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EFFICACY OF VINEGAR, THYME WATER AND vB_EcoM-P34 PHAGE IN INHIBITING *Escherichia coli* O157:H7 IN LETTUCE

Tuba Sakin Şahin[1*], Rabia Erol[2], Zeliha Yıldırım[2]
[1]Niğde Ömer Halisdemir University, Bor Vocational School, Deparment of Food Processing, Niğde, Turkey
[2]Niğde Ömer Halisdemir University, Faculty of Engineering, Department of Food Engineering, Niğde, Turkey

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ABSTRACT
The antibacterial activity of thyme water, apple vinegar, grape vinegar, and vB_EcoM-P34 phage against *Escherichia coli* O157:H7 ATCC 35150 and their potential to be utilized for decontamination on lettuce were examined in this study. Lettuce samples were treated with thyme water, apple or grape vinegar solutions (10, 25, 50%), vB_EcoM-P34 phage (about 10^8 PFU/mL) or tap water for 30 min after inoculation of lettuce with *E. coli* O157:H7 at the level of 5.66 log. Depending on the concentration, vinegars and thyme water reduced the bacterial count in lettuce by between 0.78 and 2.69 logs. The most effective reduction was achieved by treatment with vB_EcoM-P34 at 3.23 log. Bacteriophage was the most suitable method for decontamination, followed by grape vinegar, apple vinegar, and thyme water at 50%. However, high concentrations of vinegars and thyme water caused a sharp odor and a slight lightening in the color of the lettuce.
Key words: Acetic acid, bacteriophage, thyme water, *E. coli* O157:H7, lettuce

SİRKE, KEKİK SUYU VE vB_EcoM-P34 FAJININ MARULDA *Escherichia coli* O157: H7'nin İNHİBE EDİLMESİ ÜZERİNE ETKİNLİĞİ

ÖZ
Bu çalışmada kekik suyu, elma sirkesi, üzüm sirkesi ve vB_EcoM-P34 fajının *Escherichia coli* O157:H7 ATCC 35150'ye karşı antibakteriyel aktivitesi ve marulda aynı bakteriye karşı dekontaminasyon için kullanılma potansiyeli incelenmiştir. Marul örnekleri, 5.66 log düzeyindeki *E. coli* O157:H7 ile inoküle edildikten 30 dakika sonra kekik suyu, elma sirkesi, üzüm sirkesi (%10, %25, %50), vB_EcoM-P34 fajı (10^8 POB/mL) ve musluk suyu ile yıkanmıştır. Konsantrasyona bağlı olarak elma sirkesi, üzüm sirkesi ve kekik suyu maruldaki bakteri sayısını 0.78 ve 2.69 log arasında azaltmıştır. Bakteri sayısında en etkili azalma 3.23 log olarak vB_EcoM-P34 fajı ile muamele sonucu elde edilmiştir. Marulda dekontaminasyon için bakteriyofaj uygulaması en uygun yöntem olarak belirlenirken bunu sırasıyla %50 konsantrasyonda hazırlanan üzüm sirkesi, elma sirkesi ve kekik suyu izlemiştir. Fakat yüksek konsantrasyonda uygulanan sirkeler ve kekik suyu marulda keskin bir koku ve hafif düzeyde renk açılmasına neden olmuştur.
Anahtar kelimeler: Asetik asit, bakteriyofaj, kekik suyu, *E. coli* O157:H7, marul

[*] Corresponding author / Yazışmalardan sorumlu yazar
✉: tuba.sakin@ohu.edu.tr ☎: (+90) 388 225 2304 🖷: (+90) 388 311 8437

Tuba Sakin Şahin; ORCID no: 0000-0002-0597-1872
Rabia Erol; ORCID no: 0009-0005-0906-0944
Zeliha Yıldırım; ORCID no: 0000-0002-6155-6921

INTRODUCTION

Raw vegetables have a significant part in our diet due to their high fiber and nutrient content. However, such foods can be contaminated with pathogenic and toxigenic microorganisms such as *Salmonella*, *E. coli* O157:H7, *E. coli* O104:H4, *Staphylococcus aureus,* and *Listeria monocytogenes* during processes including harvesting, transporting, processing, and marketing. Lettuce consumed raw and used in the preparation of various salads almost all over the world is among the risky food groups due to possible pathogenic bacterial contamination. The surface of lettuce is conducive to the growth of enteric pathogens. Lettuce has been noted to be a common source of outbreaks and is most often contaminated with *E. coli* O157:H7 (Elias et al., 2019; Turner et al., 2019). Pathogens and/or toxins are transmitted directly to humans when poorly washed vegetables are consumed and can cause serious health problems. Surface decontamination in raw products is crucial for the shelf life of products, product safety and human health (Sethi et al., 2020).

