



Comparative Efficacy of Various Vinegars in Reducing Bacterial Load and Inhibiting Foodborne Pathogens



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Abstract

Background: Although leafy vegetables are utilized extensively in Iraqi cuisine, yet it loads various microbial contaminants that may lead to foodborne diseases, food poisoning, and food spoilage. **Objectives:** The purpose of the present study was to evaluate the use of natural products (4 vinegar types) in sterilization and disinfection of leafy vegetables. **Methodology:** This laboratory-based experimental study was conducted in the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. This study was carried out from November 2024 to January 2025. The isolation of pathogenic bacteria from leafy vegetables collected from the Baghdad city market. Using well diffusion techniques, the presented work sought to assess the antibacterial properties of four vinegar types-grape, date, white vinegar, and apple cider and the impact of vinegar concentration, type, and exposure duration on the bacterial load in leafy vegetables by Pour plating on PCA (Plate Count Agar). **Results:** (6) *Salmonella typhi*, (10) *Pseudomonas aeruginosa*, and (6) *Shigella* species were isolated from 25 distinct leafy vegetables basal, celery, parsley, watercress, leek, spinach, and mint. *Salmonella Typhi* showed sensitivity to vinegar through inhibitory diameters ranging between 25, 28, 35, and 35 mm, and *Shigella* (15, 20, 28, 28 mm) for date, grape, apple, and white vinegar, respectively. *Pseudomonas aeruginosa* was resistant to all vinegar types. The microbial load regarding food has been examined using six vinegar concentrations, such as 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, and 6.0%, as well as exposure times like 10, 20, and 30 minutes for each one of the four types of vinegars such as grape, date, apple, and white. With regard to each one of the vinegar samples, aerobic colony counting has been carried out through pour plating on PCA (Plate Count Agar). On the bacterial load, white vinegar has the strongest effect, followed by apple, grape, and date. A significant factor in reducing the bacterial load is extending the treatment period and raising the vinegar content. **Conclusion:** *Pseudomonas aeruginosa* is resistant to all types of vinegar; however, vinegar samples from dates, grapes, cider apples, and white vinegar have antibacterial activity against the two studied bacteria, *Salmonella Typhi* and *Shigella* species. Date vinegar was the least effective at lowering the number of bacteria, whereas white vinegar was the most successful. Vegetables and fruits could be safely disinfected with vinegar, although the type of vinegar, concentration, and exposure duration all affect antibacterial activity. [Bangladesh Journal of Infectious Diseases, June 2026;13(1):96-103]

Keywords: Bacterial load; vinegar; foodborne pathogens; antibacterial activity

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Introduction

Microorganisms can be defined as one of the factors contributing to food spoilage, as well as food-borne illnesses, which impact both developing and developed nations worldwide. Numerous saprophytic as well as pathogenic microorganisms are known to lead to food spoilage, which in turn leads to food-borne illnesses¹. Only with an efficient sanitation process could food be reliable and clean. There are several widely utilized techniques for food sanitation, and one of the most popular ones for household food sanitation is using vinegar². To attain a high degree of safety against foodborne pathogenic microorganisms, natural alternatives are required³. One of the natural products with sterilizing qualities is vinegar, sometimes referred to as acetic acid. The fermentation of alcoholic beverages, primarily wine, produces vinegar, an acidic liquid⁴.

Approximately 5.0% to 20.0% acetic acid ($\text{CH}_3\text{-COOH}$), water, and several trace chemicals, including flavorings, make up vinegar, a liquid⁵. The fermentation process involving ethanol through microorganisms produces acetic acid. Grain vinegars, which use sorghum, wheat, rice, or other grains as their raw materials, and fruit vinegars, which use fruits like apples, grapes, and dried dates as their raw materials, are the two primary categories of vinegars depending on their raw materials⁵⁻⁶. Moreover, alcohol as well as sugar can be fermented to produce vinegars. Conventional fermented fruit vinegars as well as grain vinegars have several physiological properties, including anti-infection, antibacterial, antioxidative, lipid metabolism, blood glucose, anticancer properties, and weight reduction. Polyphenols, organic acids, and melanoidins are the primary components that give vinegars their anti-infection and antibacterial properties³⁻⁴.

