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Antibacterial activities of the commercial apple vinegar and red grape vinegar against some isolates of plant pathogenic bacteria

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Abstract \

The aim of this study was to evaluate the inhibitory effect of the commercial apple vinegar and red grape vinegar against four plant pathogenic bacterial isolates*; Pseudomonas savastanoi*, *Pseudomonas syringae*, *Erwinia amylovora* and *Erwinia carotovora*. Results showed that all bacterial isolates were sensitive to commercial apple and red grape vinegar at all tested concentrations (25%, 50%, 75% and 100%). The maximum inhibitory effect of apple vinegar was recorded against isolate *Erwinia amylovora* (8.00, 10.00, 10.02 mm) at concentrations (50%,75% and 100%) respectively comparing to other isolates which showed minimum inhibition, results showed that bacterial isolate *Erwinia amylovora* was also higher sensitive to red grape vinegar (7.21, 8.00, 9.00 mm) at concentrations (50%, 75% and 100%) respectively comparing to other isolates that showed minimum inhibition. These results explained that apple vinegar had stronger inhibitory effect than red grape vinegar on isolate *Erwinia amylovora*. Results revealed that apple and red grape vinegar had weak inhibitory effect at concentration (25%) on bacterial isolates tested; *P. savastanoi*, *E. amylovora*, *P.*

syringae and *E. carotovora*. This kind of studies represents the alternative methods which are generally less dangerous and less expensive than chemical agents, by using such organic acids (acetic acid) at recommended doses of controlling phytopathogenic bacteria and fungi, instead of using the excessive chemicals and pesticides that cause environmental, health risks as well as ecological imbalance on the useful microorganisms.In addition of its economicly high expensive.

Key wards: Vinegar, Apple and Red grape, Phytopathogenic bacteria, Antibacterial activities.

Introduction \

The earliest known use of vinegar extended to more than 10,000 years ago, described as natural antimicrobial substance. Vinegar have antibacterial properties active against most bacteria and fungi, that makes it useful for a number of applications. The organic acids of vinegar, mainly acetic acid pass into cell membranes of microorganisms leading to bacterial cell death due to change in pH of the cytoplasm. Vinegar applied as an antimicrobial agent, it was shown to be effective in reducing spoilage bacteria. The vinegar products can be applied as natural antimicrobial agents that can increase the safety, shelf -life, and quality of food products. Vinegar is an acidic sour liquid that is made from the fermentation of an alcoholic beverage mainly wine that contains sanitizing properties **(4, 11, 20)**. Vinegar is comprised mainly of acetic acid, typically 4-18% acetic acid by mass, which is prepared in households by the fermentation of many types of fruits. This solution is also commercially available, cheap and easily found in markets (**11**).

The total acidity of vinegar is expressed as an acetic acid content which is the major organic acid in vinegar. Acetic acid is a monocarboxylic acid. It has a pungent odor and flavor. It is generally regarded safe for general purposes and miscellaneous usages. According to Malicki et *al*., 2004, organic acids are considered weak acids, that their antimicrobial effect is mainly caused by its undissociated forms **(8)**. They passively diffuse through the bacterial cell wall, internalizing into neutral pH dissociating into anions and protons. Release of the protons causes the internal pH to decrease, which exert inhibitory effects on bacteria (**15, 17,18**).

