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DETERMINATION OF ANTIBACTERIAL EFFICIENCIES OF COMMERCIAL PLANT ESSENTIAL OILS AND TANNINS AGAINST PLANT PATHOGENIC BACTERIA

C. C. Başaran[1*†], İ. Bektaş[2†] and M. Küsek[1†]

[1]Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Plant Protection, Avşar Campus 46040 Kahramanmaraş Turkey
[2]Amasya University, Suluova Vocational School, Department of Plant and Animal Production, Amasya Turkey
[*]Corresponding Author's email: ceycydcey@gmail.com

ABSTRACT

Kahramanmaras region is suitable for pome fruits, vine and pepper cultivation. This study was conducted to evaluate the antibacterial effects of 6 different commercial essential oil (thyme, mint, cinnamon, clove, orange and ginger) and 3 different commercial tannin (Artutan, Artutan K and Farmatan) extracts against several isolates of different plant pathogenic bacterial species (*Rhizobium* spp. (syn. *Agrobacterium*), *Xanthomonas* spp. and *Erwinia amylovora*) by using disc diffusion method. Minimum Inhibitory Concentrations (MICs) was determined by using broth micro-dilution method for plant extracts which caused > 5 mm inhibition zones. Thyme and cinnamon essential oils were determined as the most effective essential oils but ginger essential oil was the least effective oil determined. The activity of essential oils also varied statistically according to bacterial species tested. The most susceptible isolate was determined *Rhizobium* sp. CU5-4/6 and the most resistant isolates were *Xanthomonas* spp. MB7-5 and MB6-3. *Xanthomonas* spp. isolates were found to be more resistant to essential oils than *Rhizobium* spp. and *E. amylovora* isolates. MIC value of mint and thyme essential oils against *Rhizobium* spp. isolates varied between 10-20 µl/ml; thyme 10 µl/ml for *Xanthomonas* spp. isolates and mint 20 µl / ml for *Xanthomonas* spp. MB6-6 isolate. MIC value of cinnamon essential oils against *Rhizobium* spp. isolates CU5-4/6 and CU5-4/10 was determined as 20-40 µl/ml. Clove essential oil caused inhibition at 10 µl/ml against *Xanthomonas* spp. isolates. Our results clearly shown that plant extracts may be used as an alternative control method in sustainable and organic farming against pathogens.

Key words: plant pathogen bacteria, plant extracts, antibacterial activity, MIC

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INTRODUCTION

Plant pathogenic bacterial species cause many problems before and after crop harvest. Today, chemical pesticides, antibiotics and food additives were used in the control against food and plant pathogenic bacterial species problem in agriculture and food industries (Soylu et al. 2009). However, due to the emergence of pesticide-resistant pathogen species and the negative impact of pesticides on the environment and human health, several environmentally friendly studies carried out on alternative control methods (Soylu et al. 2009, Dadaşoğlu 2016). Plant essential oils, extract and major components can be used in the control against fungal and bacterial plant diseases since they do not create residues on the product and don't have a negative effect on the environment (Belgüzar et al. 2016; Kara and Soylu, 2020). In recent years, the number of studies on the antimicrobial activities of plant extracts and essential oils from different medical and aromatic plant has been conducted against a varieties plant fungal and bacterial disease agents (Karaman et al. 2001, Meral and Karabay 2002, Yeğen et al. 2002, Şahin et al. 2003, Güllüce et al. 2003, Adıgüzel et al. 2005, Tepe et al. 2006, Soylu et al., 2010, Mengüllüoglu & Soylu 2012, Kotan et al. 2014, Dadaşoğlu et al. 2015, Görmez et al. 2015; Bozkurt et al. 2020; Kara et al., 2020). Tannin plant extracts and essential oils from thyme (*Thymus vulgaris*), mint (*Mentha spicata*), crove (*Caryophyllus aromaticum*), cinnamon (*Cinnamamum zeylanicum*), orange (*Citrus sinensis*), ginger (*Zingiber officinale*) have been reported to possess antimicrobial activities against different microbial disease agents (Altundağ and Aslım 2005, Karami-Osboo et al. 2010, Dobre &Niculita 2012, Lucas et al. 2012, Mengüllüoglu & Soylu 2012, Jafarpour et al. 2013, Badawy &Abdelgaleil 2014, Ben El Hadj et al. 2015, Habbadi et al. 2017, Bektaş et al. 2020). Kahramanmaras province of Turkey is suitable for pome fruits and grapevines farming. Pepper is regarded as the most important economic crop cultivated in this region. Diseases caused by plant pathogenic bacterial disease agents in Kahramanmaras were reported to cause significant crop losses. Pepper bacterial spot disease caused by *Xanthomonas* spp. in peppers, fire blight disease caused by *Erwinia amylovora* in pome fruits, crown gall disease caused by *Rhizobium* spp. (Syn. *Agrobacterium* spp.) were major bacterial diseases encountered in the region. It is important to control these