The health and composition advantages of vinegar have been previously demonstrated in several studies. Catechin, gallic acid, chlorogenic acid, epicatechin, syringic acid, caffeic acid, and ferulic acid are known to be present in grape vinegar, while catechin, gallic acid, chlorogenic acid, epicatechin, caffeic acid, and p-coumaric acid are known to be present in apple cider vinegar⁷. Bioactive compounds, like flavonoids, polyphenols, vitamins, and dietary fiber, are found in dates. These bioactive compounds are more easily absorbed by the body when dates are fermented to make vinegar, which improves their medicinal effects^{8,9}. The combined activity of various organic acids, alcohol, and phenolics was ascribed to their combined activity⁵.

There is certain evidence that the inclusion of phenolic compounds in fermented vinegars enhances their antimicrobial effect, although certain vinegars with an acetic acid level of just 0.1% could inhibit the growth of a variety of bacterial species^{8,10}. With the use of the well-diffusion method, the presented work intended to examine the antibacterial activity of four types of vinegar such as grape, date, white, and apple against three bacterial isolates that were extracted from leafy vegetables. It also aimed to determine the impact of four vinegar types, varying concentrations, and exposure duration on the bacterial load in leafy vegetable samples.

Methodology

Study Design: This laboratory-based experimental study was conducted in the Department of Biology at the College of Science, University of Baghdad, Baghdad, Iraq. This study was carried out from November 2024 to January 2025.

Vinegar Samples: The samples of date vinegar, apple cider vinegar, grape vinegar, and white vinegar utilized in the presented work were bought from the local store in Baghdad. With the use of pH paper, the pH of vinegars was determined.

Food samples: Various samples of leafy vegetables such as celery, basil, watercress, parsley, spinach, leek, and mint were collected from a local market in Baghdad. The leafy food samples were cut using a sterile knife, then we weighed 5 grams of leafy vegetable samples were weighed, put in 45 ml of sterilized peptone water in sterilized containers, shake them, use rinse and washing method².

Bacterial Isolation: Samples of 25 distinct leafy vegetables-basal, celery, parsley, watercress, leek, spinach, and mint-were collected at the Baghdad city market between November and January of 2024 to January 2025. A total of five grams of food sample was suspended in 45 milliliters of peptone water in sterile containers. After that, using the rinse as well as the washing method, 0.1 milliliters of diluted solution were added to a sterile Petri dish. Cetrimide agar, MacConkey agar, and Salmonella-Shigella agar (SS agar) were added separately using the pouring plate method, and the dishes were incubated for twenty-four hours at 37 degrees Celsius².

Identification of the Isolates: Isolates are identified based on their morphological and microscopic characteristics as well as the use of biochemical tests for diagnosis. The isolates regarding the bacteria that were obtained were stained with Gram stain, and their morphological

characteristics were examined on Cetrimide agar, MacConkey agar, and SS agar. The bacteria that were isolated from food samples were diagnosed using API20E^{2,11}.

Antibacterial Activity of Vinegar Samples Against Foodborne Bacterial Pathogens: The antibacterial activity of four different types of vinegars against *Salmonella typhi*, *P. aeruginosa*, and *Shigella* isolated from leafy food samples has been investigated using the well-diffusion method. The brain-heart infusion broth was injected with loopful growths from bacterial isolates and incubated for 18 hours at 37 °C. Normal saline was used for diluting the bacterial suspensions. Adjust the turbidity to produce a uniform suspension with 1.5×10^8 CFU/ml and compare it to the standard tube (McFarland number 0.5). After dipping a cotton swab into the adjustment suspension and streaking the whole Mueller-Hinton agar surface regarding the plates (for every tested bacterium), the plates were allowed to dry for five to fifteen minutes at room temperature. A cork borer was used to cut the medium into five wells (5 mm in diameter), and 20μ of each type of vinegar solution was added to each well, along with a control well (D.W.). After that, all of the examined organisms' plates were left to incubate for the entire night at a temperature of 37°C. Note the zone of inhibition for each isolate following a 24-hour incubation period. The millimeter (mm) scale was used to quantify the zone of inhibition's diameter 3,11.

