Design of Experiment: Creating an acidic solution to water household fruit plants

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STA 6833 Design of Experiment

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Project Objective

Growing a fruit plant is difficult for the average home gardener. Fruit plants prefer a specific type of soil, water, light source, temperature, and air moisture. Unfortunately, I learned this lesson the hard way. I have purchased banana and papaya plants from Amazon, planted lemon, orange, and pomegranate seeds from fruit, and bought strawberries seeds from Home Depot to plant outside. Every single one of them died within one month of sprouting. After intense research into citrus plants, I overlooked the fact that acidic soil is needed to keep the plant alive and thriving. There are many soil additives available for purchase that are mixed with water to change the pH level of the soil. The price of these items varies and will need to be repurchased regularly throughout the life of the plant. Since I consider myself a low budget gardener a cheaper alternative solution to this problem is needed. Therefore, creating a solution that changes the pH level of the soil using household items is desirable over purchasing the soil additive though Amazon. The follow up problem to this concept is "What is the best mixture to use?", "Do all water sources produce the same results?", and "Does the acid matter?" These are all great question to asks when there is a nominal fee and time is incurred for each new plant purchase. Therefore, the objective of this study is to analyze which factors significantly affect the acidic solution that could be used to water household citrus plants. The acidic solution will be measured in potential hydrogen (pH) measurements. The pH level has a range from 0 to 14. A pH from 0 to 6 is considered acidic, 7 pH is considered neutral, and 8 to 14 pH is considered alkaline. A successful solution for this experiment is a solution with a pH reading within the range of 5.5 through 6. This range is based on the guide provided by www.citrus.com website. This range will be best for the plant to absorb potassium, phosphorus, calcium, manganese, zinc, and iron in the soil. One digital pH meter reader was bought from Amazon.com and will be used to measure the pH levels during the experiments.

Factors for Design

Controlled Factors

The factors that are expected to influence the pH outcome of the acidic solution are the liquid base, temperature, acid, volume size, and time lapse. The liquid base will represent different levels of a manually processed water source. The two-water source that will be compared are store brand commercial bottle water from a local HEB store and natural river water from the San Marcos river. It is a common practice that commercially processed water has many minerals removed and added for human consumption. On a molecular level, this difference could be significant to the solution. The next factor of interest is the temperature of the solution and its influence on the ph. The temperature will be measured in Celsius with the one level

set to 24 Celsius (Room temperature) and other level set to 53 Celsius (slight boil). I hypothesize that the temperature of the solution may produce a chemical reaction at different temperature levels. A digital thermometer was purchased from Amazon to provide an accurate reading of the degree. The next factor to investigate is the acid. This factor will have two levels, one acid is lemon juice which represents a natural ingredient and the other is distilled vinegar, a commercially processed acid. One teaspoon of each will be added to the solution. The acid is expected to have an interaction in the solution by lowering the pH level but may also face a degradation affect due to the time lapse factor. The next factor in the design is the volume size of the solution. The volume of the solution will range in two sizes, one cup and three cups. Standard measuring cups will be used to measure the liquid. The hope is that a larger solution will fit within the target range, thus can extend over a large range of plants. The last factor to investigate is time lapse. The time allowed for the chemical reaction to change will be 15 minutes and 30 minutes. Two digital timers will be used to time the events. This is an appropriate time to wait for common gardeners before watering plants. In total there are five factors in this study with each factor having 2 levels.

Uncontrolled Factors

The uncontrollable factors were minimized in this experiment due to the nature of chemical interactions, therefore, there are only a few uncontrollable items within the experiment that could have affected the pH level. Since the equipment used for this experiment are common household items in a home environment, proper sterilization could not be conductive. The residue left after cleaning with dish soap was not measured on the glass bowls used to hold solution. Any residue from cleaning may have a minimal affect in the pH readings. To counter this effect, a rise of distilled water will be used on the equipment to neutralize the pH of any remaining residue after cleaning. While not a completely uncontrolled factor, the stove oven will be used to heat the water to 53 degrees Celsius. Due to the variance of the heat, there will be a variance component of the temperature. To minimize the variance of the heat, the stove temperature was held at the level 6 heat. The next uncontrollable factor was the air temperature. This factor was not measured but is known to have an effect on the glass bowl or any container used to hold the solution. This could have a minimal effect depending on if the solution is created outdoor in extreme weather. The next factor that was not measured is the degree change from the start time of the time elapse of either fifteen minutes or 30 minutes and afterwards. Since it is not expected to give a plant heated water and some cooling is expected.