plant pathogenic bacterial species with alternative control methods to chemical control. For this reason, the present study was conducted to investigate *in vitro* antibacterial activity of some plant extracts and essential oils against plant pathogenic bacterial disease agents such as *Xanthomonas* spp., *E. amylovora* and *Rhizobium* spp which were recorded as the most important bacterial disease agents encountered in the region.

MATERIALS AND METHODS

Isolation and maintenance of plant pathogens: Plant sample which infected by *Rhizobium* spp. were collected from the vineyard areas in June-September as described by Argun (2001). Bacterial suspension were prepared from these fresh gall tissues and streaked on King's B (KB) and Luria Broth Agar (LBA) medium as stated by Küsek (2007). The petri dishes were incubated at 25+1° C for 2-4 days. The pure cultures of *E. amylovora* and *Xanthomonas* spp. species were previously isolated from diseased pear and pepper plants growing in the region. Bacterial cultures were obtained from the stock and incubated in Nutrient Broth **(**NB) medium. *E. amylovora* and *Xanthomonas* spp. were grown on Sucrose Nutrient Agar (SNA) and NA medium, respectively. Typical *Rhizobium* spp. colonies growing in King B and LBA medium, which appeared as cream coloured partially transparent, flat around, round and convex, were selected from petri dishes and purified by streaking on PDA+$CaCO_3$ or King B medium. The pure colonies obtained were kept in 30% glycerine and YDCA medium until they were used. The plant pathogenic bacteria isolates used in the study are given in Table 1.

Table1. Plant pathogenic bacterial isolates used in the study.

Plant Pathogen Bacteria	Host Plant	Origin	Isolate No.
Erwinia amylovora	Quince	Şahin Kayasi/Göksun	U1-6-1
Erwinia amylovora	Apple	Andirin	H1-3
Erwinia amylovora	Pear	Afşin	U-AF-2
Erwinia amylovora	Apple	Göksun	G-7
Erwinia amylovora	Quince	Döngel/Göksun	D-10
Xanthomonas sp.	Pepper	Türkoğlu	MB3-6
Xanthomonas sp.	Pepper	Minehüyük/Türkoğlu	MB4-0
Xanthomonas sp.	Pepper	Türkoğlu	MB5-3
Xanthomonas sp.	Pepper	Balikalan/Gaziantep	MB6-6
Xanthomonas sp.	Pepper	Balikalan/Gaziantep	MB7-5
Rhizobium sp.	Grapevine	Türkoğlu	CU5-4/6
Rhizobium sp.	Grapevine	Bertiz/Dulkadiroğlu	CU5-4/4
Rhizobium sp.	Grapevine	Kazma Bağlari/Onikişubat	CU5-4/10
Rhizobium sp.	Grapevine	Tekirdağ	Ref.Culture

Plant essential oils and tannins: In this study commercial tannins [Artutan (65% ± 2 tannins; Valonia isolated from *Quercus ithaburensis* ssp. *macrolepis*), Artutan K (63-65% tannin, isolated from scarlet, *Pinus brutia*) which belongs to the catechol tannin group) and Farmatan (soluble in water and isolated from chestnut tree, Castanea sativa Mill.)] and essential oils from thyme (*Thymus vulgaris*), mint (*Mentha spicata*), clove (*Caryophyllus aromaticum*), cinnamon (*Cinnamamum zeylanicum*), orange (*Citrus sinensis*), ginger (*Zingiber officinale*) were purchased from Sigma Aldrich and used in *in vitro* antibacterial activity studies.

