<u>Comparing the Effects of Various Solutions on the Lifespan of Cut Flowers</u> Lydia Huntsman, Veerpal Kingra, Sabrina Moore lydia.huntsman16@gmail.com, kingraveerpal@gmail.com, moore.sabrina1999@gmail.com

Abstract:

The objective of this study was to test sugar, a sugar-vinegar solution, and a sugar-bleach solution (sugar, sugar + white vinegar, and sugar + bleach) in their effectiveness of preventing carnation flower petals from wilting. The larger implications for this report will allow us to be able to give individuals the ability to keep their flowers alive for longer after being cut. We performed an one-way ANOVA test on the four groups which gave us a p-value of 0.0082. A Tukey-Kramer test revealed that the base group (bleach + sugar solution) was statistically significant from the water group (p-value= 0.0174), sugar group (p-value=0.0193), and the acid group (p-value= 0.0124). We predicted that the base group was more effective. Therefore, we suggest that perhaps the pH effects were overpowered by the characteristics of bleach itself. The bleach could be acting as a strong antimicrobial solution such that when added to the solution, it decreases the amount of bacteria in the xylem allowing for a greater absorption of water by the carnations, preventing wilting.

Introduction:

Preserving the lifespan of cut flowers allows people to appreciate the natural beauty of flowers for longer. Cutting a flower from its stem does not immediately kill the flower; wilting is a process of an increase in 1-aminocyclopropane-1carbocylic acid synthase activity, ethylene-forming enzyme activity, ethylene production, and membrane permeability (Woltering et al., 1993). The objective of this study was to compare the effects of four different solutions on the lifespan of cut flowers in order to determine which is the optimal solution to extend their lifespan.

We used four different testing groups, with a different solution used for each. Our control group was placed in tap water with nothing added to it. This allowed us to compare the effects of water alone to water with substances added. One of these additions was sugar, as when sugar is added to cut flowers, the loss of solutes is delayed (Nichols & Acock, 1979). It has been

suggested that this is because sugar maintains the integrity of cell membranes. (Nichols & Acock, 1979). Sugar will not change the pH of a solution (Gillespie).

As bleach and vinegar have both been cited as solutions that can delay wilting (Reader's Digest), we also chose to test the effect of solutions made with each of these substances. As vinegar is a low pH solution and bleach is a high pH solution, this allowed us to compare the effects of a high pH environment and a low pH environment on cut flower lifespans. Low pH limits the number of bacteria in the stems, which prevents blockage of the vascular system and allows water to reach the flower faster (Doom & Perik, 1990). A low pH solution of Aminooxyacetic acid (AOA) combined with sugar was found to suppress flower drooping and delay the senescence of open flowers (Rattanawisalanon et al., 2003). Contrarily, the ability of water to move through stems has been found to be decreased in a high pH environment (Reid, 2009).

Plants wilt as a consequence of not having enough water, leading to the cells collapsing, hence the wilting that we observe (Petruzzello). We predicted that if being placed in a low pH environment would allow water to travel through the plant stem faster, preventing wilting (Doom & Perik, 1990) then the flowers placed in a mixture of water, white vinegar and sugar should have the lowest percent of wilted petals at the end of the experiment compared to the other groups. Our null hypothesis was that there would be no difference in means between the four groups.

Materials and Methods:

