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Antibacterial activity of plant essential oils obtained from *Satureja* species against *Xanthomonas phaseoli* pv. *phaseoli* and *Xanthomonas citri* subsp. *fuscans*

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ABSTRACT: In this study, the antibacterial effects of essential oils obtained from different *Satureja* species (*Satureja cuneifolia* Ten., *Satureja spicigera* (C. Koch) Boiss., *Satureja thymbra* L., *Satureja hortensis* L. and *Satureja cilicica* P.H. Davis) against *Xanthomonas phaseoli* pv. *phaseoli* (Smith) Vauterin and *Xanthomonas citri* subsp. *fuscans* (Burkholder) Starr & Burkholder, which cause common leaf blight in bean plant, were tested. Essential oils were found to significantly inhibit the growth of bacterial strains of both disease agents *in vitro*, and the lowest concentrations that prevent bacterial growth were determined for both pathogens. The effects of essential oil applications on seed germination, number of infected cotyledons and disease severity were also evaluated. It was determined that essential oils of *S. cuneifolia* and *S. spicigera* has no negative effects on seed germination while essential oils of *S. hortensis*, *S. thymbra* and *S. cilicica* caused a little decrease in seed germination compared to the control. As a result of *S. cuneifolia* + pathogen and *S. spicigera* + pathogen applications, no infected cotyledons were detected, and it was determined that the disease development caused by two pathogens was prevented by 100%.

Keywords: Essential oil, *Satureja* spp., antibacterial activity, *Xanthomonas phaseoli* pv. *phaseoli*, *Xanthomonas citri* subsp. *fuscans*, bean

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INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is an economically important legume plant cultivated in many parts of the world (Yu et al., 2000; Popovic et al., 2012). It is one of the most consumed legumes in the world due to its rich nutritional and protein content. Plant residues of bean are used in the animal feed industry, plays a role in soil improvement and is widely grown due to its adaptation to different climatic conditions and its diversity (Ofuya and Akhidue, 2005; Voisin et al., 2014).

Bean production is limited by various biotic and abiotic factors (Mourice and Tryphone, 2012; Beebe et al., 2013). Common leaf blight, caused by seed-borne *Xanthomonas phaseoli* pv. *phaseoli* (Smith) Vauterin (Xpp) and *Xanthomonas citri* subsp. *fuscans* (Burkholder) Starr & Burkholder (Xcf) (Chen et al., 2018) are a highly-devastating diseases commonly seen around the world. It is one of the main biotic factors affecting the yield of beans and causes yield losses ranging from of 10-45% on average in the bean production (Gillard et al., 2009; Popović et al., 2010; Francisco et al., 2013). Depending on the density of the inoculum, host sensitivity and environmental conditions that support the progression of the disease, the yield loss can reach 100% (Opio et al., 1996). The presence of *Xanthomonas phaseoli* pv. *phaseoli* and *Xanthomonas citri* subsp. *fuscans* in Turkey has been reported by early studies. (Demir and Gundagdu, 1994; Kahveci and Maden, 1994; Dönmez 2004; Bastas and Sahin,2017). Bean bacterial pathogens also cause serious yield and quality losses in bean production in Turkey (Bastas and Sahin, 2017).

Both pathogens cause the same symptom in leaves, stems, pods and seeds of the plants. However, Xcf is reported to be more aggressive (Opio et al., 1996). The disease begins in the form of spots that have absorbed water on the underside of the leaves. Later, the center of the stains dries up and turns brown, and a narrow, thin lemony-yellow halo is observed around the stains. Over time, the lesions expand irregularly and the spots combine to turn into a sign of blight (Agrios, 2005). The disease symptom is seen as water-absorbed sunken areas on the stem and these areas grow and expand as reddish lines. Affected stem often crack and water-soaked cankers appear around these cracks (Belete and Bastas, 2017). Symptoms in pods are generally circular, slightly-sunken and dark red-brown in color. The pathogen is found in the seed coat or hilum, and causes a buttery-yellow- or brown-colored symptom in white bean seeds. The seeds affected by the disease shrink and show weak germination. In humid weather, a yellowish bacterial exudate forms on the pod, leaf and stem that dries later (Saettler, 1991; Schwartz et al., 2005; Okechukwu and Ekpo 2008).