Several studies have shown that using only water may not be adequate to reduce the microbial load in raw vegetables like lettuce, and living bacteria may still be present on the surface of the vegetable (Gülmez et al., 2006; Poimenidou et al., 2016). Chlorine, chlorine dioxide, ozone, peroxyacetic acid, hydrogen peroxide, and trisodium phosphate are disinfectants commonly used in the decontamination of vegetables. However, there are various restrictions on their use, and some of them are prohibited by legal regulations (Chinchkar et al., 2022). In addition, consumers do not take kindly to the use of chemicals in foods. These factors have made using decontamination products of natural origin more popular. The potentials of using vinegar types, lemon juice, thyme water, extracts of some other plants (rosemary, rose, clove, etc.) and essential oils in the decontamination of vegetables are being investigated. Also, bacteriophages are another natural antimicrobial agent that can be used for decontamination of vegetables. Successful results regarding the effect of bacteriophages against foodborne pathogens have been reported by various researchers in recent years (Guo et al., 2021; Yamaki et al., 2022, Zhang et al., 2023; Wojcicki et al., 2023). vB_EcoM-P34 phage isolated from slaughterhouse waste water had lytic activity against different *E. coli* O157:H7 serovars (Yıldırım et al., 2018; Yildirim et al., 2021).

E. coli O157:H7 is an important pathogen that causes diseases such as diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura in humans. The transmission routes of this bacterium are mostly considered to be poultry, livestock, and meat products obtained from them, but vegetables such as baby spinach, carrots, cabbage, lettuce and tomatoes have also been associated with *E. coli* O157:H7 infections (Puligundla and Lim, 2022; Singha et al., 2023). Due to the fact that it is a healthy alternative, consumers prefer salads made with raw vegetables in their daily diet, so they are likely to encounter this pathogen frequently.

This study was designed to reveal the antibacterial effect of apple vinegar, grape vinegar, thyme water, and bacteriophage against *E. coli* O157:H7 and to determine whether they provide an effective decontamination on raw lettuce.

MATERIALS AND METHODS

Bacterial strain and bacteriophage

vB_EcoM-P34 phage specific for *E. coli* O157:H7 ATCC 35150 (Yıldırım et al., 2018; Yildirim et al., 2021) and the bacteria *E. coli* O157:H7 ATCC 35150 were taken from our culture collection. *E. coli* O157:H7 was grown in brain heart infusion broth (BHI) (Lab M, Lancashire, UK) and stored at -80°C in BHI broth with 20% glycerol. For preparation of the bacteriophage, nutrient broth (Lab M, Lancashire, UK) was used.

Preparation of vinegar and thyme water samples

Apple and grape vinegar used for decontamination was purchased from a local market in Niğde. The acetic acid content of both vinegars is stated as 4-5% in the label information and there are antioxidant sodium metabisulfite,

sulfur dioxide in the vinegars. Thyme water, on the other hand, was purchased online and it was stated in the label information that its content was 100% thyme water. Solutions of different concentrations (10%, 25% and 50%) of apple vinegar, grape vinegar and thyme water to be used in the study were prepared using sterile water. Prepared solutions (10 mL) were taken and the pH values were measured with a digital pH meter (Hanna, HI 2211).

Preparation of vB_EcoM-P34 bacteriophage infecting *E. coli* O157:H7 ATCC35150

For phage preparation, phage sample (200 µL) and test bacteria *E. coli* ATCC 35150 (200 µL) were added to nutrient soft agar (0.7% agar) at 45-50 °C. Then the soft agar spread on petri dishes containing solidified Nutrient agar and left to incubate at 35°C for 24 hours. At the end of the incubation, soft agar was stripped from the surface of the petri dish with a Drigalski loop using SM buffer. The collected soft agar was kept at 25 °C for 1 hour in the shaking incubator. At the end of the period, it was centrifuged (25 min at 8000 × g) and the agar was removed. The phage containing filtrate obtained was passed through a membrane filter (0.45 µm pore diameter) and purified from *E. coli* ATCC 35150. Titre of the bacteriophage sample was determined by double layer agar and used for decontamination at the level of 10^9 POB/mL in the study.

