 2, 4 and 6 were statistically different from group 1, 2 and 3 (p¡0.01). Group 1 and 3 are also statistically different from group 5 (p¡0.01). This, like the previous graphs, is showing groups 2, 4 and 6 having the most growth while group 5 has the least efficient growth.

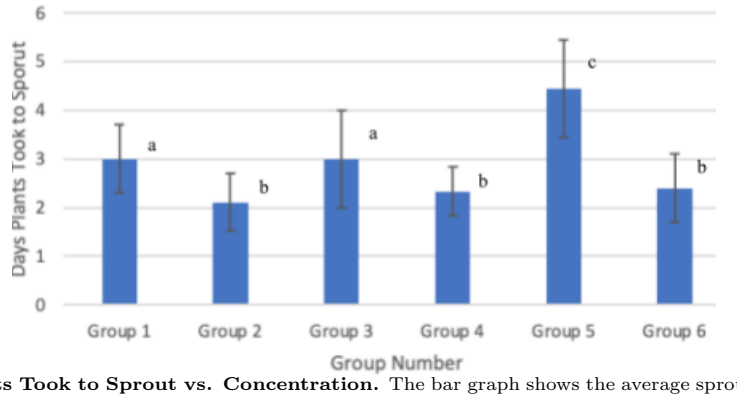

Figure 10. **Days Plants Took to Sprout vs. Concentration.** The bar graph shows the average sprouting rate for all different concentrations. As mentioned, each group contained 10 plants and were given different amounts of carbonated water. The error bars represent +/- SEM. Different letters on each bar represent groups that were significantly different from each other. ANOVA and Tukey HSD tests were completed. Group 2, 4 and 6 were significantly different from group 1 and 3 (p¡0.05) as well as group 5 (p¡0.01). Groups 1 and 3 were also significantly different from group 5 (p¡0.05). This shows that groups 2, 4 and 6 had the fastest sprouting rate while group 5 had the longest sprouting rate.

took between 2-3 days for Groups 2, 4, and 6 to sprout, it took 4.5 days for Group 5 to sprout. Groups 1 and 2 sprouted in between these groups.

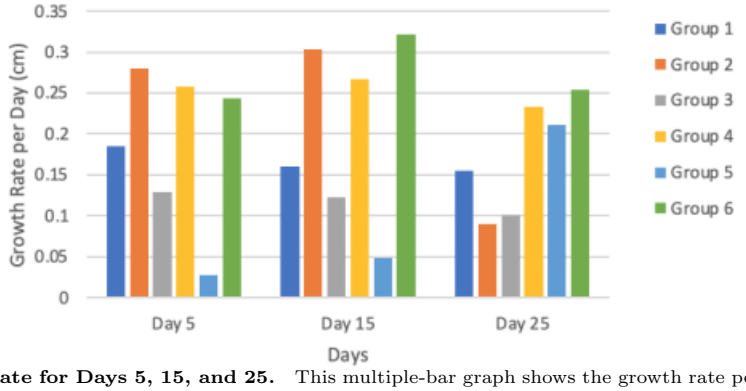

Figure 11. **Growth Rate for Days 5, 15, and 25.** This multiple-bar graph shows the growth rate per day on days 5, 15, and 25 in centimeters per day for all six groups. Each group had 10 kale plants and was given various concentrations of carbonated water as well as different amounts of the buffer. Error bars were not used due to the graph only showing a rate as no averages or standard deviation was calculated. Similarly, statistical tests were not calculated for the data due to each point making up one value instead of a group. The rate was found by using the previously mentioned equation and the average height from the three days selected, i.e., $(Height2 - Height1)/(Time2 - Time1)$.

Fig. 11 shows varying growth rates. On Day 5, Group 2 had the highest growth rate followed by Groups 4 and 6. On Day 15, Group 6 had the highest rate followed by Groups 2 and 4, respectively. On Day 25, Group 6 had the highest growth rate followed by Groups 4 and 5. For the most part, Groups 2, 4, and 6 had the highest growth rate throughout.

4. Discussion/Conclusions

The group that received 75% carbonated water as treatment had the best growth qualities followed by the group that received pure deionized water and the group that received 100% carbonated water. Overall, these three groups showed significnatly better properties compared to the groups that received the buffer. Therefore, the sodium bicarbonate buffer had no positive impact on the growth of the kale. In fact, it proved to be detrimental to the plants by slowing down its growth. As seen in the graphs, the groups with just carbonated water and no buffer had the largest heights, root lengths, number of leaves, and masses. Therefore, our hypothesis was not supported as the groups with the buffers did worse than the groups without the buffer. The plants in all groups grew at similar steady rates except for group 5 which showed minimal growth throughout the whole experiment with the smallest mass and fewest leaves. This is very significant because it showed that the buffer did not enhance the growth of the kale in our experiment. The results of this experiment actually support the results of our previous experiment, proving that 75% carbonated water enhances plant growth.

There were some limitations to the experiment. For instance, the measurements might have some errors, specifically the plant heights because the plants started to tilt over making it harder to measure the height. Similarly, the root lengths were harder to measure because they were harder to separate. Another problem that arose was trying to keep the EC of our solution below 1. Adding the smallest amounts of MaxiGro sometimes led to an EC above 1, which resulted in the solution needing to be remade.

Future research can explore why the bicarbonate buffer hindered plant growth when it was supposed to balance the pH of the carbonated water. Although, it can be inferred that the sodium in the buffer was what yielded these negative results. Research shows that excessive amounts of sodium can actually stunt plant growth. Excessive salts in the soil reduce plants' uptake of water and while it won't show other symptoms, plant growth is still stunted [30] This same idea could be true with hydroponics. This is a possible explanation for our results.

5. Future Directions

Our future research would be to find another way, possibly another buffer that could help solve our problem and enhance plant growth at the same time. All in all, the data reinforced our previous experiments and shows that a 75% concentration of carbonated water best promotes the plant growth of kale, however, shows that the buffer did not help in stabilizing the pH and enhancing the plant growth.

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Conflict of Interest

Authors of this article declare that they have no conflict of interest.

Human Studies/Informed Consent

No human studies were carried out by the authors for this article.

Animal Studies

No animal studies were carried out by the authors for this article.

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