



Tarım Bilimleri Dergisi  
Tar. Bil. Der.

Dergi web sayfası:  
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences

Journal homepage:  
www.agri.ankara.edu.tr/journal

## Antibacterial Activity of Different Kefir Types Against Various Plant Pathogenic Bacteria

Bilgin TAŞKIN<sup>a</sup>, Ahmet AKKÖPRÜ<sup>b</sup>

<sup>a</sup>Van Yuzuncu Yil University, Faculty of Agriculture, Department of Agricultural Biotechnology, 65080, Van, TURKEY

<sup>b</sup>Van Yuzuncu Yil University, Faculty of Agriculture, Department of Plant Protection, 65080, Van, TURKEY

### ARTICLE INFO

Research Article

Corresponding Author: Bilgin TAŞKIN, E-mail: bilgintaskin@yyu.edu.tr, Tel: +90 (432) 225 11 04

Received: 20 December 2018, Received in Revised Form: 04 February 2019, Accepted: 05 May 2019

### AUTHORS ORCID ID:

(Bilgin TAŞKIN: 0000-0002-9772-7438), (Ahmet AKKÖPRÜ: 0000-0002-1526-6093)

### ABSTRACT

Kefir is a probiotic, dairy product produced by the fermentative activity of a diverse range of lactic acid bacteria, acetic acid bacteria, and yeast. In this study, we revealed the antimicrobial spectra of five types of kefir supernatants (EG, AN, KF, KY and SD) from different regions of Turkey fermented for 24 and 48 h against seven plant pathogenic bacteria and one bacterial biocontrol agent *in vitro* and *in vivo* for the first time. *In vitro*, antibacterial activity was investigated by the disk diffusion agar method. Their antibacterial potencies varied according to the type of kefir and the fermentation time. Also,

we showed that the antimicrobial activity of kefir could be attributable to antimicrobial substances in supernatants rather than the low pH. *In vivo*, studies using the most potent kefir type on cucumber and common bean with their pathogenic bacteria in the climate chamber showed no remarkable decrease in diseases but revealed an increase in some plant growth parameters. The application resulted in an increase of 22% in shoot fresh weight, 20% in shoot dry weight, 79% in root fresh weight and 113% in root dry weight in common bean, on the other hand, 25% in shoot fresh weight, 34% in root fresh weight and 30% in shoot dry weight in cucumber.

Keywords: Kefir; Probiotic; Plant pathogens; Biological control

© Ankara Üniversitesi Ziraat Fakültesi

## 1. Introduction

Bacteria cause diseases in plants which are generally characterized by morphological symptoms such as galls and overgrowths, wilts, leaf spots, specks and blights, soft rots, as well as scabs and cankers. In general, the most common and devastating plant pathogenic bacteria belong to genera *Erwinia*, *Pectobacterium*, *Agrobacterium*, *Pseudomonas*, *Ralstonia*, *Burkholderia*, *Acidovorax*, *Xanthomonas*, *Clavibacter*, *Streptomyces*, *Xylella*, *Spiroplasma*, and *Phytoplasma* (Bull et al 2010). The diseases caused by bacteria in essential crop plants cause financial problems on a global scale. For example, The U.S. government spent US\$1 billion to eradicate citrus canker disease during 1995-2005, while the Indian government spent more than INR20 million to combat bacterial blight diseases in pomegranate during 2003-2008. These and other examples indicate the importance of controlling bacterial plant diseases throughout the world (Borkar & Yumlembam 2017).

There are many approaches to controlling the plant bacterial diseases but the most commonly used one is chemical control (Kannan & Bastas 2015). The chemical management of individual bacterial diseases has been largely driven by the use of copper compounds and the antibiotics (Sundin et al 2016). However, as a result of extensive use of these compounds, bacteria have started to evolve resistance either through mutation or the acquisition of a resistance gene(s) (Förster et al 2015; Sundin & Wang 2018). Also, the use of antibiotics in plant disease control is banned in European

Union because of potential impacts on human health and the transfer of antibiotic resistance into clinical pathogens (Sundin et al 2016; Sundin & Wang 2018).

Kefir is a fermented milk product that contains both mainly lactic acid bacteria (*Lactobacillus*, *Lactococcus* spp., *Leuconostoc* spp., *Acetobacter* spp. and *Streptococcus* spp.) and yeasts (*Kluyveromyces* spp., *Torula* spp., *Candida* spp. and *Saccharomyces* spp.) (Güzel-Seydim et al 2011; Fiorda et al 2017). Various functional properties of kefir products such as cholesterol-lowering effects (Liu et al 2006) and the antitumor activities (Renner & Munzner 1991; Liu et al 2005; Güzel-Seydim et al 2006) were reported. There are also studies focusing on the effects of different kefir products. Jeong et al (2017) showed the antimicrobial activities of the exopolysaccharide (EPS) produced by a *Lactobacillus kefirianofaciens* strain which is a member of the microflora of kefir granules against *Listeria monocytogenes* and *Salmonella enteritidis* in their study.