Determination of antibacterial activities: The antibacterial sensitivity test was carried out according to the disc diffusion method as described by Bozkurt et al. (2020). 1 g of each tannin was dissolved in 2 ml of 70% alcohol and vortexed by adding 8 ml of sterile distilled water. In disc diffusion method, 10 µl of tannin samples and 1, 2, 5 and 10 µl/ml of essential oil samples were taken and added on each disc separately. Streptomycin antibiotic was used as positive control. Four different discs treated with essential oils or tannins were placed at equal distance on each petri plates (Figure 1). The prepared petri plates were incubated at 25±1ºC for 48 hours. The zone of inhibition diameter (mm) formed around the discs was measured with the help of a calliper (Torq 150 mm Digital Calliper). The experiment was repeated twice with 3 replications each.

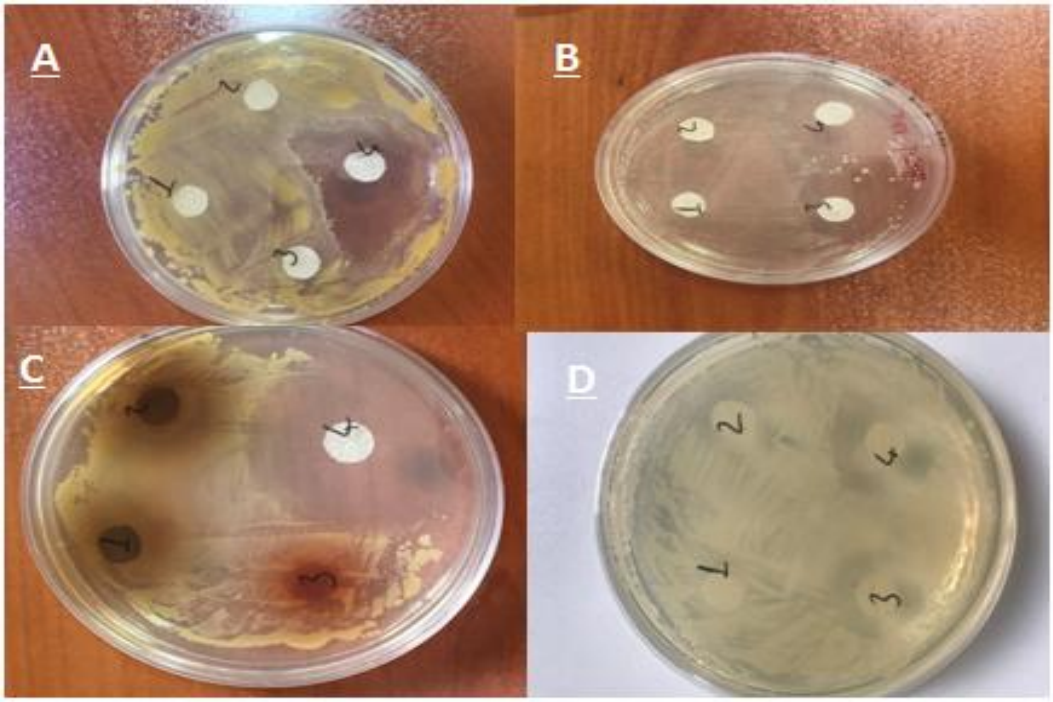

Figure 1. Inhibition zones caused by essential oils and tannins as determined by disc diffusion method. (A) *Xanthomonas* spp.; (B) *E. amylovora*; C1: Farmatan; C2: Artutan; C3: Artutan K; C4: Control of antibiotic; (D) *Rhizobium* spp.; 1:1 µl/ml; 2: 2 µl/ml; 3:5 µl/ml and 4:10 µl/ml).

Determination of minimum inhibition concentration (MIC): Minimum Inhibition Concentrations (MIC) were determined for tannins and essential oils, which caused an inhibition zone of 5 mm and above, by using the broth micro dilution method. For this purpose, two-fold dilution series were prepared from essential oils mixed with 0.01% dimethyl sulfoxide (DMSO) and tannins extracts to NB medium in concentrations up to 16.0 µl/ml, starting from 0.10 µl/ml (Habbadi et al. 2017). Since DMSO has no antibacterial effect, DMSO was used as negative control and antibiotic streptomycin was used as positive control in NB medium. Each tubes were inoculated with 10 µl bacterial suspension at the concentration of $1x10^8$ and incubation at 25±1°C for 24 hours. 100 µl of the bacterial cultures from each tube was spread and observed for any growth to determine MIC. Trials were set up in duplicate and streptomycin antibiotic was used as positive control.