Design of Experiment

This experiment will utilize a half-fractionated 2^5 resolution V design with ten repeats. This fractionated design was chosen because of the low cost of the items used and the desire to analysis as many two-way interactions as possible. The uncoded and coded design can be seen in the graph to the right. There will be a total of 26 test runs. The additional 10 repeat test are needed to evaluate the small difference in the change of the ph. The runs that will be repeated are experimental numbers 3, 5, 7, 8, 9, 10, 12, 14, 15, and 16. These numbers were chosen at random. The defining relationship is *I=A B C D E (I = Water Temp Acid Size Time)* and design generator is *E = A B C D (Time = Water Temp Acid Size).* Internally in the design the one-way interactions are not alias with the two-way interactions and the two-way interaction are alias with the three-way interaction as can be seem in the figure to the right. There are ten two-way interactions available for the study. They are (Water × Temp), (Water × Acid), (Water × Size), (Water × Time), (Temp × Acid), (Temp × Size), (Temp × Time), (Acid × Size), (Acid × Time), (Size × Time). Since I am not sure which or how many factors will be significant in the experiment, I have chosen to include as many two-way interactions as possible in the initial model. Each factor has 2 levels that are coded as 1 or -1. The assignment of the factor levels was chosen at random. In the factor level water base, river is coded as 1 and bottle water is -1. In the temperature factor, 24 Celsius is coded as -1 and 53 Celsius is 1. In the acid factor, lemon is coded as -1 and vinegar is coded as 1. In the size factor three cups is coded as -1 and one cup is 1. Lastly, in the time factor fifteen minutes is coded as -1 and thirty minutes is 1. The test runs will be randomized using SAS as shown in the coded factor graph to the right. The equipment used for this experiment includes one stainless steel 8-inch extra-long probe digital thermometer, two 8-inch saucepan, one Digital PH Test Pen 0.01 Accuracy, 2 plastic measuring teaspoons, 2 plastic measuring one cups, 2 clock timers, 2 two cup glass bowls, and 2 four cup glass bowls. The test runs are randomized and will be completed as listed in the next section in batches of four.

Acidic Solution Coded Factors

Obs	Water	Temp	Acid	Size	Time
1	River	24 C	Lemon	3 Cups	30 Min
2	River	24 C	Vinegar	3 Cups	15 Min
3	Bottle	53 C	Lemon	1 Cup	15 Min
4	River	24 C	Lemon	1 Cup	15 Min
5	River	53 C	Vinegar	3 Cups	30 Min
6	River	53 C	Vinegar	1 Cup	15 Min
7	Bottle	53 C	Lemon	3 Cups	30 Min
8	Bottle	24 C	Lemon	3 Cups	15 Min
9	Bottle	53 C	Vinegar	3 Cups	15 Min
10	River	53 C	Lemon	3 Cups	15 Min
11	Bottle	24 C	Vinegar	3 Cups	30 Min
12	Bottle	24 C	Lemon	1 Cup	30 Min
13	Bottle	24 C	Vinegar	1 Cup	15 Min
14	River	53 C	Lemon	1 Cup	30 Min
15	Bottle	53 C	Vinegar	1 Cup	30 Min
16	River	24 C	Vinegar	1 Cup	30 Min

Acidic Solution Coded Factors

Obs	Water	Temp	Acid	Size	Time
1	1	-1	1	-1	1
2	-1	-1	1	-1	-1
3	1	-1	1	1	-1
4	-1	-1	-1	1	-1
5	1	-1	-1	-1	-1
6	-1	-1	-1	-1	1
7	-1	1	-1	1	1
8	1	1	1	1	1
9	-1	1	1	-1	1
10	1	-1	-1	1	1
11	1	1	-1	1	-1
12	-1	-1	1	1	1
13	-1	1	-1	-1	-1
14	-1	1	1	1	-1
15	1	1	-1	-1	1
16	1	1	1	-1	-1