We purchased 12 cut *Dianthus caryophyllus* (carnations) flowers from the Save-On-Foods Dunbar grocery store. We then made a 2% sugar solution by dissolving 1.28 Tbsp of Rogers brand white sugar into 32 oz of boiling water. This solution was set aside to cool. We made an acidic solution by mixing 2 Tbsp of Western Family brand white vinegar into 8 Tbsp of water, creating a 5x dilution. Using a homemade litmus paper, we estimated the pH of this solution to be between 3 and 4. Then, we made a 10x bleach dilution by mixing 1 Tbsp of Clorox brand bleach with 9 Tbsp of water. Using a homemade litmus paper again, we estimated the pH of this solution to be between 10 and 11. We then took 12 identical Heineken 330 ml beer bottles, and labeled 3 bottles as controls, 3 as sugar, 3 as acid and 3 as base, numbering the bottles from 1-3 in each respective group. We filled the control bottles with 3 Tbsp of tap water. We filled the sugar bottles with 3 Tbsp of our cooled sugar solution. The acid bottles were filled with $\frac{1}{2}$ tsp of our vinegar solution and the remaining 2.8 Tbsp was filled using the sugar water. Similarly, the bleach bottles were filled with ¹/₂ tsp of the bleach dilution and 2.8 Tbsp of sugar solution. We then placed one cut flower in each of the bottles. Since the flowers had multiple flowers per stalk, we decided to measure the wilting of the tallest one in each case to maintain consistency. These were also marked on the stalk with a sharpie to prevent any possible confusion during data collection. The bottles were placed on the window sill of our kitchen in a bright, sunny place, to ensure that they all received the same conditions. Figure 1 provides an image of a sample experimental set-up. The bottles were rotated randomly every day in order to reduce the effects of any potential variables such as access to sunlight or position in the kitchen. Additionally, we changed the solutions in each bottle every three days (as recommended by the woman who sold us our flowers at Save-on Foods). In order to determine which solution was the most effective, we recorded the percent of individual petals wilted each day. Amount of wilting was measured based on the change in texture of the petals, as they became more wrinkled. Based on our observations, the first petals to wilt were typically those at the base of the flower, closest

to the stalk, and the wilting gradually spread up the flower. Therefore, this is where one should look for the first signs of wilting if one were to recreate this experiment. We ended our experiment when the petals of one flower were 100% wilted, which occurred after 11 days of observation.

All statistical analysis was done on R-studio. We log transformed our data from day 11 of our experimental period in order to normalize it. We then used a one-way ANOVA test on the transformed data in order to determine whether our results were significant. Once it was determined that results were significant, a Tukey-Kramer test was used to determine where differences in means between the groups were found.



Figure 1. A flower from each of the four groups being compared on the last day of data collection. This image shows the experimental set up for this study, including flowers, bottles, labels and position on windowsill. Markings on stalks not visible. From left to right: control 1: 100% wilted, sugar 2: 57% wilted, acid 2: 85% wilted, base 2: 20% wilted.

Results:

Results from the Duration of the Study





An Analysis of Variance (ANOVA) test was performed on the log transformed data, which resulted in a p-value of 0.0241. In order to determine which of the groups differed from each other, we ran a Tukey-Kramer test. The Tukey-Kramer test revealed that the base group (bleach + sugar solution) was statistically significant from the water group (p-value= 0.0174), sugar group (p-value=0.0193), and the acid group (p-value= 0.0124). No other groups showed a statistically significant difference.



Figure 3. Log-transformed percent wilted flowers for all experimental groups. The lines above and below data points indicate 95% CI.

Discussion:

Based on the results of our ANOVA test, our p-value of 0.024 indicates that we can reject our null hypothesis that there are no differences in group means, as it is below the alpha value of 0.05. We chose this significance level as it is a standard alpha value used in scientific research. The results of our Tukey-Kramer test show that the only group which had a significant difference in the log values for percent wiltedness after 11 days of observation was the base group. This is exhibited in Figure 3, which shows that the 95% confidence intervals of the other 3 groups overlap, while the base group shows no overlap with the others. Additionally, our Tukey Kramer test determined that the mean of the bleach group was statistically different from the water group (p-value= 0.0174), sugar group (p-value=0.0193), and the acid group (p-value= 0.0124). There were no other statistically significant differences between any of the other groups. These results differed significantly from our hypothesis, which was that if a low pH environment allow water to travel through the plant stem faster, preventing wilting, then the flowers placed in a mixture of water, white vinegar and sugar would have the lowest percent of wilted petals at the end of the experiment compared to the other groups. This hypothesis was developed based on the results of previous studies (Doom & Perik, 1990; Jones, 2001).