Statistical analysis: Subsequently, ANOVA test was performed and the differences of means were compared according to the $P<0.05$ significance level according to the DUNCAN multiple comparison test by using SPSS Statistic programme (SPSS Inc., Ver. 20.0, Chicago, IL).

RESULTS AND DISCUSSION

Isolation of plant pathogens: Five *E. amylovora* (U1-6-1, H1-3, U-AF-2, G-7, D-10) isolates were isolated from pome fruit with the characteristics of the development of domed, bright, white mucoid colonies in the SNA medium. After identification these isolates were stored in 30 % glycerol at -20˚C as a stock culture. Five isolates of *Xanthomonas* spp. (MB3-6, MB4-0, MB5-3, MB6-6, MB7-5) isolated from pepper fields with the characteristics of typical yellow round mucoid colony development in NA medium. After identification these isolates were stored as pure culture. Three *Rhizobium* spp. isolates (CU5-4/4, CU5-4/6, CU5-4/10), which were obtained from fresh galls as a result of the surveys carried out in Kahramanmaras vineyard areas and formed a cream coloured, partially transparent, flat around, round and convex colony. After identification these isolates were also stored as pure culture. All these isolates were used in antibacterial assays.

Determination of antibacterial activities: The concentrations of 1, 2, 5 and 10 µl / ml of different essential oils and tannin extracts were used for antibacterial activities by using disc diffusion method and the inhibition zone diameters caused by each treatment on the petri plates were measured. These concentrations were given in Figure 1, and the calculated averages and standard errors were given in Table 2. Thyme and clove essential oil inhibited bacterial growth the most against tested bacterial isolates. It was determined that tannin and ginger prevented bacterial growth the least.

Table 2. Inhibition zone diameter (mm) caused by tannins and essential oils by using disc diffusion methods.