Acidic Solution Coded Factors

The FACTEX Procedure

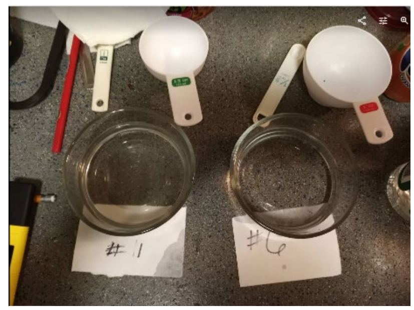
Aliasing Structure
Water
Temp
Acid
Size
Time
Water*Temp = Acid*Size*Time
Water*Acid = Temp*Size*Time
Water*Size = Temp*Acid*Time
Water*Time = Temp*Acid*Size
Temp*Acid = Water*Size*Time
Temp*Size = Water*Acid*Time
Temp*Time = Water*Acid*Size
Acid*Size = Water*Temp*Time
Acid*Time = Water*Temp*Size
Size*Time = Water*Temp*Acid

Execution of Experiment

A pre-experimental test was completed on April 4,2021. The test consisted of the four runs listed below. The pH of the liquid base and the resulting solution pH was recorded to ensure that there would be a change in the pH level. The result of that run is listed here. Since there was a noticeable difference in each pH, I continued to complete the fractionated experiment.

Water	Size	Acid	Temp	Time	Pre-pH	Post-pH
River	1 Cup	Lemon	53 C	15 min	8.4	5.9
River	3 Cup	Vinegar	24 C	30 min	8.6	5.3
Bottle	1 Cup	Vinegar	53 C	15 min	7.4	4.3
Bottle	3 Cup	Lemon	24 C	30 min	7.2	4.5

The fractionated experiment was conducted on April 7, 2021. Since the experimental test runs were completed in batches of four, seven batches are needed to complete the experiment. The first batch was completed with no observable odd behavior from the instruments or pH reading. The photo of the right shows one of the test runs used in the experiment. The liquid based is selected and is measured by the digital thermometer to ensure the temperature is achieved. The stove is used for the liquid base to reach 53 C degree as seen in the next image. The liquid base was collected from the holding containers and added to the glass bowls by the measurements listed for that specified test. The timers were set for the waiting period and the pH was recorded as shown. The second batch of experimental runs were completed without any issues and the results were recorded. On the third batch of test runs (9 – 12) the liquid distorted the labeling for the experimental test run and I added the wrong acid to the wrong container. Test run #11 recorded a pH reading of 7.8 which seemed a little too high considering the acid that was added or lack of one being added. Due to this error, I repeated this batch. I was able to complete the fourth batch of solution but then the pH reader begins to malfunction and was unable to hold a pH reading for a solution. This issue may have been due to some distilled water not properly drying between rinses. The PH meter was cleaned with distilled water and properly dried as recommended by the owner guide and the test runs continued. This caused me to completely miss my time lapse setting for my solution and this batch had to be repeated. The remaining batch runs were completed with no odd issues from user or equipment. The experiment was completed in five hours. The results of the test can be viewed in the graph to the right.



	RUN	TEMP	WATE	ACID	SIZE	TIME	pH
1	1	-1	-1	-1	-1	1	3.33
2	14	-1	-1	-1	1	-1	6.87
3	7	-1	-1	1	-1	-1	3.03
4	8	-1	-1	1	-1	-1	3.59
5	20	-1	-1	1	1	1	3.82
6	4	1	-1	-1	-1	-1	5.92
7	5	1	-1	-1	-1	-1	5.63
8	17	1	-1	-1	1	1	5.93
9	10	1	-1	1	-1	1	5.89
10	11	1	-1	1	-1	1	7.13
11	23	1	-1	1	1	-1	3.77
12	24	1	-1	1	1	-1	4.06
13	2	-1	1	-1	-1	-1	6.43
14	3	-1	1	-1	-1	-1	6.84
15	15	-1	1	-1	1	1	7.82
16	16	-1	1	-1	1	1	6.93
17	9	-1	1	1	-1	1	7.01
18	21	-1	1	1	1	-1	6.07
19	22	-1	1	1	1	-1	6.46
20	6	1	1	-1	-1	1	5.69
21	18	1	1	-1	1	-1	6.73
22	19	1	1	-1	1	-1	6.84
23	12	1	1	1	-1	-1	4.83
24	13	1	1	1	-1	-1	4.77
25	25	1	1	1	1	1	6.13
26	26	1	1	1	1	1	6.76