Rodrigues et al (2005) conducted a study to examine the antimicrobial activity of the kefir and kefiran which is the polysaccharide extracted from kefir beads on various types of human pathogenic bacteria. Ulusoy et al (2007) studied the antibacterial effect of 24 h and 48 h fermented kefir produced from commercial starter culture against several food pathogens. Kim et al (2016) reported a study to elucidate the optimal fermentation time and conditions for antimicrobial activities of kefir products from different origins against food-borne pathogens. They found that the spectra and potencies of kefir products varied according to the type of kefir and the fermentation time. In addition to those, Marquina et al (2002) reported that the consumption of kefir caused to decrease in *Enterobacteriaceae* and *Clostridia* population in the mucosa of mice bowel. Furthermore, other than the main ones mentioned above various functional properties such as improving lactose tolerance, stimulation of immune system and control of irritable bowel symptoms are exhibited by kefir (Güzel-Seydim et al 2011).

Although kefir has been shown to have an antibacterial effect on human and food pathogens in several studies, there is no evaluation of such application for plant pathogenic bacteria, to the best of our knowledge. We investigated the *in vitro* antimicrobial activities of kefir products from different origins fermented for 24 and 48 h against seven plant pathogenic strains and one bacterial biocontrol agent. Additionally, we studied the effects of the kefir product on common bean and cucumber diseases and plant growth parameters in a climate chamber. This study aimed to elucidate the antimicrobial activities of kefir products against plant pathogenic bacterial strains and evaluate the potential of this dairy product to be used in plant pathology for the first time.

## 2. Material and Methods

### 2.1. Kefir and supernatant preparation

Five types of kefir grains, i.e., SD, AN, EG, KY and KF, were used in this study. Among them, kefir SD is the one which is being sold commercially and the others were collected from private households in a different region of Turkey. Starter grains (5 g) were continuously cultured in pasteurized, low-fat milk for 15 days prior to experiments. The medium was changed at 24 h intervals and the grains washed with sterile water. For antibacterial activity tests, viable kefir grains, 10% w/v, were inoculated in pasteurized, low-fat milk and cultured at 25 °C for 24 and 48 h. At the end of the fermentation process, the grains and milk were separated using a sterilized plastic filter (2-mm pore size).

For antimicrobial activity tests, kefir milk was centrifuged at 6,000 g for 10 min and the supernatant was sterilized by filtration using a 0.45-µm pore-size syringe filter (Millipore Co., USA). The pH of the filtered kefir supernatant was determined with a pH meter.

### 2.2. Bacterial strains

All bacterial strains were provided by the bacteriology laboratory in the Department of Plant Protection, Faculty of Agriculture, Van Yuzuncu Yil University. The pathogens; *Pseudomonas syringae* pv. *syringae* (*Pss*), *Pseudomonas syringae* pv. *lachrymans* (*Psl*), *Pseudomonas syringae* pv. *tomato* (*Pst*), *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), *Xanthomonas euvesicatoria* (*Xe*), *Erwinia amylovora* (*Ea*), *Clavibacter michiganensis* ssp. *michiganensis* (*Cmm*) and the biocontrol agent of plant diseases, *Bacillus* spp. 66/3 (66/3), were used as the test strains in this study. All strain cultures were grown either in Nutrient Broth (NB) broth (Difco, Detroit, MI, USA) or on Nutrient Broth agar plates at 25 °C.

### 2.3. *In vitro* antibacterial activity

Antibacterial activity of kefir supernatants was evaluated using the disk diffusion method as described by the National Committee for Clinical Laboratory Standards (NCCLS 2002). Cell suspensions were prepared with the spectrophotometric method and the final bacterial cell concentration applied on the agar surface was approximated to  $10^6$  CFU. Sterile test disks (Bioanalyse Co., Ltd., Ankara, Turkey) were applied to the agar surface previously inoculated with 0.1 mL bacterial suspension. 20  $\mu$ L of each kefir supernatant was directly dropped onto the surface of disks. The plates were incubated for 48 h at 25 °C, and the inhibition zones were measured. All experiments were done in nine replicates. The presence of a clear zone around the disks was considered as inhibition.