Essential Oils	Dose	Bacterial isolates											Mean value
		CU5-4/4	CU5-4/6	CU5-4/10	Ref- Av.	U1-6-1	H1-3	G-7	MB3-6	MB4-0	MB6-6	MB7-5	
Thyme	1 µL	8.1 ±0.2	8.0 ±0	7.3 ±0.2	6.0 ±1.3	8.6 ±0	10.0 ±0.6	9.3 ±0.7	0 ±0	4.3 ±4.3	8.0 ±0.2	0 ±0	9.13 A
	2 µL	10.5 ±0	9.6 ±0.1	10.0 ±0	14.3 ±0.5	10.5 ±0	10.8 ±0	10.3 ±1.0	1.3 ±0.3	8.8 ±0.8	9.3 ±0.3	0 ±0	
	5 µL	11.2 ±0.1	12.0 ±0	11.4 ±0.1	13.4 ±0.4	11.3 ±0.4	13.9 ±1.7	12.1 ±0.2	2.5 ±0.5	11.0 ±0.1	11.0 ±1.5	0.4 ±0.4	
	10 µL	11.7 ±0.4	13.4 ±0.4	13.1 ±0.5	16.8 ±1.0	12.0 ±0.4	14.0 ±0.1	13.5 ±0.1	5.7 ±0.2	12.0 ±0	11.7 ±1.8	3.2 ±2.3	
Clove	1 µL	1.5 ±1.5	7 ±1	7.1 ±3.2	5.7 ±5.7	0 ±0	0.6 ±0	2.8 ±2.8	0 ±0	8.0 ±0	6.8 ±0.2	0 ±0	7.99 B
	2 µL	11 ±1.4	8.8 ±0.5	9.0 ±2.5	12.7 ±2.1	3.9 ±1.3	6.9 ±0	8.1 ±0.3	5.3 ±4.3	9.6 ±0.1	7.9 ±0.4	0 ±0	
	5 µL	11.3 ±0.3	12 ±0.8	10.5 ±1.6	12.8 ±0.1	5.7 ±1.5	11 ±1.4	10.9 ±0.5	3.8 ±3.8	10.3 ±0.3	10.7 ±1.3	1.1 ±1.0	
	10 µL	14.7 ±1	12.7 ±0	11.9 ±1.6	15.1 ±0.2	9.3 ±0.3	14.7 ±0.5	12.7 ±1.1	8.1 ±5.6	12.0 ±0.3	12.8 ±1.6	4.5 ±1.4	
Cinnamon	1 µL	10.7 ±2	11 ±0	8.0 ±0	6.8 ±0.4	1.3 ±1.3	6.0 ±2.1	8.1 ±0	0 ±0	4.4 ±3.5	9.6 ±2.3	0 ±0	8.87 A
	2 µL	13.2 ±0.4	10.2 ±0.2	9.5 ±0.3	13.0 ±0.1	2.5 ±2.5	10.3 ±0.5	9.6 ±0.1	1.7 ±0	9.9 ±0.5	10.3 ±1.6	0 ±0	
	5 µL	13.1 ±1.1	13.4 ±1.2	10.2 ±0.2	13.6 ±2	4.9 ±1.1	10.9 ±0.8	10.4 ±0.1	2.1 ±2.1	12.9 ±0.9	12.3 ±0.6	0.6 ±0.6	
	10 µL	16.3 ±0.7	16.4 ±1.2	11.4 ±0.4	18.9 ±2	6.5 ±0.8	13.7 ±1.7	12.0 ±0.3	3.9 ±1.4	15.5 ±0.6	13.4 ±0.5	2.2 ±1.4	
Orange	1 µL	1.2 ±1.2	3.8 ±3.8	2.6 ±0.1	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	6.3 ±0.5	6.3 ±0.1	0 ±0	3.96 D
	2 µL	1.1 ±1.1	7.5 ±3.7	4.4 ±1.3	0.5 ±0.5	0 ±0	0 ±0	0 ±0	0 ±0	11.9 ±0.4	7.6 ±0.7	0 ±0	
	5 µL	5.5 ±1.5	9.2 ±0.8	6.0 ±2	2.8 ±1.3	0 ±0	0.7 ±0.7	0 ±0	0 ±0	14.6 ±1.5	12.0 ±0	0 ±0	
	10 µL	7.4 ±3.1	10.1 ±4.2	8.7 ±0.9	6.4 ±0.2	0.4 ±0.4	1.0 ±0.8	0.1 ±0.1	0 ±0	14.7 ±0.6	13.9 ±0.5	7.4 ±7.4	
Mint	1 µL	8.0 ±0.7	5.2 ±5.2	0 ±0.04	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	5.7 ±0.7	0 ±0	0 ±0	5.32 C
	2 µL	11.1 ±2	8.9 ±3.2	5.3 ±1.7	0 ±0	0 ±0	0.1 ±0	2.3 ±0.5	0 ±0	5.9 ±3.3	7.5 ±0.5	0 ±0	
	5 µL	11.4 ±1	10.0 ±3.7	8.4 ±1.1	0.02 ±0	3.8 ±0.2	10.9 ±0.4	6.0 ±1.7	0 ±0	10.7 ±1.8	9.3 ±0.7	0 ±0	
	10 µL	13.5 ±0.8	13.1 ±1.7	11.0 ±0.9	3.0 ±1.6	7.1 ±0.2	13.5 ±2.7	10.3 ±1.9	0 ±0	13.2 ±2.6	15.3 ±1.5	0 ±0	
Ginger	1 µL	0 ±0	3.2 ±1.3	0.2 ±0.2	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	1.01 F
	2 µL	0 ±0	4.9 ±1.9	4.2 ±1.7	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	
	5 µL	0 ±0	7.3 ±1.2	5.0 ±0.5	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	
	10 µL	0 ±0	9.1 ±1.2	10.7 ±1.3	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	
Tannin	Farmatan	7.5 ±5	0 ±0	0.8 ±0.6	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	8.3 ±1.5	6.6 ±0.7	0 ±0	3.14 E
	Artutan	3.2 ±3.2	0.3 ±0.3	0.8 ±0.8	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	4.9 ±2.0	2.4 ±1.1	0 ±0	
	Artutan K	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	
Streptomycin	10 µL	11.5 ±1.3	11.0 ±2.2	12.0 ±2.9	0 ±0	0 ±0	10.0 ±0.8	9.8 ±0.7	10.0 ±0.9	14.2 ±0.7	14.1 ±0.4	10.8 ±3.4	$F_{60.308}$=14.06; p<0.01

According to the results of variance analysis in Table 3, significant statistical difference was found between essential oils. Thyme and cinnamon essential oils were found as the most effective oils were, while the ginger essential oils was found to be the least inhibition oil ($F_{6,308}$= 227.517; p<0.01).