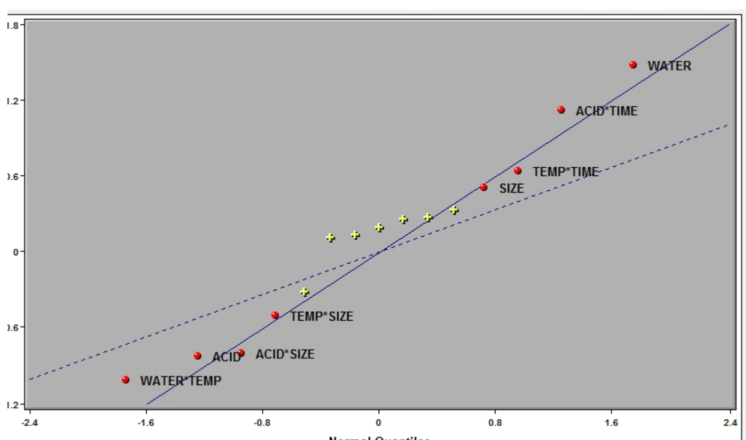
Analysis of the Experiment

Dependent Variable: pH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	44.49180385	2.96612026	16.74	<.0001
Error	10	1.77215000	0.17721500		
Corrected Total	25	46.26395385			

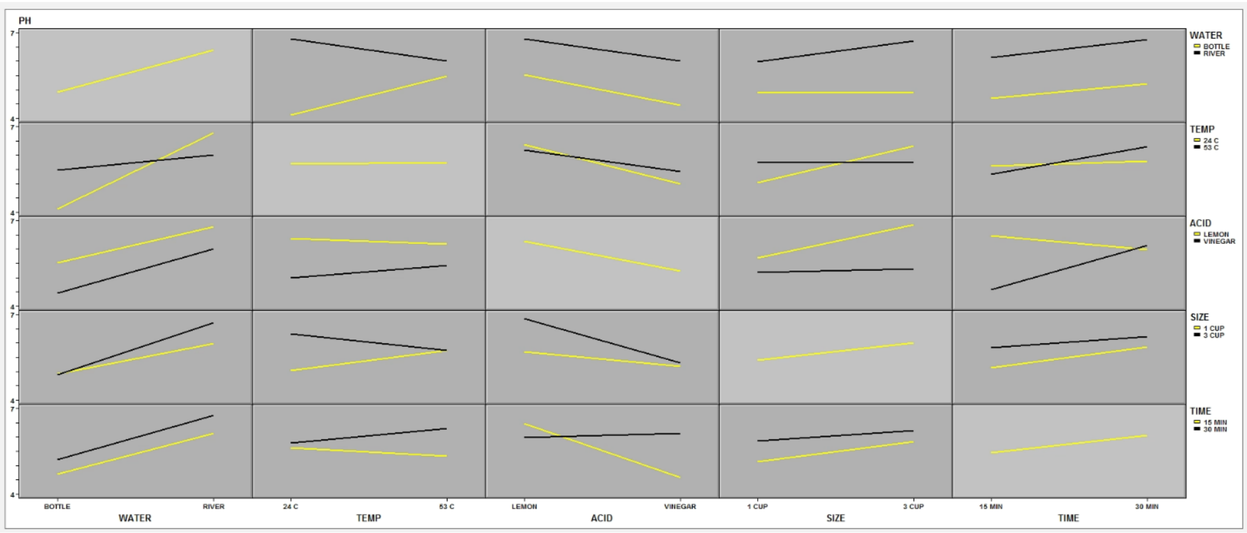
Source	DF	Type III SS	Mean Square	F Value	Pr > F
WATER	1	12.11700227	12.11700227	68.37	<.0001
TEMP	1	0.13865682	0.13865682	0.78	0.3972
ACID	1	3.62538409	3.62538409	20.46	0.0011
SIZE	1	1.71627500	1.71627500	9.68	0.0110
TIME	1	0.28000227	0.28000227	1.58	0.2373
WATER*TEMP	1	6.36120227	6.36120227	35.90	0.0001
WATER*ACID	1	0.51711136	0.51711136	2.92	0.1184
WATER*SIZE	1	0.11505682	0.11505682	0.65	0.4391
WATER*TIME	1	0.48720227	0.48720227	2.75	0.1283
TEMP*ACID	1	0.15245682	0.15245682	0.86	0.3755
TEMP*SIZE	1	1.27500227	1.27500227	7.19	0.0230
TEMP*TIME	1	2.13400227	2.13400227	12.04	0.0060
ACID*SIZE	1	4.09920227	4.09920227	23.13	0.0007
ACID*TIME	1	7.75320227	7.75320227	43.75	<.0001
SIZE*TIME	1	0.47465682	0.47465682	2.68	0.1328