To see whether the antimicrobial activity of kefirs could be attributable to low pH value or not, we conducted a control experiment. For this, lactic acid solution (Sigma-Aldrich, USA), acetic acid solution (Merck, Germany), and absolute ethyl alcohol (JT Baker, USA) were diluted with sterilized distilled water. The pH of the diluent was adjusted to 3.5 for both acid solutions. Ethyl alcohol was diluted to 2.0% v/v. All solutions were sterilized by filtration using a 0.45- $\mu$ m pore-size syringe filter before use. Antibacterial activity of control solutions was evaluated as mentioned above.

### 2.4. *In vivo* antibacterial activity

In this part of the study, kefir AN fermented for 48 h which was one of the most potent kefir types on *Psl* and *Xap* in *in vitro* tests were investigated in climate room. Bacterial blight disease of common bean (*Phaseolus vulgaris* cv. Gina) caused by *Xap* and angular leaf spot disease of cucumber (*Cucumis sativus* cv. Gordion F1) caused by *Psl* were tested in a climate chamber.

### 2.5. Cultivation of plants, application of pathogens and kefir supernatant

Pesticides free cucumber and bean seeds were planted in 250 mL volume containers filled with sterile peat and left in a climate room at  $24 \pm 2$  °C with 60% humidity and 14-h light conditions. During the study, nutrition required by seedlings was provided as recommended by Akköprü & Özaktan (2018).

The supernatant was diluted with sterile distilled water at a rate of 1 to 3 just before application to prevent phytotoxicity due to its low pH. The supernatant was applied to the plants three times in two different ways. First, the suspension was applied twice to the seedlings by drenching method with 10 mL plant<sup>-1</sup> and 20 mL plant<sup>-1</sup> 48 h prior to the pathogen application. The second, the suspension was sprayed onto plants 24 h prior to the pathogen application.

For the application of pathogens, 48-h *Psl* and *Xap* cultures grown on Nutrient Broth medium were prepared in suspension with  $10^8$  cfu mL<sup>-1</sup> and 0.01% Tween 80 was added as a surfactant. Pathogens were inoculated with a hand sprayer 24 h after last application of kefir supernatant. The *Psl* suspension was applied to the cucumber seedlings when the second true leaves began to open (Akköprü & Özaktan 2018). The *Xap* culture was applied to the bean seedlings during the trifoliolate leaf period (Akköprü et al 2018). Immediately after pathogen application, the plants were left in high relative humidity for 48 hours.

### 2.6. Determination of disease severity and plant development parameters

The diseases severity ratings were based on the infected leaf area. Three weeks after *Xap* application, disease symptoms on common bean plants were assessed by scale 1-5 (1: no disease symptoms, 2: a few necrotic spots or  $\leq 5\%$ , 3: 6-25%, 4: 26-50%, 5:  $\geq 50\%$  and defoliation) (Abbasi et al 2002). 14 days after *Psl* application, disease symptoms on cucumber plants were assessed by scale 1-6 (1: no disease symptoms, 2: a few necrotic spots or  $< 10\%$ , 3: 10-25%, 4: 26-50%, 5: 51-75%, 6:  $> 76\%$  or fallen or dead leaves) (Akköprü & Özaktan 2018). The disease index (1) and % efficacy (2) were calculated using the following formulas;

$$\text{Disease index} = \frac{\sum (\text{Rating number} \times \text{Number of leaves in the rating})}{\text{Total number of leaves} \times \text{Highest rating}} \times 100 \quad (1)$$

$$\text{Efficacy\%} = \frac{\text{Control value} - \text{treatment value}}{\text{Control value}} \times 100 \quad (2)$$

The effect of Kefir supernatant on cucumber and common bean were determined in the 15<sup>th</sup> day and 21<sup>st</sup> day, respectively. After the root parts of the seedlings cut from the root collar were washed, roots and shoots were weighed separately to determine fresh weight. Then they were dried at 65 °C for 72 h and weighed again to obtain dry weights.

### 2.7. Statistical analysis

All *in vitro* experiments were performed in nine replicates. *In vivo* experiments were set up according to completely randomized with ten replicates. SPSS 17.0 package was used for statistical analysis.

## 3. Result and Discussion

### 3.1. *In vitro* antibacterial activity

Antimicrobial spectra of the kefir supernatants for 24 h and 48 h against the plant pathogens are presented in Table 1 and 2, respectively. Antimicrobial activity generally increased along with prolonged fermentation time in all types of kefir; this effect was evaluated by using Student's t-test and found statistically important (Table 3). This test indicated that all kefir types showed higher antibacterial activity against *Pss* and *Xe* after 48 h fermentation compared with 24 h fermentation. On the other hand, not all kefir types showed a statistically significant increase in antibacterial activity with time against all bacterial strains. For example, the antibacterial activity of kefir types increased with prolonged fermentation time against *Cmm*, except for kefir KF. It did not exhibit a statistically significant increase in antibacterial activity with time against this pathogen. The similar situation was also observed for *Ea*, *Pst*, *Psl*, *Xap* and *66/3* (Table 3).