Quantification of Bacteria: Six different vinegar concentrations (1.0%, 2.0%, 3.0%, 4.0%, 5.0%, 6.0%), exposure times (10 min, 20 min, 30 min) for each of the four types of vinegars (date, grape, apple, white) were tested for microbial load of food. Pour plating on PCA (Plate Count Agar) has been used for performing aerobic colony counting for each vinegar sample as well as concentration. First, 0.5 ml (1.0%) of vinegar has been added to one container, followed by 1 ml (2.0%) in the other container, and so on until we reached 3 ml (6.0%). This process has been repeated for all four types of vinegar, resulting in six containers of sample with varying concentrations such as 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, and 6.0%. Next, with the use of a micropipette, we remove 0.1 mL of the sample diluent, put the inoculum in the center of petri dish, pour the cooled agar medium (PCA) over it, and mix it both clockwise and counterclockwise to homogenize it. Take into account adding a sample to the Petri dish without vinegar and considering it as a control. Following the above steps prepare the control dish first for the vinegar then we wait 10

minutes before the second inoculation, then after the second addition we also wait another 10 minutes, so the time that the samples were exposed to will be 20 minutes, then after third, we wait another 10 minutes, so the total will be 30 minutes, these steps are applied to all types of vinegar^{12,13}. After completing these steps, we wait for the agar to harden completely and incubate the plates at 37°C for 24 h. After 24 hours, we take the Petri dishes out of the incubator and notice the presence of bacterial colonies in them. Count the bacterial colonies using the colony counter. The experiment was repeated three times for each type of vinegar.

Statistical Analysis: Data were statistically analyzed using the program Statistical Analysis System –SAS (V 21.0). Significant differences between the averages were compared using the Duncan test (22) polynomial.

Ethical Considerations: Formal ethics approval was granted by the local ethics committee. All data were collected anonymously and were analyzed using the coding system.

Results

Isolation and Identification of the Isolates: Following their diagnosis by biochemical tests in API20E system, ten isolates of *P. aeruginosa*, six isolates of *Shigella*, and six isolates of *Salmonella typhi* have been obtained from samples of leafy vegetables. The Gram stain method was used for confirming each one of the isolates. FeS deposition caused *Salmonella* bacteria to grow in colonies on S.S. agar that had a black center. On S.S. agar, the colorless *Shigella* colonies seem transparent. Because *P. aeruginosa* bacteria produce fluorescein dye, colonies on cetrimide agar look yellow-green, indicating the presence of the bacterium.

pH Measurement of Vinegar Samples: The vinegar samples' pH values have been as follows: 3.5 for grape vinegar, 2.6 for white vinegar, 4 for dates vinegar, and 3 for apple cider vinegar.

Antibacterial activity of Dates, Grape, Apple cider, and White Vinegar Against *Salmonella typhi*, *Shigella*, and *Pseudomonas aeruginosa*: Using the well diffusion method, the antibacterial properties

Table 1: Diameter of Inhibition Zone of the 4 Vinegar Types Towards Isolated Foodborne Bacteria

Bacteria	Date Vinegar	Grape Vinegar	Apple Vinegar	White Vinegar
<i>Salmonella typhi</i>	25 mm	28mm	35mm	35 mm
<i>Shigella</i>	15 mm	20 mm	28 mm	28 mm
<i>Ps. aeruginosa</i>	-	-	6mm	8mm

of apple cider, dates, grape, and white vinegar were tested against *Shigella*, *P. aeruginosa*, and *Salmonella typhi* (Table 1).

