INTERNATIONAL BACCALAURATE PROGRAMME

EXTENDED ESSAY

SUBJECT: BIOLOGY

TOPIC: Investigating the antibacterial effect of different vinegar types; white vinegar, apple cider vinegar, vinegar of grapes, and vinegar of date palm on *Escherichia coli* bacteria, by comparing their colony numbers by counting the colonies formed on the EMB growth plates after incubation with the vinegar types and 18-hour growth period.

RESEARCH QUESTION: Do different types of vinegar; white vinegar, apple cider vinegar, vinegar of grapes, and vinegar of date palm have an antibacterial effect on *Escherichia coli*, measured through comparing their colony numbers by counting the colonies formed on the EMB growth plates after 18-hour growth period when the bacteria are incubated with 100μL with the vinegar type?

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INTRODUCTION

Every day in our lives we eat vegetables or fruits. Before we eat them, we often wash them by simply running tap water over and gently scrubbing them with our bare hands. However, these fruits or vegetables have a chance of being contaminated by bacteria, especially the bacteria *Escherichia coli* (*E. coli)*.[1] Therefore, just washing them with tap water might not quite risky if the food are contaminated. Vinegar or acetic acid is a commonly used disinfectant that is used in house cleaning to wash fruit and vegetables.[2] There has been researched that showed vinegar had an antibacterial and an antiviral effect, but only for some type of bacteria and viruses.[2,11,12] Vinegar has an antibacterial effect on *E. coli*, but does the antibacterial effect change through the change of different vinegar types, or all vinegars have the same effect over the bacteria?

Research Question

Do different types of vinegar; white vinegar, apple cider vinegar, vinegar of grapes, and vinegar of date palm have an antibacterial effect on *Escherichia coli*, measured through comparing their colony numbers by counting the colonies formed on the EMB growth plates after 18-hour growth period when the bacteria are incubated with 100μL with the vinegar type?

Background Information

1. ***Escherichia coli:***

E. coli are the predominant nonpathogenic facultative flora of the human and animal intestine, and it is usually harmless, but some types of the bacteria can cause food poisoning or infections.[3] The *E. coli* are a diverse group and have a mutualistic relationship with its host, usually preventing colonization of pathogenic bacteria. However, because it has such a diverse group some types of the bacteria (such as *E. coli O157:H7*) are pathogenic rather than spending a mutualistic life. If the bacteria lives in the intestines, then how does it contaminate fruit or vegetables? *E. coli* is expelled to the environment within fecal matter.[4] That fecal matter gets mixed with fertilizers and soil. Thus, the vegetables and fruit we eat.

When *E. coli O157:H7* enters the digestive system, there is a possibility it can cause serious issues more than common diarrhea and stomach pain. After a three-four day of incubation period, the symptoms start to show, and they usually get worse through the week if not treated due to its rapid

reproduction. Some of these symptoms are diarrhea; mild-severe-bloody, stomach pain, tenderness or cramping, nausea and vomiting and fever.[5] In serious cases the bacteria can affect the central nervous system; can cause urinary tract infections, respiratory illness, or bloodstream illness.[5] In young children and older adults, the bacteria can lead to kidney failure. Therefore, even though it seems like a harmless type of bacteria, some types can lead to serious health problems. But it is generally treatable with antibiotics, rest, and fluids to help prevent dehydration and fatigue.

The easiest and the most efficient way to prevent any illness from *E. coli* is simply to prevent it as much as possible. And the prevention process deeply relies on hygiene. Washing hands is crucial before eating to prevent getting the bacteria in your digestive system. Avoiding raw, uncooked meat; unpasteurized milk, daily products and juices is also helpful.[6] Moreover, since these bacteria can also contaminate water, it is useful to avoid swallowing water while swimming.[1] Lastly, washing vegetables and fruit before consuming is very important to prevent illness from the bacteria. Vinegar is commonly used to wash and sanitize. But how effective is vinegar, and which type of vinegar is the most efficient to prevent the colonization?

2. *E. coli* ATCC 25922™:

E. coli ATCC 25922™, is a type of *E. coli*, produced by the American Type Culture Collection[7], with the specific intent for it to be used for experimenting safely. It is a live culture recommended for antibiotic susceptibility testing and quality control. The culture's biosafety level is detected to be Biosafety Level 1, again by the ATCC.[8] Biosafety levels are safety levels determined to conduct the experiments with the correct precautions for different microorganisms, so the experimenter or the environment won't be contaminated by the agent used in the experiment. The levels go from 1 to 4, 1 being the safest and 4 being the most dangerous. BSF-1 (BioSafetyLevel-1) are infectious agents or toxins not known consistently to cause disease in healthy adults and Standard Microbiological Practices are sufficient with no need for special equipment.[9] Because of these reasons, this bacterium is chosen for the research question in terms of safety and usage.

3. Vinegar:

Vinegar is a fermented solution containing 5-8% acetic acid and it has been used for centuries as a cleaning agent, medicine and for the preservation of food.[10] Vinegar is made though a two-step fermentation method: first yeast feed on fruit sugar (which type of vinegar it is), which ferments to alcohol. Then, the alcohol is exposed to the acetic acid bacteria *Acetobacter* and oxygen to ferment again for months or weeks. Vinegar is antibiotic because of the acetic acid it contains.[11] The acetic acid prohibits the environment that is needed for production of the bacteria because it damages the bacteria and even kills it by chemically changing the proteins and the fats that make up the bacteria and destroying their cell structures.[12] Therefore, the growth of a bacterial colony becomes much slower compared to its natural environment.