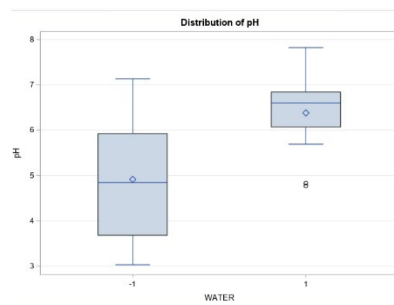
The result from the analysis is shown in the graph to the right. The assumption for this model is that the observations are independent, identical, and randomly distributed, the variance is constant, and the error is random. The model that will be used is that PH equals to water, acid, size, time, and temperature with the added factors of the accompanying two-way interaction. The result of the model shows a F- value of 16.74 and a P-value of less than 0.0001. The model is significant at a significant p-value of less than 0.05 with indicates that at least one of the factors is significantly different from zero. The one-way factors that show significance at a p-value less than 0.05 using the Type III Sum of Squares are Water with a p-value of less than 0.0001, Acid with a p-value of 0.0011, and Size with a p-value of 0.0110. The two-way factors that show significance p-value less than 0.05 using the Type III Sum of Squares are (Water × Temp) with a p-value of 0.0001, (Temp × Size) with a p-value of 0.0230, (Temp × Time) with a p-value of 0.0060, (Acid × Size) with a p-value of 0.0007, and (Acid × Time) with a p-value of less than 0.0001. In total five two-way interactions are significantly different from zero. The alias three-way interaction that are also significant are (Acid × Size × Time), (Water × Acid × Time), (Water × Acid × Size), (Water × Temp × Time), and (Water × Temp × Size). The QQ plot presents similar results from the Sum of Squares graphs III. As can be seen in the Normal Quantiles plot, the significant factors are (Water × Temp), Acid, (Acid × Size), (Temp × Size), Size, (Temp × Time), (Acid × Time) and Water and are shown to lay on both side of the plot as significant.



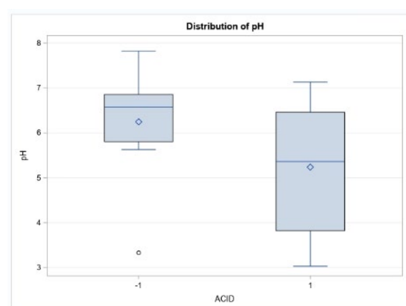
The interaction plot was generated by the SAS Design of Experiment component. This plot graphical displays the interactions indicated in the Type III Sum of Squares. The important items to note from this graph are the nonsignificant interaction. Water, Size, and Temp show parallel lines rising to the right and Acid decreasing to the right. This displays the rise and fall of pH as the factors vary.