**Table 1- Antibacterial spectrum of five types of kefir supernatants fermented for 24 h against eight bacterial strains. The results represent the mean zone diameters (in mm) using the disk diffusion method**

Kefir Types	EA Sig. 0.0001	PST Sig. 0.0001	PSL Sig. 0.0001	XAP Sig. 0.0001	XE Sig. 0.0001	66/3 Sig. 0.0001	CMM Sig. 0.001	PSS Sig. 0.001
EG	9.78±0.15 <sup>ab</sup>	8.33±0.24 <sup>b</sup>	9.33±0.26 <sup>c</sup>	10.20±0.15 <sup>b</sup>	7.00±0.01 <sup>c</sup>	6.40±0.18 <sup>bc</sup>	8.33±0.24 <sup>c</sup>	8.00±0.29 <sup>bc</sup>
KY	9.89±0.11 <sup>ab</sup>	7.11±0.26 <sup>c</sup>	9.78±0.15 <sup>bc</sup>	9.50±0.33 <sup>b</sup>	6.11±0.33 <sup>d</sup>	5.67±0.24 <sup>c</sup>	9.33±0.41 <sup>b</sup>	7.33±0.67 <sup>c</sup>
AN	10.67±0.75 <sup>a</sup>	10.11±0.39 <sup>a</sup>	11.00±0.65 <sup>ab</sup>	9.56±0.50 <sup>b</sup>	7.67±0.17 <sup>c</sup>	6.44±0.44 <sup>bc</sup>	6.11±0.48 <sup>d</sup>	8.22±0.70 <sup>bc</sup>
KF	8.60±0.71 <sup>b</sup>	8.89±0.35 <sup>b</sup>	11.33±0.67 <sup>a</sup>	5.00±0.01 <sup>b</sup>	13±0.29 <sup>a</sup>	8.50±0.90 <sup>a</sup>	16.20±0.28 <sup>a</sup>	11.40±0.96 <sup>a</sup>
SD	9.78±0.40 <sup>ab</sup>	10.9±0.35 <sup>a</sup>	10.00±0.17 <sup>bc</sup>	9.22±0.49 <sup>a</sup>	9.67±0.33 <sup>b</sup>	7.56±0.80 <sup>ab</sup>	6.44±0.18 <sup>d</sup>	9.89±0.26 <sup>ab</sup>

\*Mean values followed by the same letter were not significantly different based on the Duncan's Multiple Range Test at P< 0.05 significance level.

**Table 2- Antibacterial spectrum of five types of kefir supernatants fermented for 48 h against bacterial strains. The results represent the mean zone diameters (in mm) using the disk diffusion method**

Kefir Types	EA Sig. 0.001	PST Sig. 0.001	PSL Sig. 0.001	XAP Sig. 0.001	XE Sig. 0.0001	66/3 Sig. 0.001	CMM Sig. 0.001	PSS Sig. 0.001
EG	10.00±0.65 <sup>c</sup>	9.33±2.06 <sup>c</sup>	12.00±0.69 <sup>c</sup>	8.00±0.26 <sup>cd</sup>	16.89±0.89 <sup>a</sup>	6.56±0.24 <sup>d</sup>	15.78±0.68 <sup>b</sup>	8.67±0.37 <sup>d</sup>
KY	10.00±0.17 <sup>c</sup>	10.3±0.50 <sup>c</sup>	10.22±0.37 <sup>d</sup>	9.00±0.17 <sup>c</sup>	12.78±0.70 <sup>b</sup>	9.44±0.50 <sup>c</sup>	10.11±0.35 <sup>c</sup>	10.78±0.64 <sup>c</sup>
AN	15.44±0.40 <sup>a</sup>	15.67±1.66 <sup>a</sup>	18.22±0.87 <sup>a</sup>	16.11±0.79 <sup>a</sup>	15.33±0.53 <sup>a</sup>	13.89±0.98 <sup>a</sup>	7.89±0.26 <sup>d</sup>	13.56±1.04 <sup>b</sup>
KF	13.33±0.60 <sup>b</sup>	12.67±1.00 <sup>b</sup>	15.89±0.62 <sup>b</sup>	6.67±0.37 <sup>d</sup>	16.17±1.05 <sup>a</sup>	11.78±0.43 <sup>b</sup>	18.78±0.36 <sup>a</sup>	16.22±0.88 <sup>a</sup>
SD	12.89±0.56 <sup>b</sup>	15.33±0.28 <sup>a</sup>	14.22±0.41 <sup>b</sup>	13.44±0.44 <sup>b</sup>	17.44±1.02 <sup>a</sup>	12.11±0.35 <sup>b</sup>	10.11±0.72 <sup>c</sup>	14.89±0.48 <sup>ab</sup>

\*Mean values followed by the same letter were not significantly different based on the Duncan's Multiple Range Test at P< 0.05 significance level.