Vinegar samples were effective against both *Salmonella Typhi* and *Shigella* species isolated from food (Figure I, Figure II), while *Pseudomonas aeruginosa* was resistant to all vinegar types with a very weak inhibition zone (Figure III).

White and apple vinegars were more efficient than grape, date vinegar was the least effective. The antibacterial effect of vinegars may be attributed to their low PH values and their acidity. White Vinegar is the most effective, with a 23.67 mm inhibition zone ($p = 0.0153$), the time effect significance is $p = 0.0488$ (Table 2).

Table 2: Zone of Inhibition Analysis

Vinegar Type	Mean Inhibition Zone (mm)
Date Vinegar	13.3± 0.59
Grape Vinegar	16.0± 0.00
Apple Vinegar	23.0± 1.01
White Vinegar	23.7± 0.58

F-stat: 0.3986 P-value: 0.7578; Not statistically significant ($p \geq 0.05$)

This indicates that the differences observed in the diameter of inhibition zones among the four vinegar types are likely due to random variation rather than a true inherent difference in their antimicrobial properties. Although White and Apple vinegar showed larger physical zones (23.67mm vs 13.33mm), the high degree of similarity in results



Figure I: Showing the Antibacterial effect of vinegar against *Salmonella typhi* by well diffusion method (A: apple vinegar, G: grape vinegar, D: date vinegar, W: white vinegar, C: control (D.W))

across the bacterial strains tested suggests that, for outward inhibition, these vinegars perform comparably.



Figure II: The antibacterial effect of vinegar against *Shigella* by well diffusion method (A: apple vinegar, G: grape vinegar, D: date vinegar, W: white vinegar, C: control (D.W))

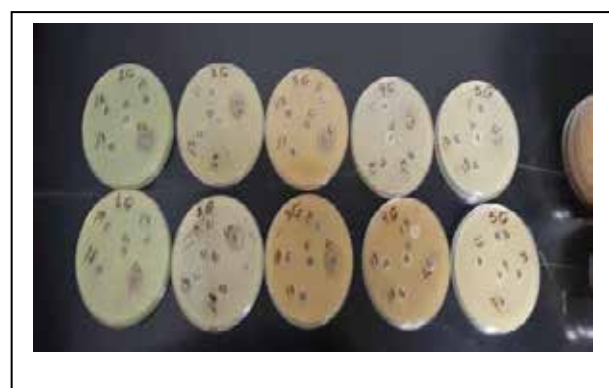


Figure III: The Antibacterial Effect of Vinegar Against *Pseudomonas aeruginosa* isolates by well diffusion method

Effect of Date, Grape, Apple, and White Vinegar Concentration and Exposure Time on Microbial Load of Leafy Vegetables: From the results shown in Table 3, Table 4, Table 5, and Table 6. White vinegar was the most effective in reducing bacterial load for leafy vegetables, as the bacterial load decreased from 550×10^4 to 460×10^4 CFU/ml (16.3%) for 1.0% vinegar concentration in 30 min, to a 72.7% decrease in bacterial load from 550×10^4 to 150×10^4 for 6.0% vinegar concentration in 30 min. On the other hand, Date vinegar was the least effective in reducing bacterial load for leafy vegetables, as the bacterial load decreased from 550×10^4 to 535×10^4 (2.7%) for 1% vinegar concentration in 30 min, to a 36.0% decrease in

bacterial load from 550×10^4 to 350×10^4 for 6.0% vinegar concentration in 30 min. Apple vinegar was more effective in 4.0%, 5.0%, and 6.0%; the percentage decrease in bacterial load in 30 min was 25.5%, 45.0%, and 63.6%, respectively. Grape vinegar shows a moderate effect on bacterial load,

ranging between 6.3% and 45.0% decrease in 1.0% to 6.0% vinegar concentration in 30 min. The antibacterial effect for all vinegar types increases in direct relationship with increases of vinegar concentration and exposure time.