Determination of antimicrobial activities of vinegar, thyme water, and vB_EcoM-P34 bacteriophage samples to *E. coli* O157:H7 ATCC 35150

Spot, disc diffusion, and well diffusion methods were used to determine the antibacterial activities of thyme water, bacteriophage, apple and grape vinegar samples against *E. coli* O157:H7 ATCC 35150. Antimicrobial activity of vinegar and thyme water samples prepared by diluting 10, 25, and 50% and without any dilution (100%), and vB_EcoM-P34 at 10^9 POB/mL were determined. Actively growing *E. coli* ATCC 35150 culture (20 µL) was added to nutrient soft agar (0.7%), mixed, and poured onto nutrient agar (1.5% agar) previously prepared.

Spot method

Vinegar, thyme water, and bacteriophage samples (20 µL) were taken and dropped onto the nutrient soft agar (0.7%) containing the test bacteria and incubated at 35°C for 24 hours.

Disc diffusion method

Sterile discs (Oxoid Antimicrobial Susceptibility Test Discs- 6 mm diameter) were placed in tubes containing 5 mL of vinegar, thyme water or bacteriophage and kept for 15 minutes to to allow the decontamination solutions to absorb. Then, the discs were placed on the nutrient soft agar (0.7%) containing the test bacteria and incubated at 35°C for 24 hours.

Well diffusion method

Wells (6 mm diameter) were opened on soft nutrient agar containing *E. coli* O157:H7 using a reverse end of sterile Pasteur pipette, and 50 µL of the prepared vinegar, thyme water, and bacteriophage solutions were added to these wells. All petri dishes were incubated at 35 °C for 24 hours.

Preparation of lettuce samples

Lettuce samples used in the study were obtained daily from a local market in Niğde. Outer leaves of the lettuce samples were discarded, the remaining lettuce leaves were washed first with tap water and then with sterile distilled water. Excess water from lettuce samples was removed with paper towels, and lettuce leaves were kept in Biological safety cabinet under UV light for 1 hour. In order to provide a homogeneous sample; the middle vein parts of the lettuce with obvious structural differences were discarded using a sterile knife, and only the green parts were used for analysis. Lettuce samples prepared in 10 g pieces were placed in sterile petri dishes.

Inoculation of lettuce samples with *E. coli* O157:H7

E. coli O157:H7 ATTC 35150 culture (100 µL) with a cell number of approximately 10^8 CFU/mL was inoculated at several different points on the prepared lettuce samples. Bacteria inoculated lettuce samples were kept in the refrigerator overnight and *E. coli* O157:H7 cells were allowed

to adhere to the lettuce surface. The lettuce sample was covered with 30 mL (10%, 25%, 50% prepared) thyme water, apple, and grape vinegar, bacteriophage, and also tap water. Lettuce samples were kept in decontamination solutions for 30 min and at the end of this period, each sample was removed from the solutions. Lettuce samples were taken into a stomacher bag and homogenized in stomacher. Dilutions were prepared from the samples and the *E. coli* O157:H7 number was determined using BHI agar.

Effects of decontamination solutions on color of lettuce

Color analysis was performed using the Konica Minolta Chromameter CR400 (Japan) at at least three different points on lettuce samples that had been decontaminated and those that had not. Before measurements, the device was calibrated using its own standard, the white color plate.

Statistical analysis

The experiment was carried out in five replications. All data obtained as a result of the research were analyzed using the SPSS 10 package program. Duncan test was used to compare the means.

RESULTS AND DISCUSSION

pH values of vinegar and thyme water samples

The pH values of apple and grape vinegar and thyme water were found to be 3.10, 3.09 and 3.99, respectively (Table 1). Although it was not statistically significant ($P>0.05$), it was seen that the pH values of the thyme water, apple, and grape vinegar samples increased relatively as the dilution rate increased.

Antimicrobial activities of vinegar, thyme water and vB_EcoM-P34 bacteriophage against *E. coli* O157:H7

Vinegar is a product obtained as a result of the conversion of fermentable sugars to ethanol by yeasts and then the oxidation of this ethanol by acetic acid bacteria (Gökırmaklı et al., 2019). Organic acids, especially acetic acid in vinegar, pass through the cell membrane and cause the death of the target bacterial cell (Bjornsdottir et al., 2006). Acetic acid was determined to be the most efficient organic acid against *E. coli* O157:H7 (Entani et al., 1998).