Our results are consistent with those of the study conducted by Kim et al (2016). They found that the spectra and potencies of kefir against several food pathogens varied according to the type of kefir and the fermentation time. Also, Silva et al (2009) reported that the antimicrobial activities of kefir generally increased with prolonged fermentation times. However, Ulusoy et al (2007) showed no difference in the antimicrobial activities of kefir fermented for 24 or 48 h against some food-borne pathogens *in vitro*.

In general, all kefir types showed a remarkable antibacterial effect against the bacteria we used. Kefir AN was the most potent one on *Ea*, *Pst*, *Psl*, *Xap*, and *Xe*. *Xe* was inhibited by all kefir types strongly except kefir KY which gave a slightly smaller zone compared to others. On the other hand, kefir KF gave the highest activity against *Cmm*, *Pss*, and *Xe* (Table 2). Those results suggest that kefir from different origins have different antimicrobial spectra against a particular

strain. Also, the results indicate that the same kefir type may have different antibacterial potencies against different species and even subspecies.

**Table 3- Comparison of fermentation time (24 and 48 hours) on antibacterial activities of kefir supernatants against bacterial strains using Student's t-test**

Strain/Kefir	EG	KY	SD	KF	AN
EA	-0.34*±0.741	-0.56*±0.588	-4.49±0.001	-4.80±0.001	-4.27±0.001
PST	-1.38*±0.001	-10.42±0.001	-9.77±0.001	-7.80±0.001	-8.22±0.001
PSL	-3.67±0.002	-1.13*±0.283	-9.73±0.0001	-4.82±0.001	-6.70±0.001
XAP	8.06±0.0001	1.41*±0.179	-6.36±0.001	-4.47±0.001	-4.16±0.001
XE	-13.24±0.001	-6.47±0.001	-5.44±0.001	2.36±0.034	-6.27±0.001
66/3	1.48*±0.163	-10.51±0.001	-3.47±0.003	-13.18±0.001	-2.80±0.015
CMM	-9.30±0.001	-4.24±0.001	-10.68±0.001	0.05*±0.961	-12.88±0.001
PSS	-1.41±0.176	-3.73±0.002	-9.09±0.001	-3.67±0.002	-4.24±0.001

One-way variance analysis was carried out using Student's t-test ( $P < 0.05$ ) to detect a significant difference between variables; \*, means no statistically significant effect of prolonged fermentation time on antibacterial activities of kefir

The microbial composition of kefir varies according to kefir origin, the substrate used in the fermentation process and the storage conditions also (Prado et al 2015). Pintado et al (1996) found different microbiological diversity in terms of yeast and Lactobacilli species content in Portuguese kefir grains compared to some other kefir grains such as Russian, Yugoslavian and Bulgarian grains. Also, it was shown that the composition of Tibetan kefir differs from that of others such as Russian, Taiwan or Turkey kefir. (Gao et al 2012; Altay et al 2013). Since the substrate used in the fermentation, storage and handling conditions were all the same for each kefir grain in our study, it can be concluded that different kefir types have variable antibacterial spectra against the bacterial strains due to their different origins.

The probiotic species, especially lactobacilli, are known to produce a wide range of antimicrobial compounds such as organic acids (lactic and acetic acids), hydrogen peroxide, ethanol, and bacteriocins (Guzel-Seydim et al 2011; Kim et al 2015). In this study, during the fermentation process, the pH gradually decreased in all kefir samples (Table 4). However, our overall data suggested that the antimicrobial activity of kefirs could not be attributable to low pH value simply. To demonstrate this, the antimicrobial activities of lactic and acetic acid solutions against the test strains were also investigated. All strains were resistant to both acid solutions at pH 3.5. The growth of test strains was inhibited by kefir supernatants, although the lowest pH value of kefirs in our study was 3.67. Additionally, since some yeast strains produce ethyl alcohol during kefir fermentation, we wanted to use ethyl alcohol solution (2% v/v) to see the antibacterial activity of it on the test strains. There are variations between 0.01% and 0.1% (v/v) among the reported ethanol contents of kefir (Guzel-Seydim et al 2000). The growth of all strains, however, was not affected by even higher ethyl alcohol solution. Therefore, we concluded that the antimicrobial activity of kefir could be attributable to the effect of antimicrobial substances in the kefir supernatants rather than the low pH.