The acidity of the vinegar types is not affected by the change of its base material. However, its sugar type and concentration vary through the base material. The fruit-based vinegars contain more sugar while vinegars like white vinegar contain relatively less sugar. This could eventually lead a causation in the growth of bacteria in the experiment because the sugar would be food for the bacteria to consume and grow. It is found in research that the presence of glucose and sodium chloride in vinegar increases the growth of bacteria colonies.[13] I chose white vinegar, apple cider vinegar and vinegar of grapes because they are the most used vinegars on the market, and I chose vinegar of date palm to create more diversity on my variables because this vinegar type has the highest amount of sugar and carbohydrates among the most common types of vinegar.

Type of Vinegar	Sugar(g)	Carbohydrates(g)	Salt(g)	Acidity
White Vinegar	0	0.7	0	4-5%
Apple Cider Vinegar	0	0.1	0	4-5%
Vinegar of Grapes	1.15	1.5	0.03	4-5%
Vinegar of Date Palm	2.6	3	0.05	4-5%

Table 1: Nutrient Ingredients of the Vinegar[14]

[14]Fersan® (The Bottle of the Vinegars)

Hypothesis

To examine if different vinegar types have a relation with bacteria production, a null hypothesis is created related to the hypothesis of the experiment:

H_0: There is no difference between the effects of same concentration of different vinegar types: White Vinegar, Apple Cider Vinegar, Vinegar of Grapes, and vinegar of Date Palm with positive control on *E. coli*.

H_1: There is a difference between the effects of same concentration of different vinegar types: White Vinegar, Apple Cider Vinegar, Vinegar of Grapes, and vinegar of Date Palm with positive control on *E. coli*.

As apple cider vinegar has the least amount of sugar and carbohydrates, the most effective results are expected from the apple cider vinegar as an inhibitor of growth, or as an antibacterial effect over *E. coli* since the acidity is constant for all vinegar types. Therefore, apple cider vinegar will produce the least number of colonies after incubation with the it, when compared to the other vinegar types used in the experiment. This is expected because the bacteria produce more in the presence of glucose.

Variables

Independent Variable	The independent variable in this investigation is the 100μL of type of material used for the first fermentation process, which defines the type of the vinegar. The most common types of vinegars are used based on the popularity of their usage in disinfestation of fruit and vegetables. Those vinegar types are apple cider, white vinegar, vinegar of date palm and vinegar of grapes. 1000μL Distillated water is also used as a control to compare the results of the vinegar types.
Dependent Variable	The dependent variable in this investigation is the antibacterial effect, by comparing their number of colonies on EMB growth plates, on the bacteria *E. coli.* By looking at the difference in the colony count of the variables after the period of incubation, we can deduce the antibacterial effect of the variables through their production process.

Table 2: Variables

Table 2 Continued

Controlled Variables	• The quantity of vinegar in 100μL • The amount of *E. coli* in 0.5MF • The amount of dilution with water in μL's • The sample which the bacterium is reproduced • The type of EMB growth plates and their date of production • The plantation method • The brand of the vinegar thus their acidity as 4-5% • The incubation period of each variable as 18 hours temperature in 34 C°, light, air pressure (environment)

METHOD DEVELOPMENT AND PLANNING

Method

Colony Counting:

Colony counting is a process used in microbiology used to determine the estimated number colonies that grew in the plate after plantation. To compare the number of colonies the unit CFU (Colony Forming Unit) is used. To calculate the CFU, the number of colonies counted are multiplied by the dilution coefficient.[15] And to compare the CFUs', their density is looked by dividing the CFU to the solutions' ml. This process can also be done by colony counter machines. However, in this experiment with my resources, colony counting has been done by hand by counting the colonies one by one.

Acknowledgement

This experiment has been conducted under supervision of Prof. Dr. Yakut Akyön Yılmaz and Dr. Neşe İnal at Hacettepe University. (Appendix 1)

Preliminary Experiment

A preliminary experiment has been done to deduce the most effective ratio of vinegar dilution to examine the effects on formation of colonies. Because the ratio in the first trial concluded results that cannot be compared the vinegar is diluted with water with 1/10 ratio for the experiment, different than planned. All the vinegar types worked so well that no growth could be

examined on the EMB plates. Thus, the experiment has changed accordingly. To be sure of the results and to be certain that the results were not caused by the EMB plate samples, two samples has been made. However, both samples showed that when vinegar and the bacterium are taken at a 1:1 ratio, vinegar prevents any growth of the bacterium. Therefore, the vinegar has been diluted by 1/10 ratio, because at home often a little amount of vinegar is used to clean the vegetables and the material list made its final state in *Table 3.*

Material List

Materials	**Quantity**	**Uncertainties and Units**
Distillated water	1mL x5	±0.005mL
White vinegar	100μ_L x5	±0.05 μ_L
Apple cider vinegar	100μ_L x5	±0.05 μ_L
Vinegar of grapes	100μ_L x5	±0.05 μ_L
Vinegar of date palm	100μ_L x5	±0.05 μ_L
Distillated water to distillate the vinegars	900μ_L x25	±0.05 μ_L
Escherichia coli culture	0.5MF x25	±0.005MF
EMB growth medium plates	25	
Incubator	1	
Disposable loops	25	
Microbiology pipette for 1000μ_L	1	
Microbiology pipette for 200μ_L	1	
Microbiology pipette for 20μ_L	1	
10mL test tubes	30	
MacFarland Densitometer	1	
Agar plates	5	
Bench Mixer	1	