The means and least square mean analysis are listed next. The Tukey-Kramer criteria was used with a significance level of p-value of less than 0.05. Only the factors that show as significant will be reviewed. The results from the water factor shows that natural river water and commercial processed water are significantly different in regard to the pH in the experiment. The means difference between the two is 1.4 and can be viewed to the right. The pH comparison for Acid also shows that natural lemon is significantly different from vinegar with a mean different of 1.009. The pH comparison for Size shows there is a significant different in the volume size of the solution with a mean difference of 0.6231. The two-way interaction will be reviewed based on the significant two-way factors listed above in Sum of Squares Type III. The (Water × Temp) show that all interaction are significant from each other except for (Bottle × 53 C). The (Temp × Size) interaction shows that there is a significant difference between (24℃ × 1 Cup) and (24℃ × 3 Cups). The (Temp × Time) interaction shows that there is a significant difference between (53℃ × 15 mins) and (53℃ and 30 mins). The (Acid × Size) interaction shows that there is a significant difference between (Lemon × 1 Cup) and (Lemon × 3 Cups), (Lemon × 3 Cups) and (Vinegar × 1 Cup), and (Lemon × 3 Cups) and (Vinegar × 3 Cups). This may be due to the dilution with increasing the cup size. The (Acid × Time) interaction shows that there is a significant difference between (Lemon × 15 mins) and (Lemon × 30 mins), (Vinegar × 15 mins) and (Lemon × 15 mins), (Lemon × 30 mins) and Vinegar × 15 mins), and (Vinegar × 15 mins) and (Vinegar × 30 mins). Reviewing the Least Square means reveals that the acid and time produced the greatest means difference and largest variation. This indicates that this interaction is very important to consider in building our solution. The second important factor that is seen by the Least Square means is the acid and volume size. This plays the second biggest role in creating the acidic solution. The remaining interaction are also important but these first two will be used as the building blocks for selecting the combination of factors to consider first. The estimated covariance of temp is 2.66E13 and covariance of time is 3.104E13. Referencing back to our main objective, getting a PH range of 5.5 thru 6.0, the

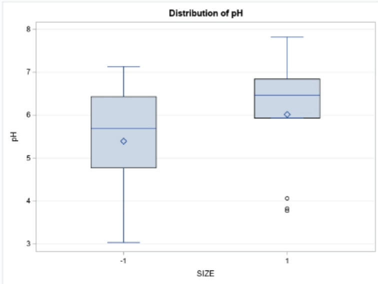




Level of WATER	N	pH Mean	pH Std Dev
-1	12	4.91416667	1.45516296
1	14	6.37928571	0.83671946



Level of ACID	N	pH Mean	pH Std Dev
-1	12	6.24666667	1.11625456
1	14	5.23714286	1.41346507



Level of SIZE	N	pH Mean	pH Std Dev
-1	13	5.39153846	1.39241305
1	13	6.01461538	1.30622621

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

TEMP	SIZE	pH LSMEAN	LSMEAN Number
-1	-1	5.07125000	1
-1	1	6.08250000	2
1	-1	5.69375000	3
1	1	5.76875000	4

Least Squares Means for effect TEMP*SIZE
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: pH

i/j	1	2	3	4
1		0.0127	0.1162	0.0724
2	0.0127		0.4334	0.5996
3	0.1162	0.4334		0.9881
4	0.0724	0.5996	0.9881	

Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

WATER	TEMP	pH LSMEAN	LSMEAN Number
-1	-1	4.33250000	1
-1	1	5.53250000	2
1	-1	6.82125000	3
1	1	5.93000000	4

Least Squares Means for effect WATER*TEMP
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: pH

i/j	1	2	3	4
1		0.0042	<.0001	0.0005
2	0.0042		0.0013	0.3777
3	<.0001	0.0013		0.0158
4	0.0005	0.3777	0.0158	

Acidic Solution Coded Factors

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

ACID	TIME	pH LSMEAN	LSMEAN Number
-1	-1	6.51625000	1
-1	1	5.58125000	2
1	-1	4.57250000	3
1	1	5.94625000	4

Least Squares Means for effect ACID*TIME
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: pH

i/j	1	2	3	4
1		0.0203	<.0001	0.1603
2	0.0203		0.0099	0.5490
3	<.0001	0.0099		0.0008
4	0.1603	0.5490	0.0008	