**Table 4- pH values of five different kefir supernatants fermented for 24 and 48 hours. Measurements were performed in supernatants at temperatures of 24±2 °C**

Kefir types	pH value according to fermentation times	
	24 h	48 h
EG	3.80	3.70
KY	3.95	3.75
AN	3.91	3.67
KF	4.50	4.20
SD	3.82	3.70

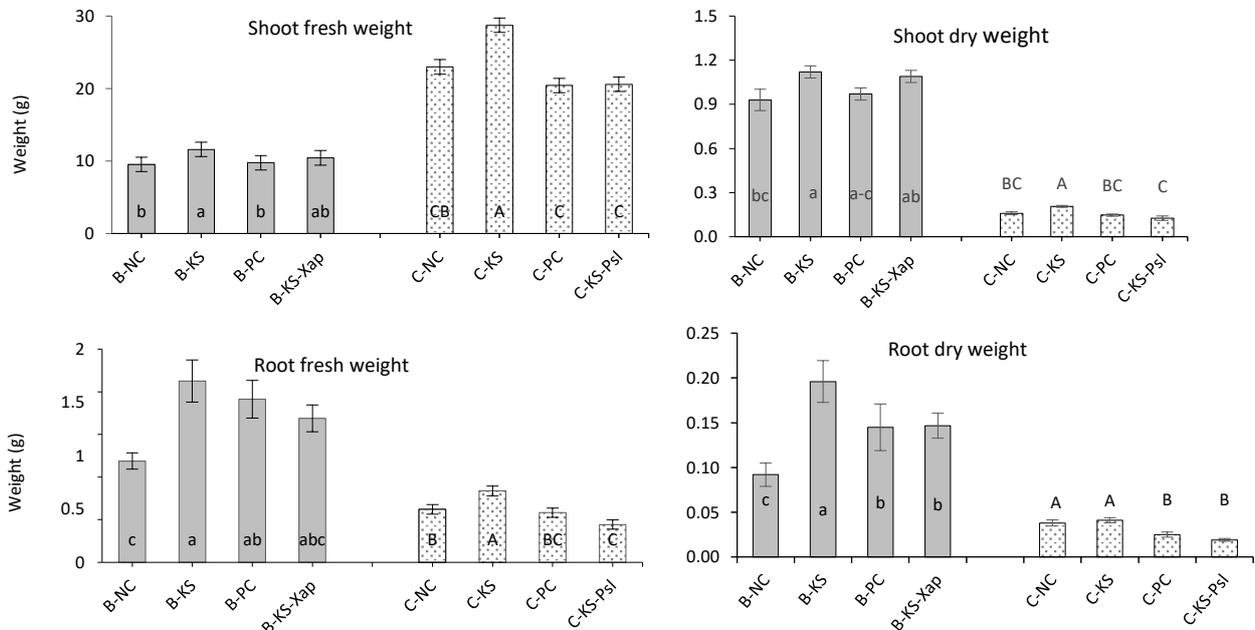
It could be postulated that the antimicrobial effect may be caused by the antagonistic action of metabolites and inhibitory compounds synthesized and released into the supernatant by various microorganisms present in kefir, and possibly interact with each other to enhance or antagonize their antimicrobial effects.

### 3.2. In planta studies

The effect of using Kefir AN, one of the most potent kefir types, on *Psl* and *Xap* were also investigated in the climate

room. *Xap* causes common bacterial blight disease in bean and *Psl* causes angular leaf spot disease in cucumber.

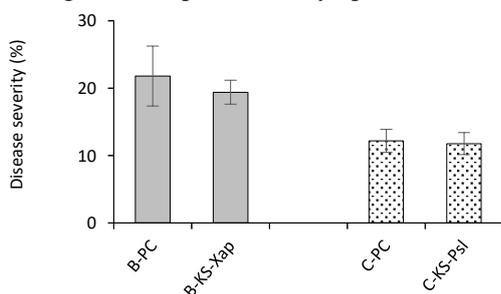
The application of kefir supernatant to bean seedlings resulted in a statistically significant increase in all plant growth parameters. It resulted in an increase of 22% in shoot fresh weight, 20% in shoot dry weight, 79% in root fresh weight and 113% in root dry weight. However, no effect on plant growth parameters was observed under disease pressure (Figure 1).



**Figure 1-** The effect of the supernatant on common bean and cucumber growth parameters with and without the disease pressure in a climate chamber. NC, negative control; KS, kefir supernatant only; C, cucumber, B, bean; PC, positive control. Mean values followed by the same letter are not significant ( $P < 0.05$ ) ( $N \geq 15$ )

On the other hand, in cucumber experiments, kefir supernatant application significantly increased at the rate of 34% in root fresh weight, 25% in shoot fresh weight and 30% in dry weight but did not cause an increase in root dry weight. However, no effect on plant growth parameters was observed under disease pressure (Figure 1).