Table 3: Material List

Table 3 Continued

A pair of plastic gloves and white coat	1	
Stirring rod (best if disposable)	25	
Glass marker pen	1	
Cotton tips	5	

Figure 1: MacFarland Densitometer

Figure 2: Microbiology Pipette

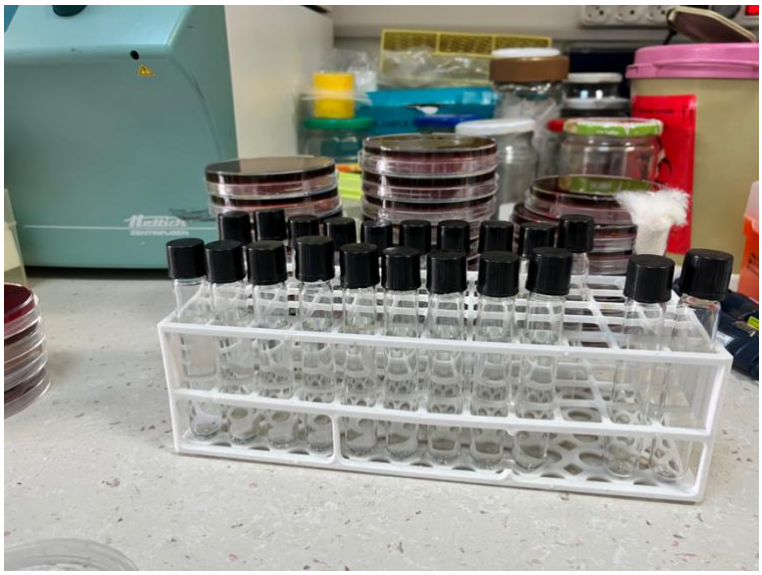


Figure 3: Test tubes and EMB growth plates

Procedure

1. Put plastic gloves and a white coat for safety. Do not touch any item before this step. After this, make sure the lab environment and the materials are disinfected and safe to use.

A- <u>Preparation of the Bacteria Solution:</u>

1. Take a test tube and put 5mL distillated water inside by the 1000 μL pipette in Figure 2. 1000 μL is equal to 5mL, so use 1000 μL.
2. Take an EMB plate with *E. coli* growth. Take a little sample from it as little as possible (tip of a cotton tip) and put it inside the tube. Use the MacFarland Densitometer in Figure 1 to measure the density of the bacterium. The density should be 0.5 MF, which is equal to 10^8 bacteria. The tip of the cotton tip should be equal to 0.5 MF. If it is less, add respectively. If it is more, than add a drop of water and adjust the density to 0.5MF. (*Figure 4*)

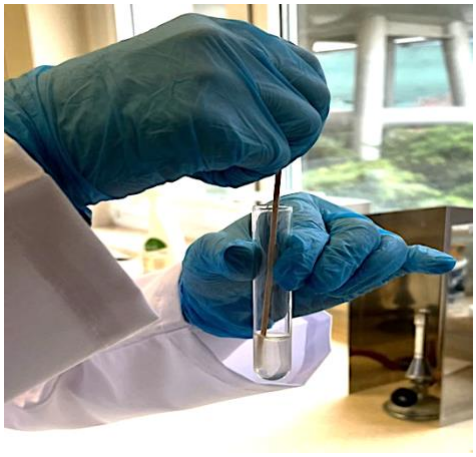


Figure 4: E. coli getting mixed inside the tube with cotton tip

B- <u>Preparation of the Vinegar Solutions & Diluting the Vinegars:</u>

1. Take 5 agar plates and put the vinegars and distilled water so it is easier to use the micropipette to such the ingredients. Number them from 1 to 5 and put the ingredients as followed: 1-distillated water, 2-white vinegar, 3- apple cider vinegar, 4-vinegar of grapes, and 5-vinegar of date palm. The amount is not relevant since, the plates' purpose it to ease the usage of the pipette while taking a sample.
2. Take 5 test tubes and mark them as 1,2,3,4, and 5, so the ingredients won't mix up. Put 1mL distillated water in the first one, this will be the positive control. Put 900 μL distillated water to the remaining four. And respectively as decided on the third step, put 100 μL of

each vinegar to the test tubes separately as their corresponding numbers from step 3. Use the 1000 μ_L pipette for 900 μ_L and 1mL and use the 200 μ_L pipette for the 100 μ_L measurements. Stir all of them so the vinegar is mixed homogenously with water.

C- <u>Mixing the Solutions Together for the Preparation of the Incubation Process:</u>

1. Put 1mL *E. coli* from the prepared test tube using the 1000 μ_L pipette to each labeled test tube separately.
2. Let them sit for 15 minutes after stirring them for homogenous distribution of *E. coli*. Do not use the same stirring item for all the tubes. Use separate ones for each tube. The stirring item can be plastic stirring rode. Dispose the rod to a biological hazard container after each use. If it is not disposable such as a glass rod, disinfectant them immediately for safety.