Organic acids have been approved by the Food Safety and Inspection Service of the United States - Department of Agriculture (FSIS) (**18**). Researchers over the world have proved the antibacterial effect of organic acids on different types of pathogenic bacteria. Organic acids such as tartaric, citric, lactic, malic, propionic, and acetic acids have been used for years for decontamination of bacteria on beef, pork, fish and poultry**(1, 2,5,6,7,8, 10, 15, 17,18,21).** Organic acids that are used to inhibit spoilage bacteria in meat are applied by spraying and dipping techniques **(5**). In a study conducted by Bradley et al., 2011, showed that the addition of each citric or acetic acids or its derivatives reduced the growth of some strains of Enterobacteriaceae **(3).** Chaff vinegar has been found to inhibit the growth of pathogenic bacteria such as *E. coli* **(7).** Vinegar assists in suppressing the anthracnose rot in tomatoes **(19).** It also assists in eliminating *Salmonella* typhimurium in carrots **(16).** Vinegar may be used also as a mixture or alone as a natural flavoring in some salad dressings **(2, 11,17)**. These salad dressings provide a harsh environment for foodborne pathogens such as *Salmonella* and *E. coli* to survive because of the acetic or citric acids effects **(2).** The bacterial effect of vinegar is stronger on bacteria but weak on fungi. Both water garlic extract and apple vinegar pickled garlic extract had strong antimicrobial activity against both bacteria and fungi **(13,14,20).** The bacteriostatic and bactericidal activities of vinegar products against *E. coli,* were independent of bacterial inoculums sizes, but was dependent of growth phase. Bacteria of logarithmic growth phase were more sensitive than those of stationary phase **(21)**. The vinegar and aqueous extracts of virgin olive oil showed the strongest bactericidal activity against *Salmonella Enteritidis* **(6)**. The vinegar showed significant antimicrobial activity against food-borne pathogens. It showed high affect on different pathogens such as *Salmonella*, *E. coli*, *Shigella* and *Klebsiella*. Meat samples were easily affected by the pathogens such as negative bacteria **(2,5,6,9**). Food-borne pathogens were less inhibited by olive oil while mostly *Salmonella*, *E. coli*, *Shigella* and *Klebsiella* were inhibited by the vinegar **(9**).

Apple cider and grape vinegar are traditional surface disinfectants, that commonly used in disinfection of fruit and vegetables at homes in Turkey. The antimicrobial activity of apple cider and grape vinegar are tested against standard strains; (*Bacillus subtilis* DSMZ 1971, *Candida albicans* DSMZ 1386,

Enterobacter aerogenes ATCC 13048, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescens* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella typhimurium* SL 1344, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* DSMZ 20044) and food isolates (*Enterococcus durans*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Listeria innocua*, *Salmonella infantis* and *Salmonella kentucky*) by minimum inhibitory concentration MIC. It was observed that grape vinegar presented the highest activity with a MIC value of 12.5 - 50 µg/mL against all microorganisms (**4).**

This study aimed to determine the antibacterial activity of commercial apple vinegar and red grape vinegar against some bacterial isolates known as plant pathogens.

Materials and Method \

Isolation of bacteria : In this study four isolates of plant pathogenic bacteria (*Pseudomonas savastanoi*, *Erwinia amylovora* strain1, *P. syringae* and *E. carotovora*) obtained from (Department of Plant Pathology, University of Szent Istvan, Pudapest, Hungary) were tested *in vitro* for inhibitory effect and antibacterial activities of two types of commercial (apple and red grape) vinegar bought from commercial supermarkets in Tripoli area, Libya.

***In vitro* antibacterial activities assay using agar plate well diffusion method :** The antimicrobial activities of the commercial (apple, red grape) vinegar on four isolates of bacteria (*Pseudomonas savastanoi*, *Erwinia amylovora*, *Pseudomonas syringae* and *Erwinia carotovora*) were assayed using agar plate well diffusion method, measuring zone of inhibition in (mm). Loop of bacterial growth from eash isolate was inoculated into nutrient agar (NA) plates and incubated at 25°C for 24 hours. Bacterial suspensions were diluted with sterile water in test tubes, adjusted by turbid metrically to approximately 10^8 cfu/ml. 1µl of each bacterial suspension was added by pipette in (NA) plates of treatments. Sterile L-shaped glass rod was streak on the surface of plates, repeated for all tested bacteria, lefted for 5 -15 minutes to dry at room temperature. Wells or holes (6mm /diameter) were cut by cork borer in NA media, wells consisted of: 50 µl distilled water as a negative control, treatment dilutions at concentrations (100%, 75%,

50%, 25%). 50 µl from eash vinegar (apple or red grape) were added, (plates were performed in triplicates). All plates of the tested bacterial isolates were then incubated at 25°C . After 24 h of incubation, each vinegar treatments were noted for zones of inhibition for all isolates. The diameters of the zones of inhibitions were measured by scale in millimeter (mm) **(12).**

Results and Discussion \

Isolation of bacteria : Four bacterial isolates were used in this study. *Pseudomonas savastanoi*, *Erwinia amylovora*, *Pseudomonas syringae* and *Erwinia carotovora*. These isolates were known as plant pathogens. They were activated in nutrient broth, pure cultures of these isolates were prepared on (nutrient agar) plates and kept in Refregirator until used.