Table 3: Mean of Microbial Load and Exposure Time of Date Vinegar

	1.0% conc.	2.0% conc.	3.0% conc.	4.0% conc.	5.0% conc.	6.0% conc.
Control	550×10^4	550×10^4	550×10^4	550×10^4	550×10^4	550×10^4
10 min	545×10^4 (0.9%)	540×10^4 (1.8%)	535×10^4 (2.7%)	520×10^4 (5.5%)	460×10^4 (16.4%)	440×10^4 (20%)
20 min	540×10^4 (1.8%)	535×10^4 (2.7%)	530×10^4 (3.6%)	500×10^4 (9.1%)	420×10^4 (23.6%)	400×10^4 (27%)
30 min	535×10^4 (2.7%)	530×10^4 (3.6%)	525×10^4 (4.5%)	480×10^4 (12.7%)	400×10^4 (27%)	350×10^4 (36%)

Table 4: Mean of Microbial Load and Exposure Time of Grape Vinegar

	1.0% conc.	2.0% conc.	3.0% conc.	4.0% conc.	5.0% conc.	6.0% conc.
Control	550×10^4	550×10^4	550×10^4	550×10^4	550×10^4	550×10^4
10 min	535×10^4 (2.7%)	530×10^4 (3.6%)	525×10^4 (4.5%)	470×10^4 (14.5%)	450×10^4 (18.0%)	450×10^4 (18.0%)
20 min	525×10^4 (4.5%)	535×10^4 (2.7%)	500×10^4 (9.1%)	450×10^4 (18.0%)	400×10^4 (27.0%)	350×10^4 (36.0%)
30 min	515×10^4 (6.3%)	530×10^4 (3.6%)	475×10^4 (13.6%)	425×10^4 (22.0%)	350×10^4 (36.0%)	300×10^4 (45.0%)

Table 5: Mean of Microbial Load and Exposure Time of Apple Vinegar

	1.0% conc.	2.0% conc.	3.0% conc.	4.0% conc.	5.0% conc.	6.0% conc.
Control	550×10^4	550×10^4	550×10^4	550×10^4	550×10^4	550×10^4
10 min	530×10^4 (3.6%)	525×10^4 (4.5%)	500×10^4 (9.1%)	460×10^4 (16.4%)	450×10^4 (18.0%)	400×10^4 (27.0%)
20 min	520×10^4 (5.5%)	500×10^4 (9.1%)	485×10^4 (11.8%)	450×10^4 (18.0%)	350×10^4 (36.0%)	300×10^4 (45.0%)
30 min	510×10^4 (7.3%)	490×10^4 (10.9%)	475×10^4 (13.6%)	410×10^4 (25.5%)	300×10^4 (45.0%)	200×10^4 (63.6%)

Table 6: Mean of Microbial Load And Exposure Time of White

	1.0% conc.	2.0% conc.	3.0% conc.	4.0% conc.	5.0% conc.	6.0% conc.
Control	550×10^4	550×10^4	550×10^4	550×10^4	550×10^4	550×10^4
10 min	520×10^4 (5.5%)	500×10^4 (9.1%)	490×10^4 (10.9%)	530×10^4 (3.6%)	350×10^4 (36.0%)	250×10^4 (54.5%)
20 min	490×10^4 (10.9%)	485×10^4 (11.8%)	450×10^4 (18.0%)	400×10^4 (27.0%)	300×10^4 (45.0%)	200×10^4 (63.6%)
30 min	460×10^4 (16.4%)	460×10^4 (16.4%)	420×10^4 (23.6%)	380×10^4 (30.9%)	260×10^4 (52.7%)	150×10^4 (72.7%)