D- <u>Plantation of the Bacteria-Vinegar Solutions & Incubation:</u>

1. Take 5 EMB growth plates and label them respectively to step 3, also add dates on them to keep in check.
2. Take tube-1 and EMB-1. Take 10μ_L sample from tube-1 by using 20 μ_L pipette and put it in the center of the EMB plate. Dispose the tip of the pipette.
3. Take a disposable loop and do the streak plate method: Using the loop, take the sample from the center to a corner of the plate slowly distribute it by moving the loop in horizontal lines while slowly moving down to the center. After reaching the center, turn the plate 60 degrees to the left and do the method again. Do this for 6 times so the whole plate has the sample equally distributed. While using the loop it is crucial to not damage the EMB. Therefore, the distribution movements should be very delicate and superficial to the plate. Holding the loop almost parallel to the plate helps. Dispose the loop after and close the EMB plate with its lid facing downwards. This prevents water forming on the plate.

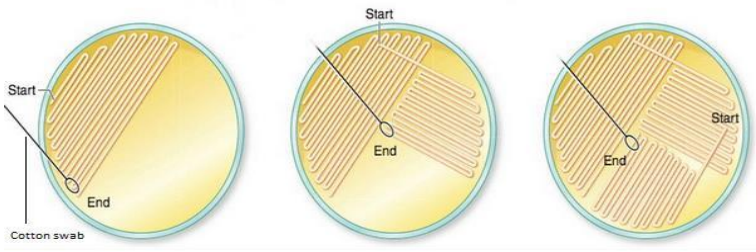


Figure 5: Streak Plate Method

4. Repeat D-2 and D-3 for the rest of the test tubes and EMB plates.
5. Put the EMB plates to the incubator in 34 C° and leave them for 18 hours.
6. Make sure every item you used are either disposed or disinfected. Dispose the gloves, put the coat to disinfestation and wash your hands with antibacterial soap.

E- <u>Collecting Data by Colony Counting:</u>

1. After 18 hours -first again wear gloves and white coat- take the EMB plates out of the incubation machine. Count the number of colonies (dots on the plate). If they are too much to be counted get help from a professional in the lab. The ones that are uncountable, in Figure 6 and Figure 7 for example, are classified as 10 000<x or if more 100 000<x, where x is the number of colonies. With the help of a professional in the lab, you can differentiate them better if you are unable to see a difference.
2. Calculate the colony forming unit to scientifically compare the results by using the formula in the introduction. For this experiment with these numbers, the dilution coefficient is 10^6.
3. To this experiment 5 times to get concise results. (Go to step 1 under the title **Procedure- A) Preparation of the Bacteria Solution**)

Risk Assessment, Environmental and Ethical Concerns:

The experiment has been conducted through Biosafety level 1 due to the bacteria used, which is *Escherichia coli* ATCC 25922. Biosafety level 1 classed bacteria are safe to work with without the risk of getting sick for an adult person typically. However, even though this safety level needs bare minimum precautions, to prevent uncontrolled distribution items used should be disposed after usage to biohazard disposable bins. At all times gloves and white coat should be worn. However, this level bacteria cannot distribute from inhalation, therefore, no need for any masks throughout the experiment. Since the dispose is done to biohazard bins, there is no concern for environmental contamination. No animals or humans are used in this experiment; therefore, this experiment has been done under ethical conditions with taking care of human safety.

DATA:

Raw Data Table:

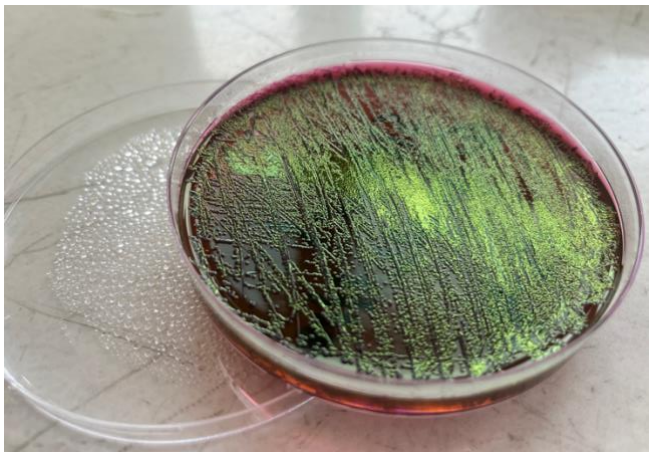
Distilled Water and Types of Vinegar	Trials	Number of Colonies Counted on EMB plates (Each colony with 10^2 bacteria)
Distillated Water (Positive Control)	**1**	x>100,000
	2	x>100,000
	3	x>100,000
	4	x>100,000
	5	x>100,000
White Vinegar	**1**	10
	2	5
	3	33
	4	1
	5	0
Apple Cider Vinegar	**1**	20
	2	80-90
	3	58
	4	24
	5	0
Vinegar of Grapes	**1**	0
	2	0
	3	0
	4	3
	5	1
Vinegar of Date Palm	**1**	≈ 10,000
	2	≈ 10,000
	3	≈ 10,000
	4	≈ 10,000
	5	≈ 1,000

Table 4: Raw Data Table of the number of colonies counted on EMB plates respectively to the different types of fluid it is incubated with.