***In vitro* testing of antibacterial activities assay using agar plate well diffusion method :** Evaluation of the inhibitory effect of acetic acid from the commercial apple and red grape vinegar on plant pathogenic bacterial isolates revealed that all bacterial isolates were sensitive to commercial apple and red grape vinegar at all concentrations tested (25%, 50%, 75%, 100%). Regarding the assay of antibacterial activity using agar plate well diffusion method, results showed that the maximum inhibition zones of apple vinegar were observed against isolate *Erwinia amylovora* (8.00, 10.00, 10.02 mm) at concentrations (50%, 75%, 100%) respectively, while the minimum inhibition zones of apple vinegar against bacterial isolates were recorded (4.22, 4.66, 5.11 mm) and has low activity against isolate *Pseudomonas syringae* at concentrations (50%, 75%, 100%) respectively compared to other treatments (Table 1 and Figure 1). On the other hand, the antibacterial activities of red grape vinegar on the bacterial isolates were assayed, isolate *Erwinia amylovora* was higher sensitive to red grape (7.21, 8.00, 9.00 mm) at concentrations (50%, 75%, 100%) respectively, comparing to other bacterial isolates. *Pseudomonas syringae* with inhibition zones was weakly (4.33, 4.16, 5.41 mm) at concentrations (50%, 75%, 100%) respectively compared to other treatments. Results explained apple vinegar had stronger antibacterial activities than red grape vinegar on isolate *Erwinia amylovora* (Table 2 and Figure 2).

Results also explained that apple and red grape vinegar were weakly effected on all isolates *Pseudomonas savastanoi*, *Erwinia amylovora*, *Pseudomonas syringae* and

Erwina carotovora at concentration (25%) compared to other treatments.

These results were in agreement with many reseachers; pointed out that the bacterial effect of vinegar is stronger on bacteria but weak on fungi and both water garlic and apple vinegar pickled garlic extracts had strong antimicrobial activity against both bacteria and fungi (**14**). Also results showed that the bacteriostatic and bactericidal activities of vinegar products against *E. coli* were independent of bacterial inoculums sizes, but was dependent of growth phase (**21**). results found that bacteria of logarithmic growth phase were more sensitive than those of stationary phase, the vinegar also showed significant antimicrobial activity against food-borne pathogens. Vinegar was highly affected pathogens such as *Salmonella*, *E. coli*, *Shigella* and *Klebsiella* (**9**), and results showed that the vinegar and aqueous extracts of virgin olive oil showed the strongest bactericidal activities against *Salmonella Enteritidis.*Food-borne pathogens were less inhibited by olive oil, while mostly *Salmonella*, *E. coli*, *Shigella* and *Klebsiella* were commonly inhibited by the vinegar (**6).** Also found that Apple cider vinegar and grape vinegar are traditional surface disinfectants, which are commonly used in disinfection of fruit and vegetables. The antimicrobial activity of apple cider vinegar and grape vinegar were tested against standard bacteria of different strains by minimum inhibitory concentration (MIC), the grape vinegar presented the highest activity with a MIC value of 12.5 - 50 µg/mL against all microorganisms, these results are in agreement with our results. We can concluded that the vinegar being cheap and can be safely, effectively and very economically used especially in elimination of multiple antibiotic resistant strains of many plants pathogenic bacteria, and can be used as alternative method of controlling antimicrobial activities (**4**). Deep studies must be carried out on different types of vinegars and different serious microbial pathogenic strains to prove their inhibitory effects on wide spectrum of microorganisms that causing economical losses. These methods of controlling plant pathogens may assist in make reduction in using chemical besticides that harm the man and its environment.

Table 1: Antibacterial activities of different concentrations of apple vinegar against bacterial isolates.

Bacterial isolates	Inhibition zone (diameter in mm)				
	Concentrations				
	control	**25%**	**50%**	**75%**	**100%**
Pseudomonas savastanoi	0.0	2.41	4.30	4.77	**5.69**
Erwinia amylovora	0.0	4.16	8.00	10.00	**10.02**
Pseudomonas syringae	0.0	2.88	4.22	4.66	**5.11**
Erwinia carotovora	0.0	3.16	4.77	4.50	**6.65**