In preventing the loss of yield and quality caused by the disease, it is important to apply methods that reduce inoculum, such as knowing host pathogen interaction, using pathogen-free seeds, crop rotation, use of resistant cultivars, eradication of weeds and removal of plant residues (Asensio-S-Manzanera et al., 2006; Zanatta et al., 2007; Bozkurt and Soylu, 2011). However, the facts that the pathogen is seed-borne, proliferating very rapidly and spreading makes it difficult to control the disease, and as a result, chemical control is seen as the most effective method, and many pesticides are used worldwide (Finizio and Villa, 2002). However, as the potency of pesticides is enriched, the strength of their side effects is increasing day by day. The unconscious use of pesticides has a great role in environmental pollution and disturbance of natural balance as well as the ensuing human, plant and animal health problems in recent years (Brent, 2004; Padovani et al., 2004; Garcês et al., 2020). In addition, the resistance developed by pathogens against pesticides and the formation of new breeds pose a serious problem and no results can be obtained from the chemical control methods (Carmona et al., 2018; Rangasamy et al., 2018). Today, as consumers become more aware of the environmental and human health hazards associated with chemical applications, the demand for organic products increases dramatically, as a result of which the global agriculture industry is experiencing a major shift towards organic agriculture. At this point, there is a growing interest in biological approaches to be applied in disease control and the potential of using plant-based essential oils instead of drugs that cause resistance in pathogens is considered (Popoola et al., 2016; Ganiyu et al., 2017). Among many plant genera and species belonging to Lamiaceae family, plants in which thymol or carvacrol are prominent in terms of essential oil components are generally known as thyme around the world and are used for such purposes (Baser, 2001; Baydar, 2007). In Turkey, about 15 plant species (*Origanum, Thymus, Thymbra, Satureja* and *Coridothymus*) are known as thyme and they are utilized in several purposes. 38 *Thymus* species (52% endemic), 23 *Origanum* species (65% endemic), 14 *Satureja* species (28% endemic), 2 *Thymbra* species and 1 *Coridothymus* species are deployed in different regions of Turkey (Baser, 1994; Baydar and Arabacı, 2013). In very recent study, antibacterial properties of essential oils and extracts obtained from several *Satureja* spp. such as *Satureja cuneifolia*, *Satureja spicigera*, *Satureja thymbra* , *Satureja hortensis* and *Satureja cilicica* against bean halo blight disease agent *Pseudomonas syringae* pv. *phaseolicola* and bean bacterial wilt disease agent *Curtobacterium flaccumfaciens* subsp. *flaccumfaciens* which led to significant losses in yield and quality on beans was investigated (Dönmez et al., 2020).

In this study, the antibacterial activity of the essential oils obtained from *Satureja cuneifolia* Ten., *Satureja spicigera* (C. Koch) Boiss., *Satureja thymbra* L., *Satureja hortensis* L. and *Satureja cilicica* P. H. Davis against Xpp. and Xcf., which causes significant yield losses and decreased seed quality in bean, were studied *in vitro* and *in vivo*.

MATERIALS AND METHODS

Material

Pathogen strains and plant species used in the study

Total of 20 pathogen strains, available at the bacterial culture collection of Asst. Prof. M. F. Donmez at Igdır University, 10 belonging to Xpp and 10 belonging to Xcf, were used in the study. As plant material, thyme (*Satureja cuneifolia* Ten), Trabzon thyme (*Satureja spicigera* (C. Koch) Boiss.), lemon thyme (*Satureja thymbra* L.), rock thyme (*Satureja hortensis* L.) and pointed (*sivri*) thyme (*Satureja cilicica* PH Davis.) species were used. As plant material, thyme (*Satureja cuneifolia* Ten) and pointed (sivri) thyme (*Satureja cilicica* PH Davis.) from Konya Selçuklu; Trabzon thyme (Satureja spicigera (C. Koch) Boiss.) from Trabzon Maçka; lemon thyme (*Satureja thymbra* L.) from Antalya Demre; rock thyme (*Satureja hortensis* L.) from Erzurum Şenkaya species were used and plants were collected during flowering between June and September in the 2014-2015 years.

Plant essential oils

Plants were dried in shady environments without sunlight, then were ground in a grinding mill and powdered, and then stored under cool storage conditions by putting them in cloth bags. Essential oils of dried plant samples (500 gr) were obtained by hydrodistillation method at 3-4 hours using the Clevenger apparatus (EM1000/CE). The essential oils obtained were dried on anhydrous sodium sulphate (Na_2SO_4). Essential oils were obtained stored a closed vial at 4 °C until used for bioassays.