Also, the application did not show any inhibitory effect on common bacterial leaf blight disease symptoms in bean and angular leaf spot disease symptoms in cucumber (Figure 2).



**Figure 2-** The effect of the supernatant on Angular leaf spot of cucumber caused by *Psl* and Common bacterial leaf blight disease of bean caused by *Xap* in climate room. The severity of diseases on bean and cucumber were assessed by scale 1-5 and 1-6, respectively. B, common bean; C, cucumber; KS, kefir supernatant; PC, positive control. Mean values followed by the same letter are not significant ( $P < 0.05$ ) ( $N \geq 15$ )

The positive effect of kefir supernatant on plant development parameters may be because of the presence of many organic and bioactive compounds in the supernatant. These compounds might have been used by plant cells and plant-associated bacteria in many ways, such as carbon and other plant nutrients sources. Also, many undefined bioactive

compounds of kefir supernatant might have affected metabolic pathways of plant cells or plant-associated bacteria which resulted in an increase of those parameters we measured.

On the other hand, we did not observe any inhibitory effect of kefir supernatant on common bacterial leaf blight disease of bean caused by *Xap* and angular leaf spot disease of cucumber caused by *PsI*. At first, we used supernatant without any dilution on plants, however; it produced slightly phytotoxicity most probably due to its low pH. Buffering the supernatant to increase the pH caused strong precipitation. Therefore, we diluted the supernatant with a 1/3 ratio with sterile distilled water before use. Phytotoxicity was prevented by this way but we could not observe any inhibitory effect on plants infected with *Xap* or *PsI*. It can be speculated that inhibitory compounds of the supernatant might have been degraded by plants itself or natural microflora on them. The other possible explanation is that dilution of the supernatant might have prevented the inhibitory compounds to exert their activities on the pathogens due to their low concentration.

Future studies should be followed to determine whether a single compound is responsible for antimicrobial properties or this was a result of antagonistic effects of various metabolites in kefir. Also, it should be created more knowledge about the key antimicrobial compounds against each pathogenic bacterium and their mechanisms. By this way, they can be used more effectively as a single compound against plant bacterial diseases without affected by undesirable conditions like low pH or dilution effect. In addition, after the isolation and identification of each microorganism in the microflora of kefir, they should be investigated as biological agents against plant pathogens.

#### 4. Conclusions

In conclusion, our results revealed different antibacterial potencies according to the type of kefir and the fermentation time. Although we could have not shown this effect in planta studies, some plant growth parameters increased significantly. There is an urgent need for the development of novel antimicrobial agents against pathogenic strains in agriculture. In parallel with the recent developments in the new generation technologies, natural product research has also gained positive momentum. With these technologies, the potential of organisms to produce natural products can be revealed. In principle, such compounds may also find applications in medicine where the antibiotic-resistance problem is also increasing. New narrow-spectrum antimicrobials like bacteriocins may contribute to agriculture's need for more sustainable and effective strategies for plant disease control.

#### Acknowledgements

This work was supported by the Scientific Research Project Units of Van Yuzuncu Yil University (Project number: FYL-2017-6263).

#### References

- Abbasi P A, Soltani N, Cuppels D A & Lazarovits G (2002). Reduction of bacterial spot disease severity on tomato and pepper plants with foliar applications of ammonium lignosulfonate and potassium phosphate. *Plant Disease* 86: 1232-1236
- Akköprü A & Özaktan H (2018). Identification of rhizobacteria that increase yield and plant tolerance against angular leaf spot disease in cucumber. *Plant Protection Science* 54(2): 67-73
- Akköprü A, Çakar K & Husseini A (2018). Effects of endophytic bacteria on disease and growth in plants under biotic stress. *Yuzuncu Yil University Journal of Agricultural Sciences* 28(2): 200-208
- Altay F, Karbancıoğlu-Güler F, Daskaya-Dikmen C & Heperkan D (2013). A review on traditional Turkish fermented non-alcoholic beverages: microbiota, fermentation process and quality characteristics. *International Journal of Food Microbiology* 167: 44-56
- Borkar S & Yumlembam R (2017). *Bacterial Diseases of Crop Plants*. Boca Raton: CRC Press
- Bull C T, De Boer S H, Denny T P, Firrao G, Saux M F L, Saddler G S & Takikawa Y (2010). Comprehensive list of names of plant pathogenic bacteria, 1980-2007. *Journal of Plant Pathology* pp. 551-592
- Fiorda F A, de Melo Pereira G V, Thomaz-Soccol V, Rakshit S K, Pagnoncelli M G B, de Souza Vandenberghe L P & Soccol C R (2017). Microbiological, biochemical, and functional aspects of sugary kefir fermentation-A review. *Food Microbiology* 66: 86-95
- Förster H, McGhee G C, Sundin G W & Adaskaveg J E (2015). Characterization of streptomycin resistance in isolates of *Erwinia amylovora* from California. *Phytopathology* 105: 1302-1310