These raw data shows that the vinegar of grapes has the greatest antibacterial effect on *E. coli* compared to the colony numbers counted on the EMB growth plates with nearly no formation of colonies. With more than 10,000 growths approximately, vinegar of date palm showed the

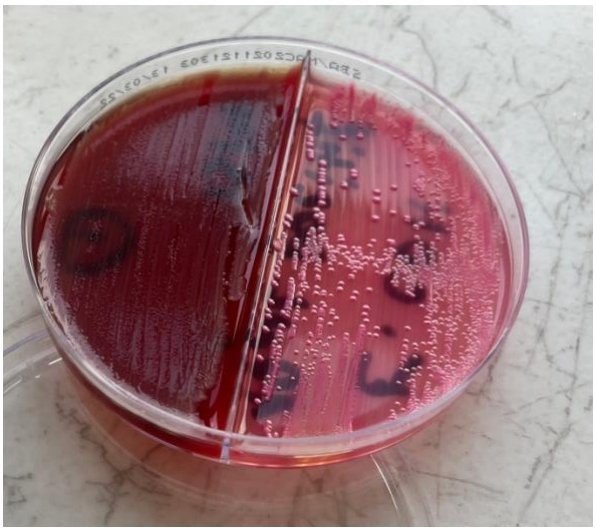
poorest antibacterial effect on the bacteria. The positive control showed expected results as growth due to the natural growth medium with no antibacterial inhibitor, such as a vinegar.

Example Images of EMB plates:



When the EMB plate is completely covered with colonies, the number of colonies is approximately more than 100,000 as in *Figure 6*. On EMB growth medium *E. coli* leaves a distinctive greenish shine, which makes the bacterium easy to differentiate.

Figure 6: Positive Control on EMB plate



On this type of EMB plates used in the experiment for colony counting, there is no reflectiveness of *E. coli* which makes it easier to count the colonies. This is the reason these types of plates were used in the experiment rather than the distinctive EMB plate showed in *Figure 6.*

Figure 7: Positive Control on EMB plate used in the experiment for colony counting

Figure 8: White Vinegar

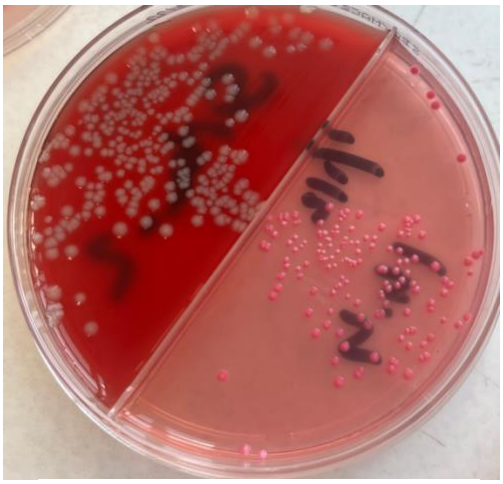


Figure 9: Apple Cider Vinegar

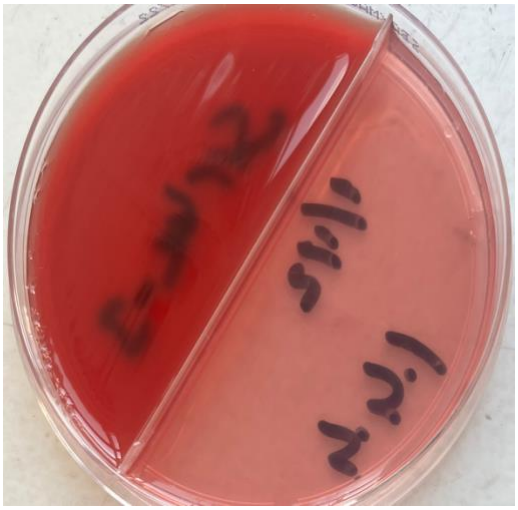


Figure 10: Vinegar of Grapes.