Method

Determination of antibacterial activities of essential oils

The bacterial strains were grown in Trypticase Soy Agar (TSA) growth medium and and bacterial concentration was adjusted to 10^8 cells / mL^{-1}(OD: $A_{660nm} = 0.15$, Spectrophotometer, Thermo). Then, 100 µl of the suspensions were transferred to TSA medium, and homogeneously spread on the TSA medium with a sterile glass drumstick. After drying the nutrient media in a sterile cabinet for 10 minutes, Oxoid standard (blank) discs (6 mm) were impregnated with essential oils (10 µl / disc) were placed at equal intervals and 1 per petri dish. The prepared petri dishes were incubated at 28 ^{o}C for 24-48 hours. Then the radius (inhibition zone) of the bacteria-free zone around the discs was measured in mm. sdH_2O was used as negative control while metilmicin (30mg) was used as positive control.

Determination of the effect of essential oil applications on seed germination and the number of Xpp and Xcf infected cotyledons *in vitro*

The Aras 98 bean seeds were surface disinfected by washing in sterile water for 30 minutes, then soaked in 95% ethyl alcohol for 5 minutes and then washed in sterile water again for removing the ethyl alcohol. Subsequently, it was kept in 10% bleach for 5 more minutes and washed 5 times in sterile water and left to dry. 2 ml from each essential oil solutions prepared in different concentrations (1/5, 1/10, 1/25, 1/50, 1/100, 1/125, 1/250, 1/500 and 1/1000 v / v) by using 10% DMSO solution were transferred to test tubes. Then, 100 µl of the 24-hour mixed bacterial strain solution (10^8 cells mL^{-1}, grown in Nutrient Broth (NB; Lab-lemco powder 1 g, yeast extract 2 g, peptone 5 g, sodium chloride 5 g, dH_2O 1 L. pH 7.4 ± 0.2) was added to these tubes. 40 seeds were added to beaker (200 ml) and these beakers were incubated for 24 hours in a hematological shaker (150 rpm) and essential oil (2 ml for each seed, total 80 ml) and pathogenic bacteria (mix of pathogen strains, 100 µl for each seed, total 4 ml) were inoculated to seed. Then the seeds were left to dry on petri dishes with sterile blotting paper. The seeds were transferred to TSA nutrients with 10 seeds placed per petri dish. The petri dishes were covered with parafilm and incubated at 28 °C for 5-10 days. Essential oil was used as negative control while bacterial solution was used as positive control. The seeds covered with the pathogen were transferred to petri dishes with blotter paper placed on the bottom and the blotter papers were saturated with sterile water and the seeds were allowed to germinate at room temperature. Petries were covered with parafilm and the number of seeds germinated was recorded daily. At the end of the 20th day, the cotyledon leaves were examined and the number of infected outlets was recorded.

Determination of the effect of essential oils on common leaf blight disease *in vivo*

Parallel to the petri trials, the seeds (Aras 98) infected with pathogen and treated with essential oil were transferred to pots and grown under greenhouse conditions (90% humidity and 24–28 °C), and were observed to record the number of germinating plants and disease severity. A scale of 1-5 was used to determine the severity of the disease (1: No disease; 2: 25% of plants are diseased; 3: 50% of plants are diseased; 4: 75% of plants are diseased; 5: All plants are diseased) (Schaad 1994). Distilled water was used as negative control and mix of Xpp/Xcf strain as positive control and applications were replicated three times.

Statistical analysis

The measurement values obtained were analysed with ANOVA using the SPSS (version 17) statistical program and the differences between the applications were determined by using Duncan's Multiple Range Test ($p \leq 0.05$).

RESULTS AND DISCUSSION

***In vitro* Antibacterial Activity of Essential Oils**

When the results of the study were evaluated, it was determined that essential oils formed inhibition zones on the tested bacteria at different rates (Figures 1A and B). *S. spicigera* essential oil was determined to have very strong bactericidal effect on strains Xpp 145, Xpp 442 and Xpp 538, while the essential oil obtained from *S. cilicica* strains has the same effect on Xpp 442 and Xpp 538. Among tested essential oils, the largest zone against Xpp was obtained from *S. cuneifolia* essential oil application with 22.90 mm (Table 1). There were significant differences between essential oils and bacterial strains used.