To evaluate the antibacterial activity of apple and grape vinegar; disc diffusion, well diffusion and spot method were used. It was determined that there was no clear zone against *E. coli* O157:H7 in apple and grape vinegar samples by spot and well diffusion methods. However, it was determined that the bacterial load decreased (turbid zone formation) with the 25%, 50% and 100% concentrations of samples (Fig. 1). Turbid zone diameters for apple and grape cider vinegar were determined to vary between 11.00-18.25 and 13.00-18.50 mm in the spot method, and between 12.25-20.25 and 14.50-19.25 mm in the well diffusion method, respectively (Table 2).

Table 1. pH values of apple vinegar, grape vinegar and thyme water samples

Sample	pH
10% Apple vinegar	3.30±0.01
25% Apple vinegar	3.20±0.01
50% Apple vinegar	3.15±0.01
100% Apple vinegar	3.10±0.01
10% Grape vinegar	3.30±0.01
25% Grape vinegar	3.20±0.01
50% Grape vinegar	3.15±0.01
100% Grape vinegar	3.09±0.01
10% Tyhme water	4.34±0.02
25% Tyhme water	4.17±0.02
50% Tyhme water	4.08±0.01
100% Tyhme water	3.99±0.01

In disc diffusion method, apple and grape vinegar at concentrations of 10, 25, 50, and 100% caused a decrease in cell density of *E. coli* O157:H7 as well as the formation of clear zones (Fig. 1). The total zone size including the turbid zone was found to vary between 9.0- 17.0 mm in apple vinegar samples and 9.50- 20.0 mm in grape vinegar samples (Table 2). The reason why clear inhibitory zone formation is observed in the disc diffusion method is thought to be due to the absorption of the tested samples by the disc and intensive application at a certain point. In the well diffusion and spot methods, the samples tested

are spread over a wide area, their concentration decreases, and their inhibitory effect weakens (Fig. 1). The results showed that grape vinegar had slightly better antimicrobial activity than apple vinegar. The quality of the vinegar is affected by many factors such as the origin of the fruit, environmental conditions, production methods, processing, and storage conditions (Ousaaid et al., 2021). The difference in organic acids and polyphenols contained in each type of vinegar leads to a variation in antimicrobial activity. It has been noted in previous literature that apple vinegar generally contains fewer polyphenols than other fruit vinegars (Kara et al., 2022).

Table 2. Antibacterial activity of apple vinegar, grape vinegar, thyme water and bacteriophage samples

Samples	Spot		Disc diffusion		Well	
	Inhibition zone diameter		Inhibition zone diameter		Inhibition zone diameter	
	Clear (mm)	Turbid (mm)	Clear (mm)	Turbid (mm)	Clear (mm)	Turbid (mm)
10% AV	-		7.00±0.02	9.00±0.01	-	-
25% AV	-	11.00±0.03	7.50±0.01	10.50±0.02	-	12.25±0.02
50% AV	-	14.00±0.01	8.50±0.01	13.75±0.01	-	16.75±0.04
100% AV	-	18.25±0.05	10.00±0.02	17.00±0.03	-	20.25±0.06
10% GV	-	-	7.00±0.01	9.50±0.01	-	-
25% GV	-	13.00±0.02	7.75±0.01	13.00±0.03	-	14.50±0.03
50% GV	-	12.00±0.03	8.50±0.02	15.50±0.04	-	16.00±0.02
100% GV	-	18.50±0.06	10.75±0.01	20.00±0.04	-	19.25±0.05
10% TW	-	-	-	-	-	-
25% TW	-	-	-	-	-	-
50% TW	-	-	-	-	-	-
100% TW	-	8.50	8.50±0.01	9.50±0.01	-	-
EcoM-P34	21.75±0.12	-	14.00±0.10	-	12.00±0.09	-