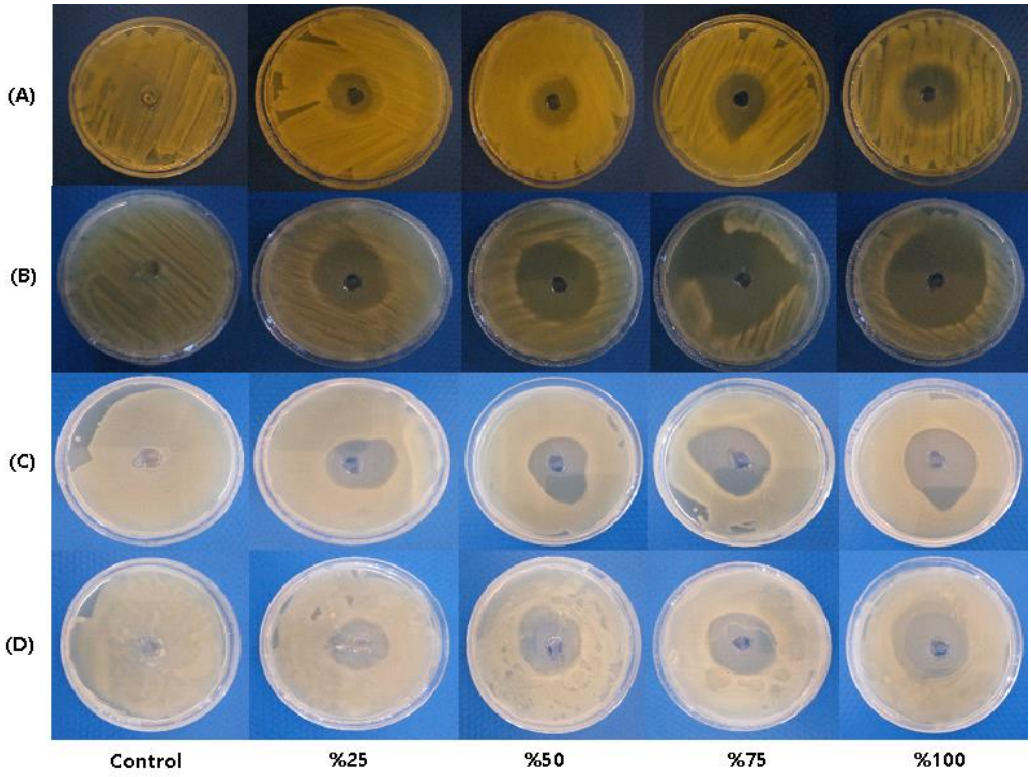
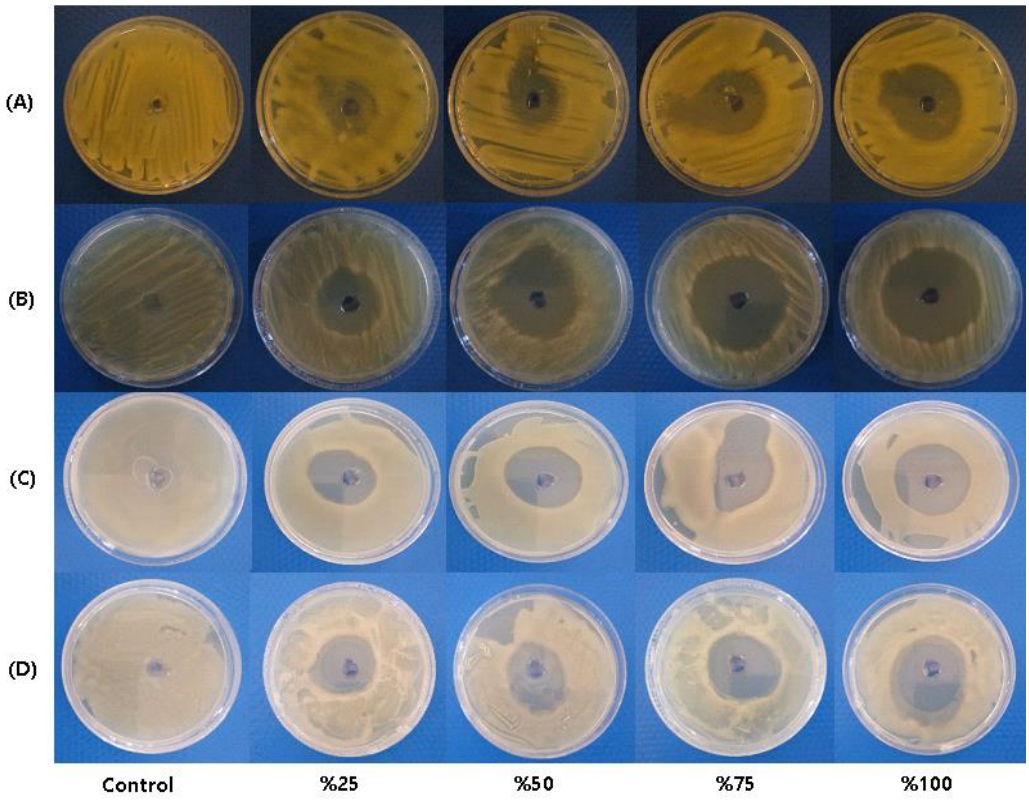