- Gao J, Gu F, Abdella N H, Ruan H & He G (2012). Investigation on culturable microflora in Tibetan kefir grains from different areas of China. *Journal of Food Science* 77: 425-433
- Guzel-Seydim Z B, Seydim A C, Greene A K & Bodine A B (2000). Determination of organic acids and volatile flavor substances in kefir during fermentation. *Journal of Food Composition and Analysis* 13: 35-43
- Guzel-Seydim Z B, Seydim A C, Greene A K & Tas T (2006). Determination of antimutagenic properties of some fermented milks including changes in the total fatty acid profiles including CLA. *International Journal of Dairy Technology* 59: 209-215
- Guzel-Seydim Z, Kok-Tas T & Greene A K (2011). Review: functional properties of kefir. *Critical Reviews in Food Science and Nutrition* 51: 261-268
- Jeong D, Kim D H, Kang I B, Kim H, Song K Y, Kim H S & Seo K H (2017). Characterization and antibacterial activity of a novel exopolysaccharide produced by *Lactobacillus kefirifaciens* DN1 isolated from kefir. *Food Control* 78: 436-442
- Kannan V & Bastas K (2015). Sustainable Approaches to Controlling Plant Pathogenic Bacteria. Boca Raton, CRC Press
- Kim D H, Chon J W, Kang I B, Kim H, Kim H S, Song K Y & Seo K H (2015). Growth inhibition of *Cronobacter sakazakii* in experimentally contaminated powdered infant formula by kefir supernatant. *Journal of Food Protect* 78: 1651-1655
- Kim D H, Jeong D, Kim H, Kang I B, Chon J W, Song K Y & Seo K H (2016). Antimicrobial activity of kefir against various food pathogens and spoilage bacteria. *Korean Journal for Food Science of Animal Resources* 36(6): 787
- Liu J R, Chen M J & Lin C W (2005). Antimutagenic and antioxidant properties of milk-kefir and soymilk-kefir. *Journal of Agricultural and Food Chem* 53: 2467-2474
- Liu J R, Wang S Y, Chen M J, Chen H L, Yueh P Y & Lin C W (2006). Hypocholesterolemic effects of milk-kefir vs soymilk-kefir in cholesterol-fed hamsters. *British Journal of Nutrition* 95: 939-946
- Marquina D, Santos A, Corpas I, Munoz J, Zazo J & Pienado J M (2002). Dietary influence of kefir on microbial activities in the mouse bowel. *Letters in Applied Microbiology* 35: 136-140
- NCCLS (National Committee for Clinical Lab. Standards) (2002). Performance standards for antimicrobial disk susceptibility test: approved standard M2-A6. 6<sup>th</sup> ed. Wayne, PA
- Pintado M E, Da Silva J L, Fernandes P B, Malcata F X & Hogg T A (1996). Microbiological and rheological studies on Portuguese kefir grains. *International Journal of Food Science & Technology* 31(1): 15-26
- Prado M R, Blandón L M, Vandenberghe L P, Rodrigues C, Castro G R, Thomaz-Soccol V & Soccol C R (2015). Milk kefir: composition, microbial cultures, biological activities, and related products. *Frontiers in Microbiology* 6: 1177
- Renner H W & Munzner R (1991). The possible role of probiotics as dietary antimutagens. *Mutation Research Letters* 262: 239-245
- Rodrigues K, Caputo L, Carvalho J, Evangelista J & Schneedorf J (2005). Antimicrobial and healing activity of kefir and kefir extract. *International Journal of Antimicrobial Agents* 25: 404-408
- Silva K R, Rodrigues S A, Filho L X & Lima A S (2009). Antimicrobial activity of broth fermented with kefir grains. *Applied Biochemistry and Biotec* 152: 316-325
- Sundin G W & Wang N (2018). Antibiotic Resistance in Plant-Pathogenic Bacteria. *Annual Review of Phytopathology* 56: 161-180
- Sundin G W, Castiblanco L F, Yuan X, Zeng Q & Yang C H (2016). Bacterial disease management: challenges, experience, innovation and future prospects: challenges in bacterial molecular plant pathology. *777Molecular Plant Pathology* 17(9): 1506-1518
- Ulusoy B H, Colak H, Hampikyan H & Erkan M E (2007). An in vitro study on the antibacterial effect of kefir against some food-borne pathogens. *Turkish Microbiology Society* 37: 103-107