Acidic Solution Coded Factors

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

TEMP	TIME	pH LSMEAN	LSMEAN Number
-1	-1	5.77000000	1
-1	1	5.38375000	2
1	-1	5.31875000	3
1	1	6.14375000	4

Least Squares Means for effect TEMP*TIME
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: pH

i/j	1	2	3	4
1		0.4734	0.2433	0.4649
2	0.4734		0.9932	0.0716
3	0.2433	0.9932		0.0246
4	0.4649	0.0716	0.0246	

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

ACID	SIZE	pH LSMEAN	LSMEAN Number
-1	-1	5.35750000	1
-1	1	6.74000000	2
1	-1	5.40750000	3
1	1	5.11125000	4

Least Squares Means for effect ACID*SIZE
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: pH

i/j	1	2	3	4
1		0.0015	0.9969	0.7542
2	0.0015		0.0014	0.0003
3	0.9969	0.0014		0.6067
4	0.7542	0.0003	0.6067	

factors levels that are significant will be based on their averages. The bottle water with an average least square mean of 4.9325, vinegar should be with a least square mean of 5.2594, and the volume size of one cup should be used with a least square mean of 5.3825. Based on ease of access and the negligible effects of the remaining factors, the temperature of 24 Celsius and fifteen-minute time lapse should be used when creating the acidic solution. This is the recommended test that should be run to get an optimal pH solution for household fruit plants.

Conclusion

The outcome of this experiment illustrates that the important factors to consider when creating an acidic solution to water citrus fruit plants is the water base, the volume of the solution, and the type of acid. When creating an acid solution, these factors should be considered. After least mean square review, it appears evident that the best combination of solution mix is (15 mins × 1 Cup × Lemon × 53℃ × Bottle water). The next appropriate test to complete would the storage capacity of the solution, i.e., extending the duration time to days and study the change in pH in long-term study. The number of days should be 3, 5, and 7 days and the test to see when the mixture should be tossed.