Figure 1. Effect of different concentrations (25%, 50%, 75%, 100%) of apple vinegar on bacterial isolates growth, (A) *Pseudomonas savastanoi*, (B) *Erwinia amylovora*, (C) *Pseudomonas syringae*, (D) *Erwinia carotovora***.**

Table 2: Antibacterial activities of different concentrations of red grape vinegar against bacterial Isolates.

Bacterial isolates	Inhibition zone (in mm)				
	Concentrations				
	control	25%	50%	75%	**100%**
Pseudomonas savastanoi	0.0	3.91	4.24	5.49	**5.94**
Erwinia amylovora	0.0	3.15	7.21	8.00	**9.00**
Pseudomonas syringae	0.0	3.10	4.33	4.16	**5.49**
Erwinia carotovora	0.0	1.50	5.66	4.27	**4.83**

Figure 2. Effect of different concentrations (25%, 50%, 75%, 100%) of grape vinegar on bacterial isolates growth, (A) *Pseudomonas savastanoi*, (B) *Erwinia amylovora*, (C) *Pseudomonas syringae*, (D) *Erwinia carotovora*.



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الملخص العربى /

النشاط المضاد للبكتيريا لخل التفاح وخل العنب الاحمر التجاريين ضد بعض العزلات البكتيرية الممرضة للنبات.

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هدفت هذه الدراسة لتقييم التأثير التثبيطي لخل التفاح و خل العنب الاحمر التجاريين ضد أربع أنواع من العزلات البكتيرية الممرضة للنبات (*Pseudomonas savastanoi, Erwinia amylovora, Pseudomonas syringae* و *Erwinia carotovora*) . أظهرت النتائج بأن جميع العزلات البكتيرية حساسة لخل التفاح و خل العنب الاحمر التجاريين عند كل التركيزات المختبرة (25% و 50% و 75% و 100%). وكان الحد الاقصى للتأثير التثبيطي لخل التفاح سجل ضد العزلة البكتيرية (*Erwinia amylovora*) (8.00 ، 10.00، 10.02 مم) عند التركيزات (50% و 75% و 100%) على التوالي مقارنة بالعزلات الاخرى التي أظهرت الحد الادنى من التثبيط. كما أظهرت النتائج ايضا ان العزلة البكتيرية (*Erwinia amylovora*) كانت اكثر حساسية لخل العنب الاحمر(7.21، 8.00، 9.00 مم) عند التركيزات (50% و 75% و 100%) على التوالي مقارنة بالعزلات الاخرى التي أظهرت الحد الادنى من التثبيط. النتائج التي تم الحصول عليها اوضحت بان خل التفاح كان أقوى من خل العنب الاحمر على العزلة (*Erwinia amylovora*)، ومن ناحية أخرى أوضحت النتائج ان خل التفاح و خل العنب الاحمر كان تأثيرهما ضعيفاً على كل العزلات البكتيرية (*Pseudomonas syringae, Erwinia amylovora, Pseudomonas savastoni* و *Erwinia carotovora*) عند التركيز (25%).

و هذا النمط من الدراسات المتمثل في استخدام الاحماض العضوية مثلاً (حمض الخليك) في مكافحة بعض البكتيريا والفطريات الممرضة للنبات يعد سبيلاً للبحث عن الطرائق البديلة لمكافحة البكتيريا و الفطريات، عوضاً عن تلك الاستخدامات المفرطة للكيماويات والمبيدات الناجم عنها مخاطر بيئية و صحية التي تخل بالتوازن البيئي للكائنات الحية النافعة، اضافة الى تكلفتها الاقتصادية الباهظة.

الكلمات المفتاحية : الخل، التفاح والعنب الاحمر، البكتيريا الممرضة للنبات، النشاط المضاد للبكتيريا.