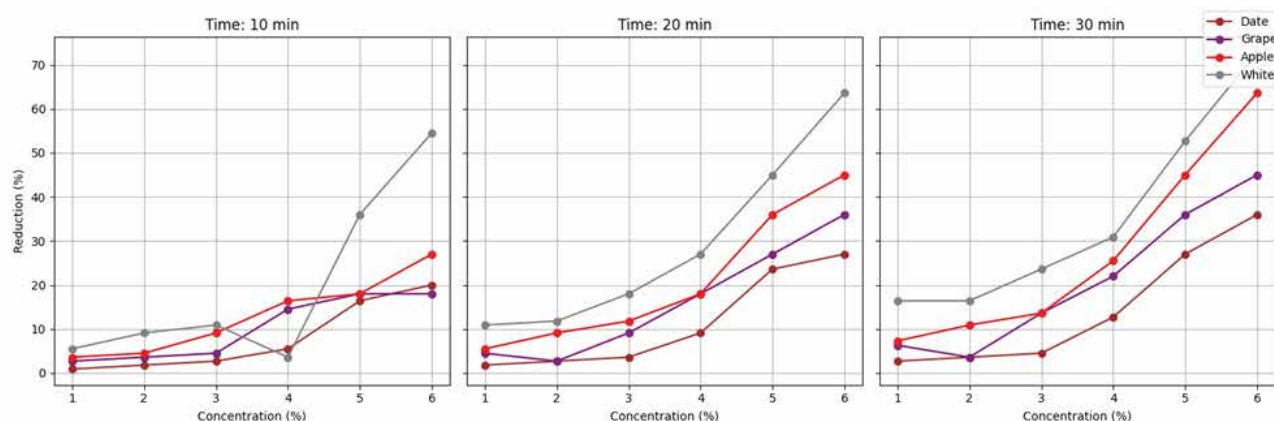


Figure IV: Reduction Percentage of Bacterial Growth at different times of Treatment with different Concentrations of Bacteria by Apple, Date, Grape and White vinegar