Table 1. Antibacterial effects of essential oils of *Satureja* species on the growth of Xpp strains

	Essential oils of *Satureja* species and inhibition zones formed (mm)				
Bacterial strains	***S.cuneifolia***	***S. spicigera***	***S. thymbra***	***S.hortensis***	***S.cilicica***
Xpp 120	21.47 b	13.20 e	17.99 a	15.44 e	11.46 h
Xpp 124	14.49 g	12.93 f	10.47 j	8.13 j	13.61 g
Xpp 135	20.43 d	19.91 b	17.01 b	18.90 a	15.47 e
Xpp 145	14.12 ı	NG	15.43 e	16.76 d	13.88 f
Xpp 256	20.58 c	14.63 d	13.05 g	12.86 ı	15.83 c
Xpp 305	14.18 h	11.02 g	15.42 d	14.24 h	15.56 d
Xpp 406	18.63 f	17.32 c	15.66 c	15.10 f	16.62 a
Xpp 436	19.96 d	20.61 a	12.45 h	17.63 c	16.37 b
Xpp 442	22.90 a	NG*	14.77 f	14.59 g	NG
Xpp 538	19.68 e	NG	12.28 ı	18.88 b	NG
Control	0.0 j	0.0 h	0.0 k	0.0 k	0.0 ı

***NG;** No Growth indicating strong antibacterial activity

It was determined that *S. cuneifolia* essential oil has also strong bactericidal effect on Xcf 264, Xcf 491 and Xcf 498, while *S. spicigera* essential oil has a bactericidal effect on strains Xcf 253, Xcf 264, Xcf 266, Xcf 490, Xcf 491, Xcf 495 and Xcf 498, and the essential oil obtained from *S. cilicica* has a bactericidal effect on Xcf 265, Xcf 266 and Xcf 498. Among tested essential oils, the highest inhibition zones were caused by *S. hortensis* against Xcf 253 strains with an inhibition zone of a radius of 21.58 mm which was followed by *S. spicigera* against Xcf 265 with an inhibition zone of 18.29 mm and against Xcf 275 with a zone of 18.12 mm (Table 2), respectively.

Table 2. Antibacterial effects of essential oils belonging to *Satureja* species on the growth of Xcf strains

	Essential oils of *Satureja* species and Inhibition Zones Formed (mm)				
Pathogen Bacteria	***S.cuneifolia***	***S. spicigera***	***S. thymbra***	***S. hortensis***	***S. cilicica***
Xcf 253	17.63 b	NG*	15.91 b	21.58 a	17.68 a
Xcf 254	17.75 a	11.11 c	16.07 a	16.96 e	11.3 g
Xcf 264	NG	0.0 d	13.37 g	18.48 b	14.79 e
Xcf 265	15.84 c	18.29 a	11.21 h	11.91 ı	NG
Xcf 266	11.69 e	NG	14.92 e	16.38 f	NG
Xcf 275	12.29 d	18.12 b	14.73 f	17.42 d	15.35 d
Xcf 490	11.48 f	NG	15.23 d	13.84 g	13.35 f
Xcf 491	NG	NG	15.66 c	17.56 c	17.04 b
Xcf 495	8.56 g	NG	8.88 ı	12.40 h	15.39 c
Xcf 498	NG	NG	7.58 j	11.65 j	NG
Control	0.0 h	0.0 d	0.0 k	0.0 k	0.0 h