AV, Apple Vinegar GV, Grape Vinegar TW, Thyme water

Aromatic water taken as a by-product during the distillation processes applied to obtain thyme oil is called "thyme water". In a study examining the composition of thyme waters in the Turkish market, it was stated that the main components of thyme waters were carvacrol (49.0-94.5%), timol (1.9-18.7%) and linalool (0.5-12.8%) (Boydağ et al., 2004). Carvacrol and thymol are reported to have antimicrobial, antiviral, antioxidant, antimutagenic, and anti-inflammatory activity (Nabavi et al., 2015). In the study, it was observed that thyme water samples prepared at 10, 25 and 50% concentrations did not form a clear inhibition zone by spot or well method, and they did not cause any decrease in bacterial density (Fig. 1). A decrease in cell density was determined in the sample where 100% thyme water was applied by spot method only. In addition, thyme water used at 10%, 25% and 50% did not give rise to a visible inhibition against *E. coli* O157:H7 by disc diffusion method. When used at 100%, it resulted in the formation of a clear inhibition zone with a diameter of 8.50. ±0.01mm (Table 2). Studies examining the antibacterial activity of thyme against *E. coli* and *E. coli* O157:H7 revealed that the inhibition zone ranged from 0 to 35 mm. Although the inhibition zones obtained in some of these studies are similar in size to those in our study (8.5–9.5 mm) (Albayrak et al., 2012). The results in the literature suggest that the extraction method of antimicrobial components and the test method used have an effect on the determination of the antimicrobial effect of thyme (Sağdıç 2003; Benli and Yiğit, 2005; Sarac and Ugur, 2008, Gonelimali et al., 2018; Qureshi et al., 2022).