Table 3. Variance analysis table by tannins and essential oils by using disc diffusion methods.

References	Type III Sum of Squares	df	Mean Square	F
Essential Oil	5169.02	6	861.50	227.52**
Concentration	2626.67	3	875.56	231.23**
İsolate	3902.17	10	390.22	103.05**
Essential oil * concent.	955.90	18	53.11	14.03**
Essential oil * isolate	3193.75	60	53.23	14.06**
Concent. * isolate	204.40	30	6.81	1.80*
Essential oil * concent. * isolate	1264.56	180	7.02	1.86**
Error	1166.25	308	3.79	
Total	38003.63	616		

*p<0,05; **p<0,01

The antibacterial activity of thyme essential oil against economically important bacterial disease agents such as, *Clavibacter michiganensis* subsp. *michiganensis*, *Xanthomonas axanopodis* pv. *vesicatoria*, *Pseudomonas syringae* pv. *tomato*, *Erwinia caratovora* pv. *atroceptica*, *Erwinia caratovora* pv. *caratovora*, *Erwinia amylovora* and *Rhizobium vitis* was previously reported by Altundağ &Aslım (2005). The antibacterial activities of essential oils also varied statistically according to bacterial isolates used in our study. Ginger essential oil did not show any antibacterial effect on *Xanthomonas* spp. and *Erwinia amylovora*, but showed antibacterial effect on *Rhizobium* spp. isolates (CU5-4/6 and CU5-4/10). In very recent study, Er et al. (2018) reported that ginger has a blocking effect on the development of *Rhizobium vitis* isolates. Among the isolates examined, the highest inhibition were displayed against CU5-4/6, while the least inhibition were recorded for MB7-5 and MB6-3 ($F_{60,308}$=14,06; p<0,01). *Xanthomonas* spp. isolates were determined to be more resistant to essential oils than *Rhizobium* spp. and *E. amylovora* isolates. In addition, among the *Xanthomonas* spp. isolates, only MB3-6 and MB7-5 isolates are more resistant than other isolates in the species. Karami-Osboo et al. (2010) stated that major compounds thymol and carvacrol present in thyme oil were responsible for strong antibacterial activivites against *E. amylovora*. Badawy &Abdelgaleil (2014) reported that the essential oil of 18 different plants were more effective against *E. carotovora var. carotovora* and *Rhizobium radiobacter*. In previous studies, thyme has been reported to be one of the most important medicinal and aromatic herbs and has antimicrobial activity (Yilmaz et al. 2014, Uçar et al. 2015). It has been determined that the inhibition zone diameters of tannin plant extracts at the single concentration of 10 µl / ml were less than the same concentrations of essential oils. However, one of the tannins (Artutan) was determined to be more effective on bacteria than other tannins tested.

Determination of minimum inhibition concentration (MIC): In order to determine the MIC, bacterial growth was measured in different concentration of essential oil amended nutrient broth by using the spectrophotometer. Following application of tannin, measurement could not be made in the spectrophotometer due to the high density of the tannin. Therefore, in tannin applications, 10 µl suspensions were taken from each tube after incubation and the measurements were made by plating on NA medium instead of measurement. Table 4 shows that different concentrations of essential oils have an inhibitory effect on bacterial growth. When the results were evaluated, the difference between essential oil, isolate and concentration was determined to be statistically significant (p<0,01).

Table 4. Analysis of variance table of essential oil MIC.

References	Type III Sum of Squares	Df	Mean Square	F
Essential Oil	24.033	6	4.006	178.130**
Concentration	7.791	4	1.948	86.622**
İsolate	1.912	6	.319	14.172**
Essential Oil * Concentration	9.165	24	.382	16.983**
Essential Oil * Concent.* isolate	13.923	144	.097	4.300**
Concent. * isolate	4.584	24	.191	8.494**
Essential Oil * isolate	9.415	36	.262	11.631**
Error	5.509	245	.022	
Total	122.227	490		

**p<0,01

Mint and thyme oils were determined more effective than other essential oils ($p<0,05$). According to the results,the lowest doses without bacterial growth were planted in NA medium and it was determined whether bacteria developed or MIC concentration values varied according to isolates and essential oils (Table 5).

Table 5. Determination of MIC values of essential oils against bacterial isolates.