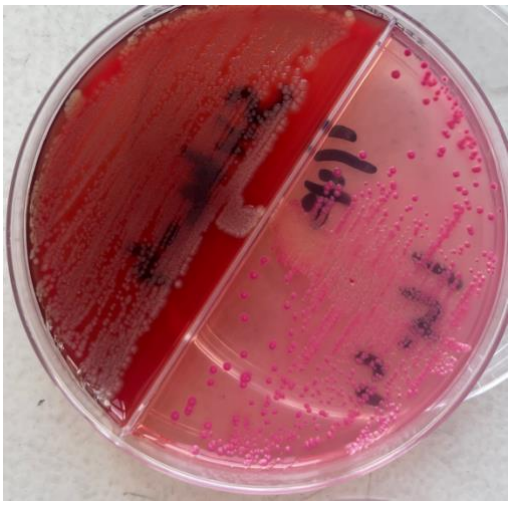


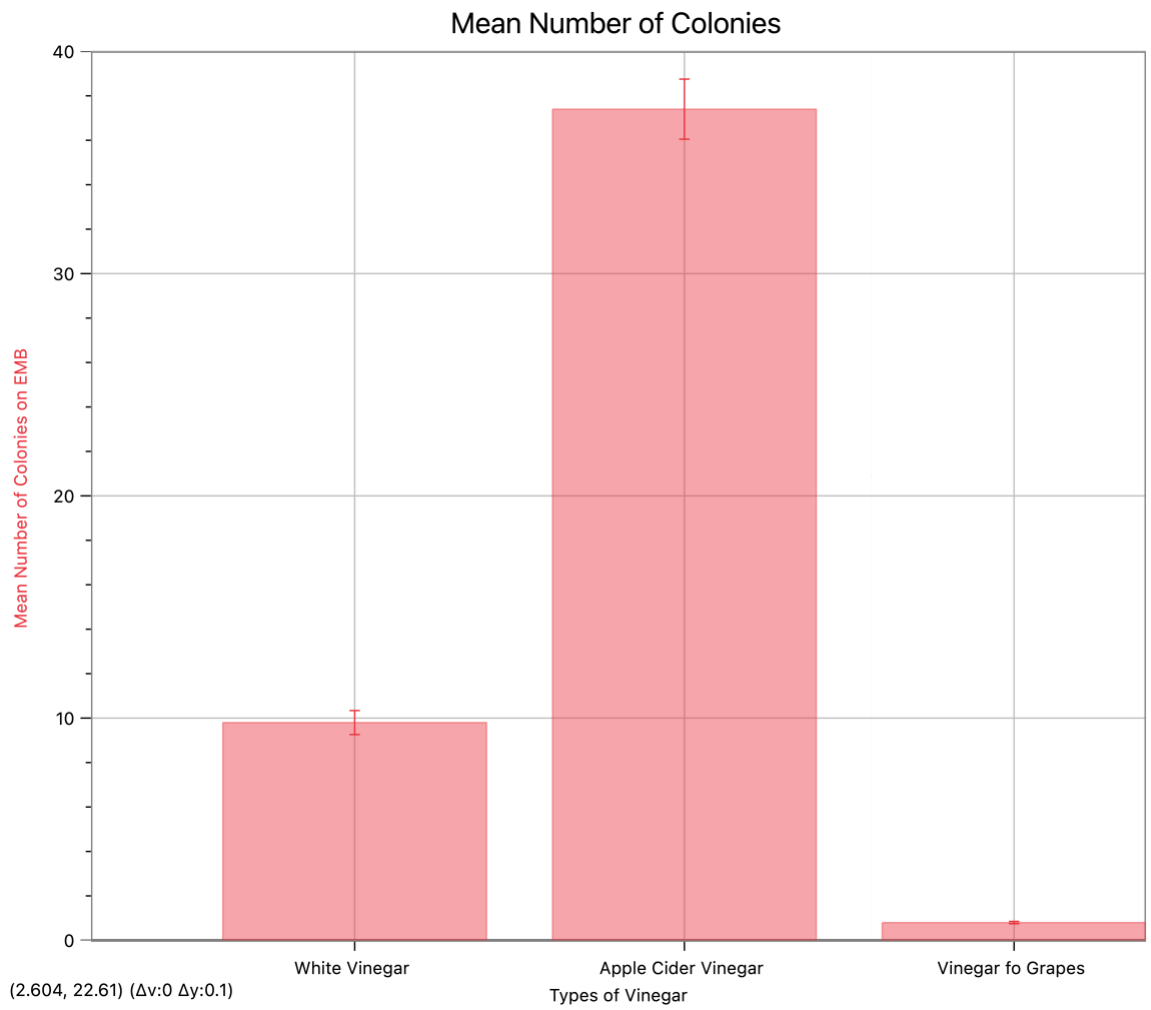
Figure 11: Vinegar of Date Palm

As shown the in the example figure vinegar of grapes, showed in *Figure 10*, had no growth at all; while in *Figure 11*, the growth has formed a coat over the medium with the effect of vinegar of date palm.