Our results did not correlate with our hypothesis. In fact, they went against much of what the literature suggested about the effects of high pH environments on cut flower survival. Studies such as Reid's (2009) found a decreased ability of water to move through stems in high pH environments. Previous studies have noted that an increase in pH actually increased the bacterial counts in the vases of cut flowers and maintaining the pH at low levels conserved the bacterial numbers to a minimum (Rattanawisalanon et al., 2003).

The optimal pH level for carnations is known to be 6-7 (Boeckmann, 2019). It is possible that carnations are less tolerant to low pH environments than other flower species in the aforementioned studies. The low pH environment induced by white vinegar may have been too far out of the carnation's optimal range. This could explain why we did not observe the results predicted by our hypothesis.

Based on the results of a 2013 study, orchids that were put in a solution containing vinegar experienced high wilting of petals, while orchids that were put in a solution containing sugar and bleach experienced less wilting of the petals. (Thwala, 2013). Additionally, bleach has shown to be effective in extending the life of many flowers species such as *Alstroemeria peruviana*, *Antirrhinum majus*, *Dianthus caryophyllus*, *Gerbera jamesonii*, *Gypsophila paniculata*, *Lilium asiaticum*, *Matthiola incana*, and *Rosa hybrida* by anywhere from 0.9-13.4 days (Macnish et al., 2008). Macnish et al. (2008) also found that bleach treatment was effective in delaying the senescence process in many flower species. Several cut flower species have been

linked to early senescence due to the accumulation of bacteria bodies in the vase water because they block the passage of water through the xylem (van Doorn, 1989). Previous studies have found that bleach can effectively reduce the number of bacteria in the xylem (Macnish et al., 2008). By reducing these bacteria, bleach may be able to extend the longevity of certain flower species by maintaining an open pathway for the water to be absorbed into the flower via the xylem.

If carnations are one of these species, it would explain why our results did not support our hypothesis. This may explain the results shown in our Figure 2, which shows the progression of % wilting in the 4 groups throughout the duration of the study; at the end of the experiment, the base group had, on average, significantly less wilting than the other groups. The bleach solution was able to prevent wilting for longer in the beginning of the experiment, as seen by the fact that the slope of the yellow line was less steep than the other groups. This further supports the idea that carnations may be one of the flower species that performs better in a basic environment.

For future studies, we could recreate this experiment while measuring the amount of bacteria present in the xylem or vase water at the beginning of the experiment and throughout its duration. This would help us determine if vinegar or bleach is more effective at reducing the number of bacteria present. By comparing this to % wiltedness data, we could gain a greater insight into whether bacterial blockage of the xylem is the factor affecting wilting, and the effects of pH on bacterial growth.

Although this study was done in a single household under the same conditions, human error may have still occurred. The progress recordings of % wiltedness were subjectively recorded by two different individuals which could have introduced bias. This could lead to

inconsistencies in the data which reduces the accuracy of the results. Additionally, although randomization of the placement of the bottles did occur in order to reduce bias, some of the flowers may have spent more time in the sun due to variable weather conditions. This would impact our results because the flowers would be exposed to different levels of sunlight which may have an effect on wilting. The use of only one flower species means that these results may be specific to carnations, and can not be extrapolated to other species. There were also varying numbers of flowers and buds in each bottle, which could have introduced another possible source of error, as it could have influenced some differences in wilting.

Conclusion:

An ANOVA test on our results after 11 days of observation resulted in a p-value of 0.0082, allowing us to reject the null hypothesis that there were no differences in group means for percent wiltedness. A Tukey-Kramer test revealed that the base group mean (bleach + sugar solution) was significantly different from the water group (p-value= 0.0174), sugar group (p-value=0.0193), and the acid group (p-value= 0.0124). No other between-group comparisons showed significant differences in means. Our results differ from our hypothesis which stated that the acid and sugar solution would be the most effective at preventing wilting, as previous studies had indicated that acid solutions allow for optimized water transport in cut flowers. The extended longevity of flowers in the base group could be due to the antibacterial properties of bleach, which would prevent bacterial blockage of the xylem and allow water to travel more efficiently up the stems.

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Appendices

Appendix 1.

The litmus paper used for this experiment was created on Jan 27th 2021.