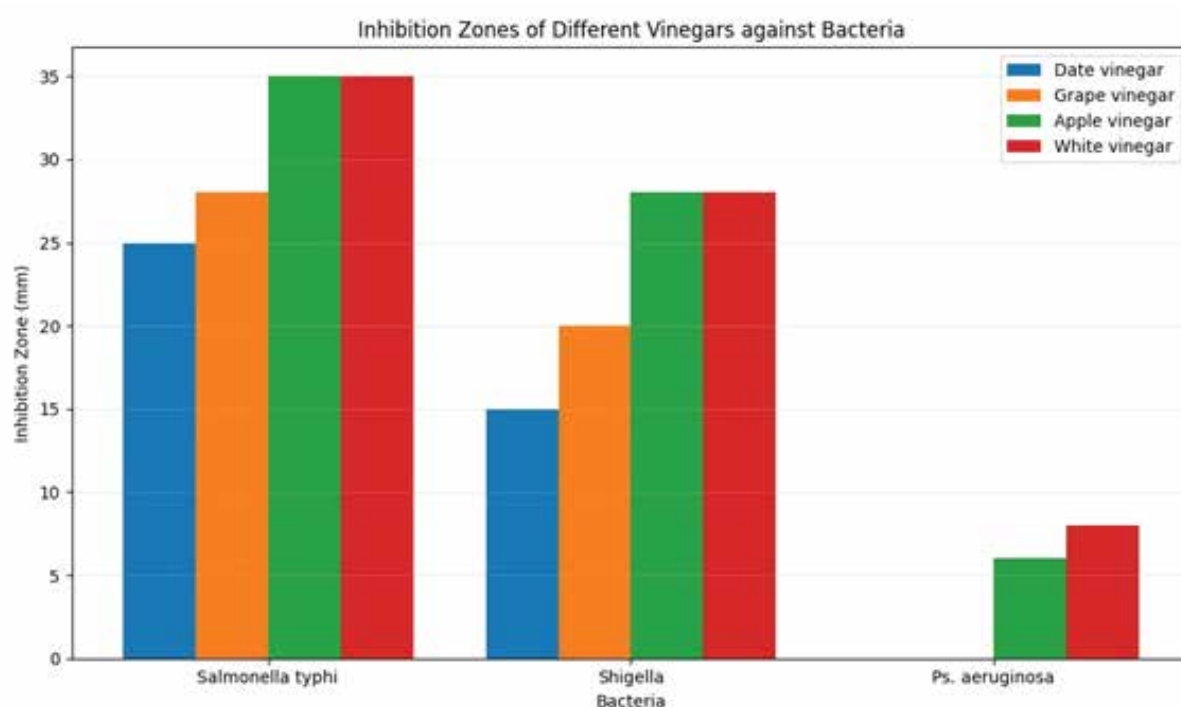


Figure V: Inhibition Zones of Bacteria by Apple, Date, Grape and White vinegar

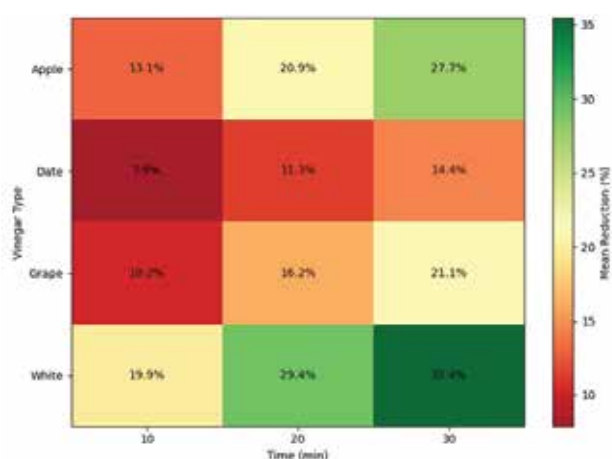


Figure VI: Heatmap of Mean Reduction Percentage of Bacteria by Apple, Date, Grape and White Vinegar

Discussion

Pseudomonas aeruginosa, *Shigella* species, and *Salmonella Typhi* are pathogenic bacteria that can lead to food poisoning; their presence should be zero according to Iraqi standard criteria². The antibacterial effectiveness regarding various vinegar varieties as well as their possible application in food sterilization were examined in numerous works. A study in Turkey² examined the antimicrobial activity of grape and apple vinegars against several bacterial isolates from food samples as well as compared them to commercial chemicals utilized for vegetable and fruit sanitation. They discovered that apple vinegar had a minimum inhibitory concentration (MIC) of 50 µ/ml and grape vinegar had an MIC of 12.5 µ/ml against

Pseudomonas aeruginosa, while *Salmonella typhimurium* had MICs of 50 µ/ml and 25 µ/ml, respectively¹. The effect of dates as well as apple vinegar on local food samples, including minced meat and poultry contaminated with pathogenic bacteria, such as *Escherichia coli* and *Salmonella typhi*, was investigated locally by Hussein². The disc diffusion approach has been applied. Date vinegar's inhibition zones have been 19 mm in diameter against *Escherichia coli* and 9 mm against *Salmonella Typhi*, whereas apple cider vinegar's inhibition zones measured 20 mm against *Salmonella Typhi* and 13 mm against *Escherichia coli*. Two types of apple vinegar with high acidity totally stop *Salmonella Typhi* from growing, out of the four types examined for antimicrobial activity⁴.

Apple cider has a very weak effect on a clinical sample of *Pseudomonas aeruginosa* with a 10 mm diameter of inhibition zone, and no effect on *Salmonella Typhi* by agar diffusion method⁵. Also, in a study, *Pseudomonas aeruginosa* isolated from the hospital environment in Hilla city shows a 10 mm diameter of inhibition zone by apple vinegar¹¹.

Due to the general trend towards reducing the use of chemicals in cleaning and sterilizing vegetables and fruits and using natural alternatives such as vinegar, salt, and other natural substances that are free of chemicals harmful to health and also have antimicrobial effectiveness. However, the question remains: are these natural substances effective against the microorganisms found in food?