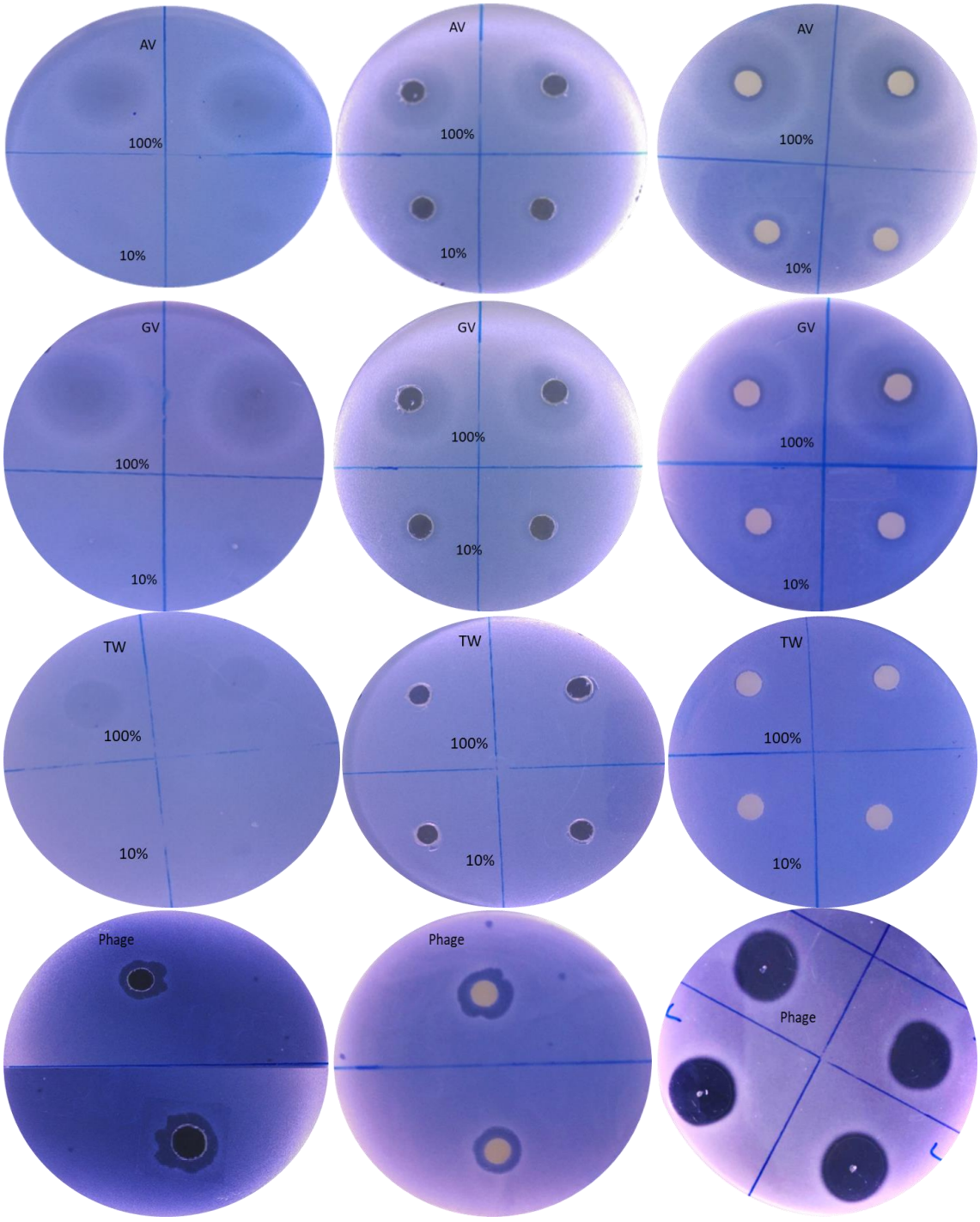


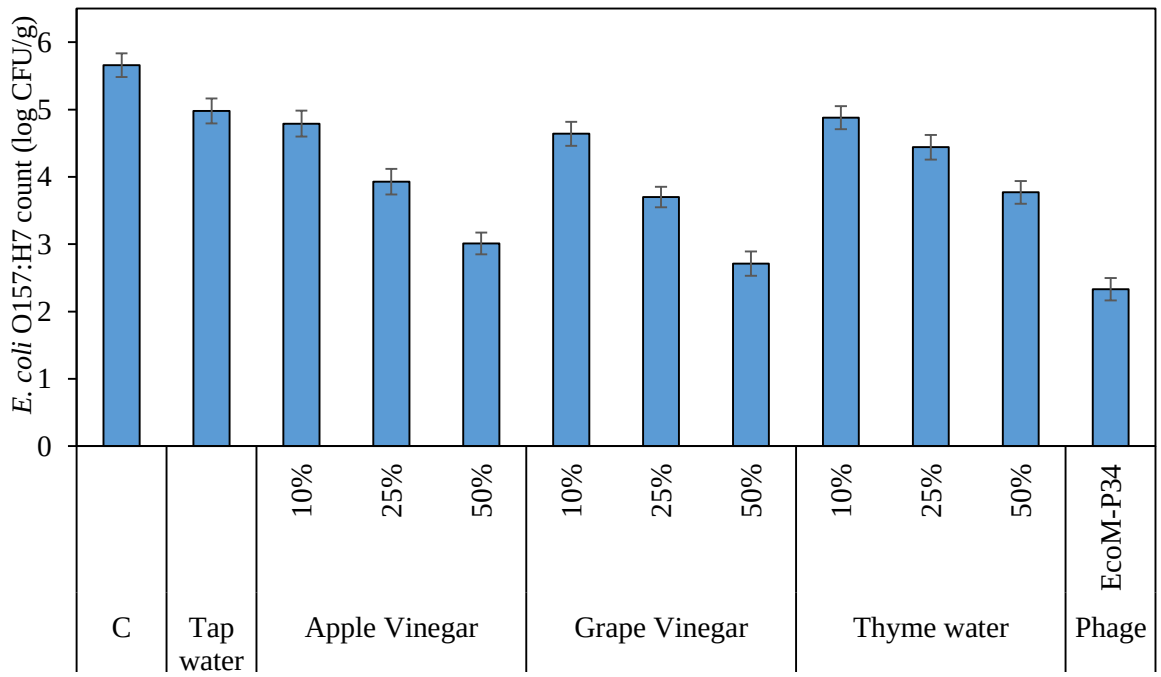
Fig. 1. Antibacterial activity of decontamination solution against *E. coli* ATCC 35155 by using spot, well and disc diffusion methods. AP, apple vinegar; GV, grape vinegar; TW, thyme water.

Bacteriophages or phages are viruses that only infect bacteria and can reproduce inside the host bacterial cell (Strauch et al., 2007). They show their antibacterial effects by lysing bacterial cells. vB_EcoM-P34 bacteriophage caused clear inhibition zone against *E. coli* O157:H7 in three antimicrobial activity tests, spot, agar well and disc diffusion methods (Fig. 1). The diameters of the clear zones formed by the spot, disc diffusion and agar well methods were 21.75, 14.00 and 12.00 mm, respectively (Table 2). Like the results of vinegar and thyme water, the spot method was the most suitable method for determination antibacterial activity of the phage. Unlike vinegar and thyme water, the vB_EcoM-P34 phage continued to exert its inhibitory effect while spreading over a wider area.