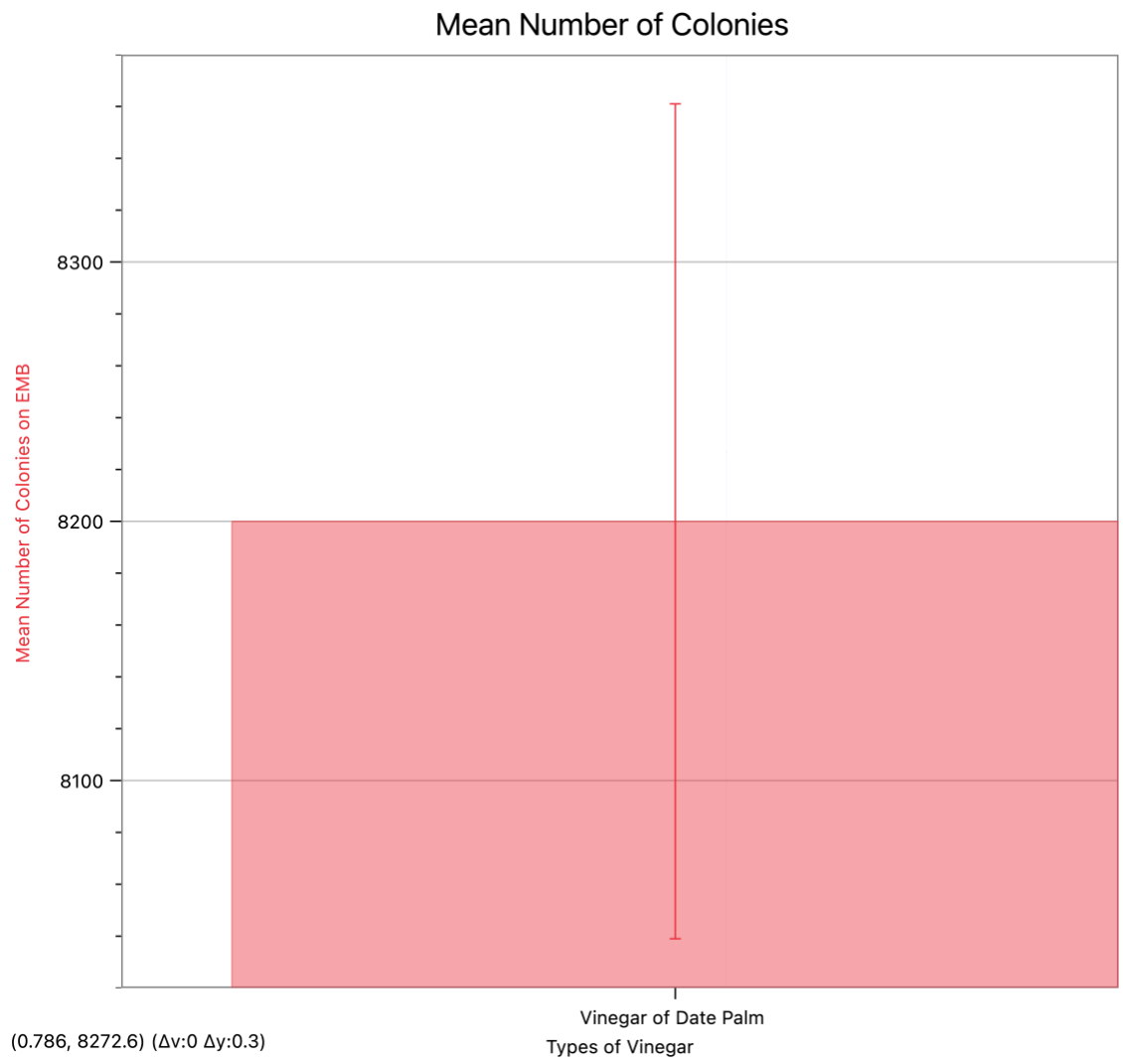
Processed Data Table:

Distilled Water and Types of Vinegar	**Mean Number of Colonies on EMB (each colony with 10^2 bacteria on average)**	**Standard Deviation**	**Standard Error**
Distillated Water (Positive Control)	x>100,000	0.0	0.000
White Vinegar	9.8	13.5	0.540
Apple Cider Vinegar	37.4	33.8	1.352
Vinegar of Grapes	0.8	1.3	0.052
Vinegar of Date Palm	≈ 8200	4025.0	161.000

Table 5: Processed Data Table with the mean number of colonies produced with standard deviations and standard error.



Graph 1: Graph of Mean Number of Colonies Produced with Error Bars representing Standard Error for White Vinegar, Apple Cider Vinegar, and Vinegar of Grapes



Graph 2: Graph of Mean Number of Colonies Produced with Error Bars representing Standard Error for Vinegar of Date Palm

Because the mean data varies from 0.8 to 100 000, it is not possible to see the mean values of white vinegar, apple cider vinegar, vinegar of grapes and vinegar of date palm in the same graph. Hence, because the vinegar of date palm is an outlier with its different value compared to the other vinegar types, it has been separated from the graph. Therefore, the values have been separated according to their values to show more clear results in the graphs. Distillated water has not been included in the graphs because it has too large values to be compared with the other results and it has not standard deviation to be shown.

ANOVA

To examine if different vinegar types have a relation with bacteria production, a null hypothesis is created related to the hypothesis of the experiment:

H_0: There is no difference between the effects of same concentration of different vinegar types: White Vinegar, Apple Cider Vinegar, Vinegar of Grapes, and vinegar of Date Palm with positive control on *E. coli*.

H_1: There is a difference between the effects of same concentration of different vinegar types: White Vinegar, Apple Cider Vinegar, Vinegar of Grapes, and vinegar of Date Palm with positive control on *E. coli*.

SUMMARY				
Groups	***Count***	***Sum***	***Average***	***Variance***
Distillated Water	5	500000	100000	0
White Vinegar	5	49	9,8	183,7
Apple Cider Vinegar	5	187	37,4	1142,8
Vinegar of Grapes	5	4	0,8	1,7
Vinegar of Date Palm	5	41000	8200	16200000

Table 6: Table of summary of Anova-single factor statistical test

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	3,8619E+10	4	9654644493	2979,58426	1,9125E-27	2,8660814
Within Groups	64805312,8	20	3240265,64			
Total	3,8683E+10	24				

Table 7: Anova-single factor test results

Because the *P-value* is smaller than prechosen alpha value as $1.91*10^{-27}<0.05=\alpha$, H_0 is rejected and H_1 is accepted. Thus, the difference in the vinegar type creates a difference in bacterial production. Since there is a significant relation between the dependent variables and the independent variables with a significant difference between the results between reproduction values of different vinegar types, there is no need to do another statistical test such as Tukey Test.

Number of colonies on average in 1mL or the CFU number:

At the beginning of the experiment 0.5 MF bacteria were taken, which is equal to 10^8 bacteria. Then the bacteria were put into 1mL of distillated water, which results of a dilution 1/1000 since 1 mL is 1000μL and 0.5 MF calculated based on microliters (μL). Therefore, after the first dilution the average number of bacteria were reduced by 10^{-3}, which results in 10^5 bacteria on average. Then after incubating the bacteria with the vinegars for 15 minutes, 10μL plantation has been done on the EMB plates. This plantation again results in a dilution by 1/1000. Thus, the total dilution is 10^6. This gives 10^2 bacteria on average on colonies counted from the EMB plates. Because the results collected from the plates were diluted with 10^6 ratios, when calculating the CFU the results should be multiplied with 10^6. The total mL of the vinegar - *E. coli* mixtures were 2mL, so the result of the multiplication will be divided into 2 to calculate 1 mL of results for CFU.