***NG;** No Growth indicating strong antibacterial activity

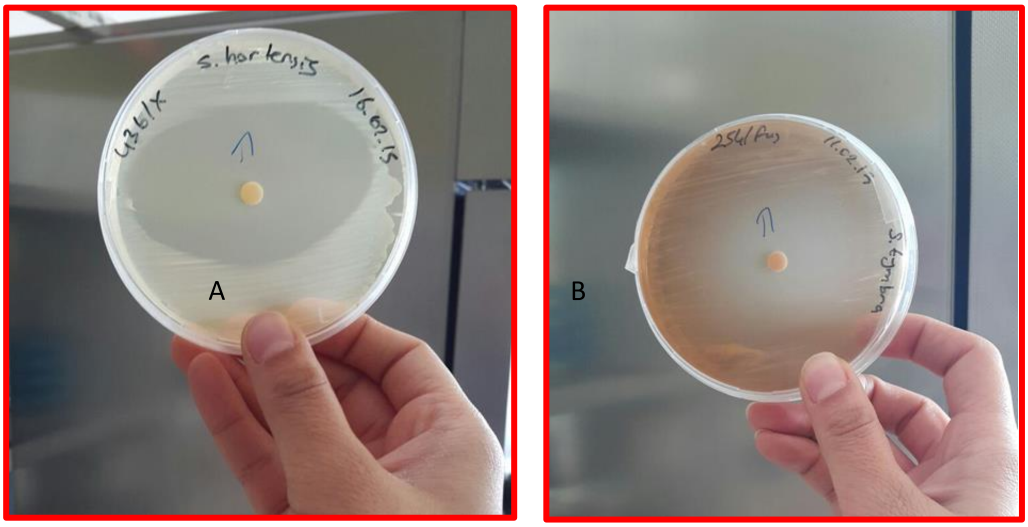

Figure 1. The antibacterial effect of *S. hortensis* L. essential oil on Xpp growth (A) and the effect of *S. thymbra* essential oil on Xcf growth (B) as shown inhibition zones around discs (arrow)

Minimal Inhibition Concentrations (MIC) of Essential Oils belonging to *Satureja* Species

The lowest concentration values at which essential oils inhibit the growth of 20 strains of two pathogens are indicated in Tables 3, 4, 5, and 6. MIC in which *Satureja hortensis* essential oil inhibits bean pathogens was determined as 62.5 µl for Xcf, 15.63 and 31.25 µl for Xpp (Table 3).

Table 3. MIC values of *Satureja hortensis* essential oil on Xpp and Xcf strains

	Satureja hortensis						
	500 µl	**250 µl**	**125 µl**	**62.5 µl**	**31.25 µl**	**15.63 µl**	**MIC**
253/fus	21.46*	10.28	8.4	7.22	-	-	**62.5**
254/fus	35.74	10.78	7.7	7.04	-	-	**62.5**
264/fus	42.66	14.64	8.7	8.66	-	-	**62.5**
265/fus	16.48	8.66	9.04	7.72	-	-	**62.5**
266/fus	32.68	11.32	8.54	7.14	-	-	**62.5**
275/fus	18.96	12.22	9.18	8.14	-	-	**62.5**
490/fus	8.44	8.18	8.22	7.26	-	-	**62.5**
491/fus	28.88	10	8.9	7.68	-	-	**62.5**
495/fus	34.98	21.98	8.54	7.04	-	-	**62.5**
498/fus	13.78	8.68	8.58	7.86	-	-	**62.5**
120/Xpp	80	80	80	80	80	80	**15.63**
124/Xpp	38.94	15.04	9.06	8.54	8.14	-	**31.25**
135/Xpp	36.04	20.96	10.48	8.22	7.66	-	**31.25**
145/Xpp	39.14	22.06	9.48	8.52	7.92	-	**31.25**
256/Xpp	40.78	15.18	8.9	8.84	7.7	-	**31.25**
305/Xpp	21.60	20	9.74	9.44	8.5	-	**31.25**
406/Xpp	31.46	18.48	9.1	8.9	7.36	-	**31.25**
442/Xpp	36.38	15.48	9.46	8.04	7.62	-	**31.25**
436/Xpp	29.74	12.14	9.04	8.4	7.54	-	**31.25**
538/Xpp	34.70	12.18	8.68	8.24	7.62	-	**31.25**

*; Inhibition zone value (r/mm)

MIC values of *Satureja thymbra* essential oil was determined to be between 15,63 and 500 µl for Xcf and between 15,63 and 250 µl for Xpp (Table 4).

Table 4. MIC values of *Satureja thymbra* essential oil on Xpp and Xcf strains

	Satureja thymbra						
	500 µl	**250 µl**	**125 µl**	**62.5 µl**	**31.25 µl**	**15.63 µl**	**MIC**
253/fus	36.56*	-	-	-	-	-	**500**
254/fus	14.92	10.3	6.86	-	-	-	**125**
264/fus	-	-	-	-	-	-	**15.63**
265/fus	9.92	8.44	7.7	6.92	6.98	6.56	**15.63**
266/fus	7.54	7.14	6.7	6.86	8.74	-	**125**
275/fus	16.92	9.34	6.66	6.7	8.04	6.3	**15.63**
490/fus	38.80	9.96	6.52	-	-	-	**125**
491/fus	29.60	13.32	10.16	8.02	9.04	9.08	**62.5**
495/fus	10.94	8.18	6.94	-	6.88	7.58	**31.25**
498/fus	27.36	13.32	11.58	8.06	8.12	6.66	**15.63**
120/Xpp	27.34	13	-	-	-	-	**250**
124/Xpp	17.48	11.8	8.44	-	-	-	**125**
135/Xpp	39.24	36	12.42	-	-	-	**125**
145/Xpp	27.50	12.4	7.22	7.2	6.72	6.4	**15.63**
256/Xpp	40.24	10.88	7.58	-	-	-	**125**
305/Xpp	32.60	11.6	8.26	6.84	-	15.63	**31.25**
406/Xpp	28.64	9.18	-	-	-	-	**250**
442/Xpp	34.66	14.12	-	-	-	-	**250**
436/Xpp	16.14	10.84	6.98	7.22	-	**6.3**	**15.63**
538/Xpp	88.86	69.16	29.38	15.08	-	-	**62.5**