We soaked $\frac{1}{2}$ of a chopped purple cabbage in boiling water for 30 minutes. Once the solution was created, we dipped a paper towel in the solution and hung it up to dry. The cabbage acts as an indicator which allowed us to approximate the pH of the vinegar and bleach solutions that we created.

Appendix 2. Raw data and observations

Control *= solutio			ution changed
Date	Sample ID	% Wilted	Observation
March 17th	1	0	None
March 17th	2	0	None
March 17th	3	0	None
March 18th	1	0	None
March 18th	2	0	None
March 18th	3	0	None
March 19th	1	0	None
March 19th	2	0	None
March 19th	3	0	None
March 20th	1	0	None
March 20th*	2	0	None
March 20th*	3	0	None
March 21st	1	10	Slight wilting present at the bottom of the flower
March 21st	2	0	0
March 21st	3	0	0
March 22nd	1	23	More wilting present at the bottom of the flower
March 22nd	2	0	None
March 22nd	3	13	Wilting present on a few bottom petals
March 23rd*	1	23	No change observed
March 23rd*	2	5	Tiny amount of wilting present on bottom flowers
March 23rd*	3	20	More wilting present along the bottom of the flower

March 24th	1	30	Even more wilting present at the bottom of the flower. One petal brown
March 24th	2	10	Very minimal increase in wilting at the bottom of the flower
March 24th	3	20	No change observed
March 25th	1	37	More wilting observed. Another petal brown
March 25th	2	10	No change
March 25th	3	40	Huge increase in wilting. Wilting spread to upper petals
March 26th *	1	80	Massive increase in wilting again. More petals brown
March 26th *	2	35	Increase in wilting present near the bottom of the flower
March 26th *	3	73	Big increase in wilting, more petals brown. Wilting spreading to middle flowers
March 27th	1	93	Almost the entire flower wilted. More of the petals brown
March 27th	2	40	Very little change
March 27th	3	73	No change
March 28th	1	100	Entire flower wilted
March 28th	2	50	Most of the bottom entirely wilted
March 28th	3	87	Increase in wilting near the tip and center of the flower

Sugar

*= solution changed

Date	Sample ID	% Wilted	Observation
March 17th	1	0	None
March 17th	2	0	None
March 17th	3	0	None
March 18th	1	0	None
March 18th	2	0	None
March 18th	3	0	None
March 19th	1	0	None
March 19th	2	0	None
March 19th	3	0	None
March 20th	1	0	None
March 20th*	2	0	None
March 20th*	3	0	None

March 21st	1	3	Slight wilting of bottom petal
March 21st	2	0	None
March 21st	3	0	None
March 22nd	1	20	Large increase in wilting present nea bottom of the flower
March 22nd	2	7	Wilting starting at the bottom petals of the flower
March 22nd	3	12	Wilting at the bottom petals
March 23rd*	1	40	Wilting commencing in the middle layer
March 23rd*	2	10	Slight increase in wilting, wilting still concentrated near the bottom of the flower
March 23rd*	3	24	Increase in wilting still near bottom
March 24th	1	55	Large increase in wilting. Spreading to middle layer
March 24th	2	13	Very slight increase in wilting
March 24th	3	28	Slight increase in wilting near bottom of the flower
March 25th	1	60	Slight increase in wilting
March 25th	2	23	More wilting present at the bottom
March 25th	3	40	Large increase in wilting.Spreading slightly more to the middle
March 26th *	1	75	Bottom and middle layer fully wilted
March 26th *	2	30	Another slight increase in wilting
March 26th *	3	48	Slightly more wilting present in middle layer
March 27th	1	90	Massive increase. All but centre of the flower wilted
March 27th	2	37	Slight increase in wilting
March 27th	3	56	Large increase. Some wilting present along the tip
March 28th	1	100	Entirely wilted
March 28th	2	57	Slight increase in wilting
March 28th	3	72	Browning plus more of the tip wilted

Acid + sugar

*= solution changed

Date	Sample ID	% Wilted	Observation
March 17th	1	0	None
March 17th	2	0	None
March 17th	3	0	None
March 18th	1	0	None
March 18th	2	0	None