-Example calculation:

- White Vinegar:

$$CFU = \frac{mean\ number\ of\ colonies\ \times\ dillution\ coefficient}{total\ mL\ of\ solution}$$

$$CFU = \frac{9.8\ \times\ 10^6}{2} = 4.9 \times 10^6$$

CFU of the results				
Distillated Water (Positive Control)	**White Vinegar**	**Apple Cider Vinegar**	**Vinegar of Grapes**	**Vinegar of Date Palm**
x>50,000 x 10^6	4.9 x 10^6	93.5 x 10^6	0.4 x 10^6	≈ 4100 x 10^6

Table 8: CFU numbers of the results

ANALYSIS AND DISCUSSION

In this experiment the antibacterial effect of different vinegar types; apple cider, white vinegar, vinegar of grapes, and vinegar of date palm on *Escherichia coli* bacteria, by comparing their colony numbers by counting the colonies formed on the EMB growth plates after incubation with the vinegar types and 18-hour growth period have been investigated. Looking at the average numbers from *Table 5*: Distillated Water- x>100000, White Vinegar 9.8, Apple Cider Vinegar 37.4, Vinegar of Grapes 0.8, and Vinegar of Date Palm ≈ 8200 mean number of colonies; vinegar

of grapes show the best antibacterial effect on *E. coli* as colony number formation after incubation with the vinegar with 0.8 colonies on average. White vinegar is the second most effective on inhibition of growth with 9.8 colonies formed. These two vinegars can be used for disinfecting vegetables and fruit compared to the other vinegar types. As for the hypothesis, apple cider vinegar did not show the most effective result, therefore, it was wrong.

Looking at the ANOVA test, the difference in the vinegar type effects the number of colonies formed, thus the production of the bacteria. Comparing the CFU units, still the most effective on inhibition of growth or the most effective disinfectant or the most antibacterial affect is shown from "Vinegar of Grapes". "Vinegar of Date Palm" with nearly %50 inhibition showed the poorest results as an inhibitor. Still, all vinegars showed that they have an antibacterial effect on *E. coli* as inhibiting the growth of colonies by at least %50 after incubation with them.

"Apple Cider Vinegar" did not follow up to its expectations in the hypothesis. However, White Vinegar as the most common disinfectant managed to inhibit %99 of the growth by forming nearly 10 colonies while the positive control resulted over 100,000 colonies on average. Even though it did not give the most effective results as "Vinegar of Grapes", %99 shows a huge impact on the inhibition of the bacterium. Therefore, all these vinegar types are effective on inhibition of the growth of the bacterium *E. coli*, thus, they have an antibacterial effect on the bacterium by preventing the growth of colonies. The comparison of the effectiveness of inhibition of growth by each vinegar type for incubation goes as followed: Vinegar of Grapes produced 0.4×10^6 CFU of bacteria, White Vinegar produced 4.9×10^6 CFU of bacteria, Apple Cider Vinegar produced 93.5×10^6 CFU of bacteria, and Vinegar of Date Palm 4100×10^6. Thus, the most effective vinegar type to inhibit bacterial growth is Vinegar of Grapes, while the least efficient is Vinegar of Date Palm.

Strengths, Limitations and Source of Error:

1- Strengths:

The experiment has given expected results from the trials. Because the experiment has been conducted in a lab specific for microbiology, the materials and instrumentals used were specific and convenient for the experiment. This benefits the experiment as creating and

all sterile environments. Moreover, the experiment has been conducted under professionally supervision from a microbiology professor, thus, further risks and unnecessary errors has been prevented. With a large sample size as 25 trials total and 5 trials for each variable precise and not random results have been exhausted. Lastly, methodology was appropriate to the experiment.

2- Limitations and Source of Error:

Because different sets of EMB plates were used as in the manufacturing dates, different trials could have been affected by the slight differences. However, there was no possible way to reduce or eliminate this type of error. Another limitation is that all the processes starting form plantation to colony counting has been done by human effort rather than machines, therefore there could be human error whilst in the process of colony counting or plantation. The last limitation is that small measurements were used which could have also lead errors that would go unnoticed. Moreover, the pipettes used could have also not take the full amount of solution and there might be slight differences for each trial and sample. However, to prevent this as much as possible the pipettes were checked for multiple times before ejaculation, and when the pipette tip was found suspicious, they were replaced with another pair to control the experiment as much as possible.

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APPENDIX

Appendix-1:

T.C.
HACETTEPE ÜNİVERSİTESİ
Tıp Fakültesi

Temel Tıp Bilimleri Bölümü
Tıbbi Mikrobiyoloji Anabilim Dalı

Sayı :
Konu :

To whom it may concern [redacted]

This is to certify that Yağmur Serttaş has done her IB diploma programme extended essay experiments in Hacettepe University Medical Microbiology Department Laboratory by herself with the supervision of Dr. Neşe İnal and myself.

Sincerely,

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