Antimicrobial effect of vinegar, thyme water and vB_EcoM-P34 bacteriophage against *E. coli* O157:H7 in lettuce

The level of *E. coli* O157:H7 inoculated and attached to lettuce samples was found to be 5.66 log CFU/g. It was observed that there was a decrease of 0.87, 1.73 and 2.65 log CFU/g in the number of *E. coli* O157:H7 in the lettuce samples applied with 10%, 25% and 50% apple cider vinegar, respectively, and a decrease of 1.02, 1.96 and 2.95 log CFU/g in the samples applied with grape vinegar at the same concentration (Fig. 2). Thyme water at the same ratios caused a decrease in the number of *E. coli* O157:H7 at the level of 0.78, 1.22 and 1.89 log CFU/g, respectively. Although grape vinegar caused a slightly more reduction in the number of bacteria than apple vinegar, there was no statistically significant difference between the two ($P>0.05$). However, there was a significant difference between vinegar applications and thyme water applications ($P<0.05$). vB_EcoM-P34 phage caused a 3.33 log CFU/g decrease in the bacterial count, and therefore it was the best decontamination method (Fig. 2). Washing with tap water was the worst among the decontamination methods since the *E. coli* O157:H7 count was decreased only by 0.68 log CFU/g after washing (Fig. 2). According to the results obtained, it can be said that the most effective decontamination method is bacteriophage, followed by prepared apple cider vinegar, grape vinegar and thyme water (prepared at 50% rate). It is also understood thyme water is not as effective as apple and grape vinegar.

Fig. 2. Inhibitory effect of decontamination methods against *E. coli* O157:H7 in lettuce. C, control



Numerous studies have reported successful results in reducing the number of bacteria in lettuce treated with vinegar (Şengün and Karapınar, 2006; Chang and Fang, 2007). It has been reported that the number of bacteria in lettuce contaminated with *E. coli* O157:H7 exposed to vinegar for various period of time decreased between 2.5 and 3.9 log (Santos et al., 2009; Poimenidou et al., 2016). Gülmez et al. (2006) could not detect *E. coli* when they kept the parsley samples in undiluted thyme water and vinegar for 1 minute. Considering these results, the decrease in the number of *E. coli* O157:H7 was similar to the literature. In a study by Elhan (2014), when carrot, cucumber and lettuce samples contaminated with *E. coli* were exposed to a solution containing 3% vinegar (0.14% acetic acid) for 30 minutes, a decrease of at least 1 log unit was observed in the number of bacteria. In studies conducted to determine the antimicrobial effect of thyme essential oil solutions on fresh vegetables, it was determined that the effect of thyme solution was less than the other methods tested (chlor dioxide, ozone, lactic acid, chlorinated water) (Singh et al., 2002; Uyttendaele et al., 2004). Different findings were achieved in investigations on the efficiency of bacteriophages in reducing the amount of *E. coli* O157:H7 in lettuce, depending on the application conditions of the phage, but phage was found to be effective in all of them (Carter et al., 2012; Boyacioglu et al., 2013; Ferguson et al., 2013; Duc et al., 2020; Lu et al., 2022). Bacteriophage washing was found to be successful and more efficient than other decontamination methods in this study.

The percentage of vinegar and thyme water that we obtained successful results in our study is high. In the literature, it is seen that vinegar and thyme water are prepared and used in different concentrations or used undiluted (Poimenidou et al., 2016; Nthenge and Liu, 2019). It has been determined in some studies that high concentration applications are successful in inhibiting bacteria, but they have a disadvantage such as causing a change in the sensory character of the applied product. Uyttendaele et al. (2004) reported that thyme essential oil solutions affected sensory properties of fresh vegetables. The samples washed with 50% apple vinegar, grape vinegar, and thyme water had an intense smell of vinegars and thyme. Similar results for parsley and lettuce were reported by Wu et al. (2000), Gülmez et al. (2006), Chang and Fang, (2007). Thyme water and vinegar can be considered as easily available products with affordable prices. The use of vinegar and thyme water in high concentrations can also increase the application cost. For these reasons, the relationship between the use of high concentrations of vinegar or thyme water as a washing solution and consumer acceptance needs to be investigated further.

Bacteriophages are suitable option for the decontamination of fresh fruits and vegetables that are not cooked before eating and are sold ready for consumption (washed-sliced). They are also natural antimicrobial source like vinegar and thyme juice. According to our results, the vB_EcoM-P34 phage was more successful in inhibiting bacteria than other treatments. However, phages are not widely used yet, and they do not have practical applications in the home environment. Commercial phage preparations are permitted to be used for biosanitation, biocontrol, and bioprotection purposes in a number of countries under legal regulations (Wojcicki et al., 2023). ListShield™ (Intralytix, Baltimore, MD, USA), PhageGuard L™ (formerly Listex™) (Micreos Food Safety B.V., Wageningen, The Netherlands), EcoShield™ (Intralytixs) and SalmoFresh™ (Intralytix) are examples of commercial phage preparations approved for use by the FDA (Svircev et al., 2018). The number of phages approved for use based on the results of scientific research is increasing day by day.