Numerous studies¹¹⁻¹² have been conducted to demonstrate the impact of vinegar on food's bacterial load. Six distinct vinegars' antimicrobial properties against foodborne pathogens as well as salad-rotting bacteria were investigated¹. A salad including cucumber, lettuce, and carrot was made for the study and inoculated with *Escherichia coli* and *Staphylococcus aureus*. Various vinegar solutions (3.0%) were applied to inoculated salads for 0, 5, 15, and 30 minutes. Consequently, a reduction of 1 log was noted, and the concentration of vinegar and the length of treatment have been found to be two crucial decontamination parameters. According to another study¹⁴, vinegar demonstrated bactericidal effects on EHEC 0157:H NGY-10. It took one minute for the bacteria to be inactivated, for the CFU to drop from 2.0×10^6 CFU/ml to less than 2.0×10^1 CFU/ml in the vinegar stock solution, such as 10.0% acetic acid. Mean microbial load ranged from 2.70×10^{10} – 3.90×10^{10} CFU/ml after washing with distilled water; 2.10×10^8 to 3.25×10^8 CFU/ml with 10% vinegar solution; 1.65×10^7 to 2.60×10^7 CFU/ml 20.0% vinegar solution and 1.10×10^7 to 1.90×10^7

CFU/ml 30.0% vinegar solution. For vinegar washes, the highest number of contaminants was found on the vegetables after washing with 10% vinegar concentration, followed by 20.0% vinegar solution, with the least contaminant found on the vegetables after washing with 30.0% vinegar solution. Increasing the concentration of vinegar solution from 10% through to 30% resulted in 93.3% to 95.0% reduction in the microbial load of the various vegetables¹³.

Eni et al¹⁵ found that increasing the concentration of the acetic acid (vinegar solution) used in washing from 0.5 to 2.5% resulted in 15.0% to 82.0% reduction in the microbial loads of the various vegetables during 0 to 10 min. All studies showed that concentration and exposure time play an important role in decreasing the bacterial load of food.

Through the action of organic acids, vinegar first prevents the growth of bacteria by destroying the bacteria's outer membrane², preventing the synthesis of macromolecules³, consuming the bacteria's energy⁴, raising intracellular osmotic pressure⁵ and promoting the production of antibacterial peptides in host cells^{2,6}. Another vinegar component with antibacterial properties is polyphenol. The main ways that the polyphenols found in vinegars have antibacterial properties are through disrupting the function of bacterial enzymes as well as damaging the integrity of the cell membrane⁶. Melanoidins, another antibacterial component, are present in vinegar. Melanoidins found in vinegar have antibacterial properties because they can chelate metal ions⁶.

Conclusion

Samples of local leafy foods have been contaminated with pathogenic bacteria such as *Shigella*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. The health of consumers may be at risk if such bacteria are found in local food samples. *Pseudomonas aeruginosa* is resistant to all types of vinegar; however, vinegar samples from dates, grapes, cider apples, and white vinegar have antibacterial activity against the two studied bacteria, *Salmonella typhi* and *Shigella* species. Date vinegar was the least effective at lowering the number of bacteria, whereas white vinegar was the most successful. Vegetables and fruits could be safely disinfected with vinegar, although the type of vinegar, concentration, and exposure duration all affect antibacterial activity.

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Conflict of interest:

The authors declared no conflict of interest.

Financial Disclosure

External funding was not obtained for this research.

Authors' contributions

Dimah Nazar Faraj designed the study and wrote the manuscript; Shaymaa Suhail Najim wrote and revised the manuscript with input from all authors; Layla Fouad Ali re-designed all tables and figures. Finalizing the article was done by the all authors together. All the authors approved the final manuscript.

Data Availability

Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate

The Institutional Review Board granted the study ethical approval. Since this was a cross-sectional study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

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