Bacterial isolates	Essential oils	MIC concentration (µl/ml)
CU5-4/4	Mint	20
CU5-4/6	Mint; Thyme; Cinnamon	20; 10; 40
CU5-4/10	Mint; Thyme; Cinnamon	10; 40; 20
MB7-5	Thyme; Cinnamon; Clove	10; 20; 10
MB4-0	Thyme; Clove	10;10
MB6-6	Thyme; Mint; Cinnamon; Clove	10; 20; 40; 10

The MIC value of mint and thyme essential oil for *Rhizobium* spp. isolates varied between 10-20 µl/ml. Antibacterial effects of cinnamon was also determined by Tanovic et al. (2007). In their study, antibacterial effect of 17 essential oils on *C. michiganensis* subsp. *michiganensis* (*Cmm*) was determined, and it was stated that essential oils obtained from thyme, cinnamon, fennel, basil plants prevent the development of *Cmm* at a concentration of 0.65 µl/ml. In *Xanthomonas* spp. isolates, clove essential oil inhibited bacterial growth at 10 µl/ml. Öksel et al. (2014) stated that the essential oil of clove has an inhibitory effect on *Cmm*. MIC values of other essential oils were determined to be higher than these oils. Similar to the disk diffusion method, the activity of essential oils varied depending on the bacterial species, concentration applied and chemical compositions of the essential oils. Tannin extracts showed inhibition properties at the higher concentrations (80 ve 160 µl/ml) than those concentartions used for essential oils. Artutan tannins was determined more effective against all isolates (*Rhizobium* spp., *Erwinia amylovora* and *Xanthomonas* spp.) than other tannins samples used in the experiment (Table 6).

Table 6. Determination of MIC values of commercial tannins against bacterial isolates.

Bacterial isolates	Essential oils	MIC concentration (µl/ml)
CU5-4/4	Artutan	80
CU5-4/6	Artutan	80
CU5-4/10	Artutan; Artutan K	80
MB7-5	Artutan; Artutan K; Farmatan	80
MB4-0	Artutan; Artutan K	160;80
MB6-6	Artutan	80
USL5	Artutan; Artutan K; Farmatan	80; 80; 160
H34	Artutan; Artutan K; Farmatan	80; 160;80
U171	Artutan; Farmatan	80

The essential oils of *O. syriacum*, *T. serpyllum* and *T. spicata var. spicata* were found to be the most promising essential oils displaying the highest antibacterial activities against three important gal-forming plant pathogenic bacterial disease agents *Rhizobium radiobacter* (syn. *Agrobacterium tumefaciens*), *Pseudomonas savastanoi pv. savastanoi* and *P.savastanoi pv. nerii* (Bozkurt et al. 2020). The effect of thyme essential oil in our disc diffusion test and MIC determination study shows parallel results with all these studies. Cinnamon essential oil also showed an antimicrobial activity on the plant pathogen bacteria. To the best of our knowledge, the activity of cinnamon essential oil against these bacterial isolates has not been investigated before. Thyme, mint, cinnamon and clove essential oils showed antibacterial effect even at low doses (10 and 20 µl) against isolates of *Rhizobium* spp. and *Xanthomonas* spp. It has been determined that essential oils show different effects on different bacterial disease agents. Differences in antibacterial activities of essential oils against different bacterial and fungal species could be due to differences in major chemical components as reported in previously published studies (Soylu et al. 2009, Mengüllüoğlu & Soylu, 2012, Bozkurt et al. 2020, Kara et al. 2020). It is thought that plant extracts of tannin have a growth inhibitory effect on the plant pathogen bacteria, but the effect would increase even more when the appropriate concentration and density of tannin are adjusted.

Conclusion: In conclusion, the most important feature of this study is the first study in which the effectiveness of 6

different essential oils and 3 different tannin extracts were determined against plant pathogenic bacteria which are problem on different cultivated plants growing in Kahramanmaras province of Turkey. When the MIC effect of the essential oils used in the study was examined, it was observed that they had the same inhibitory effect as the antibiotic application and that tannin extracts also prevent pathogen growth at appropriate concentrations. Since pathogens could gain and sustain resistance against antibiotics, alternative control methods may gain importance in organic agriculture. In addition, since some essential oils show different efficiency in different concentrations, it is predicted that more effective results can be obtained when tested with several different combinations (Görmez et al. 2015). In this direction, determining bio formulations and using antimicrobials in the disease control can reduce the negative effects of chemicals in environment.

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