Effect of vinegar, thyme water and vB_EcoM-P34 bacteriophage on the color of lettuce

The color of lettuce is one of the criteria that plays an important role in the appeal of lettuce and consequently in consumer appreciation. The color values (L*, a* and b*) of lettuce samples with and without decontamination processes are presented in Table 3. In the measurements made using Hunter method, L*, a* and b* values were determined according to the CIE color system. In

this color system, L* stands for brightness and its value is between 0 (black) and 100 (white), a* value represents the red-green color and the b* value represents the yellow-blue color values. As seen Table 3, the L* and a* values of the samples treated with apple vinegar, grape vinegar and thyme water decreased and b* values increased after the application. The most effective decontamination method on color change was grape vinegar, followed by apple cider vinegar and thyme juice. The L*, a* and b* values of the lettuce samples changed more with increasing their application concentrations. There was no significant change in L*, a* and b* values in lettuce samples treated with bacteriophage and washed only with tap water (P>0.05).

Table 3. Effect of decontamination methods on lettuce color

Samples	L*	a*	b*
Control	74.31±0.24	-17.62±0.29	32.78±0.35
10% Apple vinegar	71.26±0.07	-14.43±0.24	33.58±0.07
25% Apple vinegar	68.32±0.55*	-11.34±0.07*	36.39±0.57*
50% Apple vinegar	59.11±0.42*	-10.12±0.20*	38.25±0.55*
10% Grape vinegar	69.98±0.34*	-13.07±0.16*	33.94±0.17
25% Grape vinegar	66.99±0.47*	-11.29±0.48*	36.46±0.20*
50% Grape vinegar	57.67±0.44*	-7.42±0.14*	38.28±0.05*
10% Tyhme water	72.31±0.14	-14.14±0.09*	33.38±0.13
25% Tyhme water	70.22±0.08	-12.68±0.32*	35.49±0.07
50% Tyhme water	68.16±0.10*	-10.72±0.50*	37.26±0.21*
vB_EcoM-P34	74.16±0.05	-17.62±0.19	32.63±0.07
Tap water	73.18±0.17	-17.55±24	32.77±0.09

∗ Significant differences (P < 0.05) between control samples and decontamination solutions

The findings of Poimenidou et al. (2016) in terms of colour similar to our investigation. They reported that when lettuce samples were kept in vinegar for 5 minutes, the samples tended to look redder. They also determined that the thyme water caused the color of the lettuce to appear yellow. On the other hand, the results of our colour measurements are supporting the acceptance that bacteriophages do not change the color of food (Wang et al., 2022).

CONCLUSION

In the present study, the inhibitory effects of vB_EcoM-P34 bacteriophage and solutions prepared from apple vinegar, grape vinegar and thyme water at different concentrations against *E. coli* O157:H7 were determined by spot, disc diffusion, and well diffusion method. According to the results, it can be said that the most appropriate method for determining the inhibitory effect for thyme water, apple and grape vinegar is the disc diffusion method, and the spot method for vB_EcoM-P34 phage. In addition, the most effective method for inhibiting *E. coli* O157:H7 35150 in lettuce was vB_EcoM-P34, followed by 50% grape vinegar, 50% apple cider vinegar and 50% thyme juice. Thyme water is not as effective a decontamination method as apple and grape cider vinegar. The application of grape vinegar, apple vinegar and thyme water at 50% concentration caused changes in the color of the lettuce, and the smell of vinegars and thyme was dominant in the samples. However, no change was observed in the color and odor of the lettuce samples treated with the vB_EcoM-P34 phage.

Thyme water and especially vinegar applications are the first reliable methods that come to mind for surface decontamination of fruits and vegetables in the home environment, as they are easily accessible, easily applicable and natural products. The prevalence of bacteriophage

applications is increasing day by day. Natural agents needed for the decontamination of raw food surfaces continue to be addressed in extensive studies. With our study, we have presented information to support this field.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

The manuscript was written by TSŞ. ZY and TSŞ designed the research and interpreted the results. RE performed the experiments.

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