Appendix: SAS Code

```
*Design uncoded;
Proc factex;
factors Water Temp Acid Size Time;
size fraction = 2;
model resolution = 5;
Examine design aliasing confounding;
output out=randperm;
Title 'Acidic Solution Uncoded Factors';
run;
quit;

*Design Codede;
Proc factex;
Factors Water Temp Acid Size Time;
size fraction = 2;
model resolution = 5;
Examine design aliasing confounding;
output out=repdesign randomize
      Water cvals=('Bottle' 'River')
      Temp cvals=('24 C' '53 C')
      Acid cvals = ('Lemon' 'Vinegar')
      Size cvals = ('1 Cup' '3 Cups')
      Time cvals = ('15 Min' '30 Min');
title1 'Acidic Solution Coded Factors';
run;
quit;

proc print data=repdesign;
run;
quit;



Data Rand;
  seed = -1;
  set randperm;
  randordr = ranuni(seed);
run;

Proc Sort data=Rand out=Rand(drop=randordr);
  by randordr;
run;

proc print data=rand;
var Water Temp Acid Size Time;
run;
quit;


data report;
input RUN WATER TEMP ACID SIZE TIME pH;
DATALINES;
1      -1     -1     -1     -1     1      3.33
2      1      -1     -1     -1     -1     6.43
```

```
3     1     -1    -1    -1    -1    6.84
4     -1    1     -1    -1    -1    5.92
5     -1    1     -1    -1    -1    5.63
6     1     1     -1    -1    1     5.69
7     -1    -1    1     -1    -1    3.03
8     -1    -1    1     -1    -1    3.59
9     1     -1    1     -1    1     7.01
10    -1    1     1     -1    1     5.89
11    -1    1     1     -1    1     7.13
12    1     1     1     -1    -1    4.83
13    1     1     1     -1    -1    4.77
14    -1    -1    -1    1     -1    6.87
15    1     -1    -1    1     1     7.82
16    1     -1    -1    1     1     6.93
17    -1    1     -1    1     1     5.93
18    1     1     -1    1     -1    6.73
19    1     1     -1    1     -1    6.84
20    -1    -1    1     1     1     3.82
21    1     -1    1     1     -1    6.07
22    1     -1    1     1     -1    6.46
23    -1    1     1     1     -1    3.77
24    -1    1     1     1     -1    4.06
25    1     1     1     1     1     6.13
26    1     1     1     1     1     6.76
;
RUN;
QUIT;

proc glm;
class WATER TEMP ACID SIZE TIME ;
model ph = WATER TEMP ACID SIZE TIME WATER*TEMP WATER*ACID WATER*SIZE
WATER*TIME TEMP*ACID TEMP*SIZE TEMP*TIME ACID*SIZE ACID*TIME SIZE*TIME;
lsmeans WATER TEMP ACID SIZE TIME WATER*TEMP WATER*ACID WATER*SIZE WATER*TIME
TEMP*ACID TEMP*SIZE TEMP*TIME ACID*SIZE ACID*TIME SIZE*TIME / adjust = tukey
pdiff = all;
run;
quit;

Proc mixed data = report;
class WATER TEMP ACID SIZE TIME ;
model ph = WATER TEMP ACID SIZE TIME WATER*TEMP WATER*ACID WATER*SIZE
WATER*TIME TEMP*ACID TEMP*SIZE TEMP*TIME ACID*SIZE ACID*TIME SIZE*TIME;
random TEMP SIZE TIME ;
lsmeans WATER TEMP ACID SIZE TIME WATER*TEMP WATER*ACID WATER*SIZE WATER*TIME
TEMP*ACID TEMP*SIZE TEMP*TIME ACID*SIZE ACID*TIME SIZE*TIME / adjust = tukey
pdiff = all;
run;
quit;

title 'Graphical test of interation';
Proc glm data = report;
class WATER TEMP ACID SIZE TIME ;
model ph = WATER TEMP ACID SIZE TIME WATER*TEMP WATER*ACID WATER*SIZE
WATER*TIME TEMP*ACID TEMP*SIZE TEMP*TIME ACID*SIZE ACID*TIME SIZE*TIME;
random TEMP SIZE TIME/test ;
```

```
lsmeans WATER TEMP ACID SIZE TIME WATER*TEMP WATER*ACID WATER*SIZE WATER*TIME
TEMP*ACID TEMP*SIZE TEMP*TIME ACID*SIZE ACID*TIME SIZE*TIME / adjust = tukey
pdiff = all alpha=0.05 cl stderr;
output out=pp p=ypred r=resid student=sress;
run;


proc sort data = report;By WATER TEMP ACID SIZE TIME;
proc means data = report noprint mean var;  By WATER TEMP ACID SIZE TIME;
 var ph;
 output out = ph2 mean = av_y VAR = var_y;
 proc print data = ph2;
 var WATER TEMP ACID SIZE TIME av_y var_y;
 run;




*try two;
    /* Unbalanced Data*/
proc glm data =report;
   class WATER TEMP ACID SIZE TIME;  model ph=WATER TEMP ACID SIZE TIME
WATER*TEMP WATER*ACID WATER*SIZE WATER*TIME TEMP*ACID TEMP*SIZE TEMP*TIME
ACID*SIZE ACID*TIME SIZE*TIME/p clm solution;
   means WATER TEMP ACID SIZE TIME WATER*TEMP WATER*ACID WATER*SIZE
WATER*TIME TEMP*ACID TEMP*SIZE TEMP*TIME ACID*SIZE ACID*TIME SIZE*TIME/
hovtest=levene;
   lsmeans WATER TEMP ACID SIZE TIME WATER*TEMP WATER*ACID WATER*SIZE
WATER*TIME TEMP*ACID TEMP*SIZE TEMP*TIME ACID*SIZE ACID*TIME SIZE*TIME
/alpha=0.05 cl stderr pdiff;
   output out=one p=pred r=resid student=sres;

proc plot data=one;
   plot sres*phpred/vref=0;
   plot sres*water/vref=0;
   plot sres*temp/vref=0;
   plot sres*acid/vref=0;
   plot sres*size/vref=0;
   plot sres*time/vref=0;
run;


proc univariate data=one plot normal;
   var sres;
   probplot;
   histogram sres;
run;
ods rtf close;
quit;
```

Additional photos