March 18th	3	0	None
March 19th	1	0	None
March 19th	2	0	None
March 19th	3	0	None
March 20th*	1	0	None
March 20th*	2	0	None
March 20th*	3	15	Wilting present at the bottom of the plant
March 21st	1	0	None
March 21st	2	0	None
March 21st	3	20	More wilting present near bottom of the plant
March 22nd	1	7	Wilting near bottom most petals
March 22nd	2	5	Slight amount of wilting in bottom most petals
March 22nd	3	45	Large increase of wilting in bottom most petals. Slight browning
March 23rd*	1	17	Wilting near bottom of flower. More petals brown
March 23rd*	2	15	Wilting present near bottom
March 23rd*	3	65	Large increase in wilting. Spreading to the middle of the flower
March 24th	1	27	More brown petals plus a slight increase in wilting
March 24th	2	15	No change
March 24th	3	70	Wilting approaches middle slightly more
March 25th	1	33	Slight increase in wilting
March 25th	2	35	Large increase in wilting. More present near middle
March 25th	3	75	Slight increase in wilting
March 26th *	1	33	No change
March 26th *	2	70	Large increase. Wilting spreading towards middle + more brown petals
March 26th *	3	80	Slight increase in wilting. Moving slight towards centre
March 27th	1	40	Very slight increase. Wilting starting in middle layer
March 27th	2	75	Slight increase in wilting
March 27th	3	90	bottom + middle layer wilted
March 28th	1	65	Slight increase in wilting. Present near centre
March 28th	2	85	Bottom + middle layers all wilted

March 28th	3	95	All but the centre wilted

Bleach + Sugar		*= solution changed		
Date	Sample ID	% Wilted	Observation	
March 17th	1	0	None	
March 17th	2	0	None	
March 17th	3	0	None	
March 18th	1	0	None	
March 18th	2	0	None	
March 18th	3	0	None	
March 19th	1	0	None	
March 19th	2	0	None	
March 19th	3	0	None	
March 20th*	1	0	None	
March 20th*	2	0	None	
March 20th*	3	0	None	
March 21st	1	0	None	
March 21st	2	0	None	
March 21st	3	0	None	
March 22nd	1	0	None	
March 22nd	2	0	None	
March 22nd	3	10	Slight increase of wilting in bottom petals	
March 23rd*	1	0	None	
March 23rd*	2	0	None	
March 23rd*	3	17	Slightly more wilting present near the bottom of the flower	
March 24th	1	4	Wilting present near the bottom of the flower	
March 24th	2	0	None	
March 24th	3	17	No change	
March 25th	1	8	Slight increase in wilting near the bottom petals	
March 25th	2	4	First sign of wilting. Very slight	
March 25th	3	23	Slight increase of wilting. All wilting concentrated near the bottom	
March 26th *	1	12	Wilting spreading slightly up the flower	
March 26th *	2	12	Large increase in wilting. Now present near	

			botto
March 26th *	3	30	Wilting starting to spread near the bottom of the flower
March 27th	1	20	Slight increase in wilting starting at middle
March 27th	2	16	A very slight increase in wilting
March 27th	3	37	Most of the wilting at the bottom. Some present in the middle layer
March 28th	1	28	Slight increase in wilting
March 28th	2	20	Very small increase in wilting
March 28th	3	40	All of the bottom plus some of the middle wilted

Appendix 3

Control SD	Sugar SD	Acid SD	Base SD
25.9	21.8	15.3	10.1

 Table 1. Displays the standard deviation values calculated across the control and 3 treatment groups.

Control SD Log	Sugar SD Log	Acid SD Log	Base SD Log
Transformation	Transformation	Transformation	Transformation
0.36	0.28	0.19	0.34

 Table 2. Displays standard deviation after the log transformation on the % wilted data.

Control P-value	Sugar P-value	Acid P-value	Base P-value
0.4837	0.67	0.6369	0.7804

Table 3. Shapiro Wilk normality test on the log transformed data.



Figure 4. A qq plot of the log transformed data of % wilted from all four groups (control + experimentals).