*; Inhibition zone value (r/mm)

When MIC results of *Satureja cuneifolia* essential oil were evaluated, MIC values for Xcf strains were recorded as 31,25 - 62,5 - 125, 250 and 500 µl, while MIC values for Xpp strains were recorded as 15,63, 31,25 - 125 µl (Table 5).

Table 5. MIC values of *Satureja cuneifolia* essential oil on Xpp and Xcf strains

	Satureja cuneifolia						
	500 µl	**250 µl**	**125 µl**	**62.5 µl**	**31.25 µl**	**15.63 µl**	**MIC**
253/fus	19.56*	-	-	-	-	-	**500**
254/fus	8.70	7	-	-	-		**250**
264/fus	17.22	9.6	7.34	7	-	-	**62.5**
265/fus	-	-	-	-	-	-	**15.63**
266/fus	15.12	10.04	8.22	-	-	-	**125**
275/fus	29.06	9.86	9.02	8.3	8.04	7.48	**15.63**
490/fus	12.48	8.5	8.02	7.84	7.92	7.48	**15.63**
491/fus	23.14	15.42	9.28	8.98	8.12	8.1	**15.63**
495/fus	14.30	14.06	8.18	8.74	8.56	8.6	**125**
498/fus	14.24	9.34	-	-	-	-	**250**
120/Xpp	12.92	12.88	12.54	8.74	10.98	13.14	**62.25**
124/Xpp	27.00	14.04	8.94	8.5	7.7	7.42	**15.63**
135/Xpp	37.40	14.96	9.54	8.84	8.32	8.28	**15.63**
145/Xpp	19.26	10.14	9.34	8.72	8.28	7.8	**15.63**
256/Xpp	20.48	19.62	8.96	8.16	7.28	-	**31.25**
305/Xpp	22.24	14	9	8.2	8.04	7.8	**15.63**
406/Xpp	48.46	25.08	15.34	13.36	10.98	10.56	**15.63**
442/Xpp	38.84	18.54	12.54	9.34	8.42	8.6	**31.25**
436/Xpp	16.38	10.62	8.66	8.36	8.4	7.4	**15.63**
538/Xpp	14.18	11.26	9.44	8.62	7.86	-	**31.25**

*; Inhibition zone value (r/mm)

MIC value of *Satureja spicigera* essential oil against Xcf strains was determined to be between 15,63 and 62,5 µl. This value was found as 31,25 – 500 µl for Xpp strains (Table 6).

Table 6. MIC values of *Satureja spicigera* essential oil on Xpp and Xcf strains

	Satureja spicigera						
	500 µl	**250 µl**	**125 µl**	**62.5 µl**	**31.25 µl**	**15.63 µl**	**MIC**
253/fus	27.04*	-	-	-	-	-	**500**
254/fus	20.38	-	-	-	-	-	**500**
264/fus	39.22	8.24	7.74	-	-	-	**125**
265/fus	38.72	7.1	-	-	-	-	**250**
266/fus	30.74	12.14	7.58	-	-	-	**125**
275/fus	35.98	25.22	7.92	-	-	-	**125**
490/fus	12.88	10.06	8.24	7.02	-	-	**62.5**
491/fus	34.62	8.78	8.3	-	-	-	**125**
495/fus	32.26	8.26	7.2	-	-	-	**125**
498/fus	44.94	10.52	7.64	7.44	7.24	-	**31.25**
120/Xpp	47.78	22	21	20.3	9.7	8.3	**15.63**
124/Xpp	37.60	29.2	11.48	9.12	8.38	8.1	**15.63**
135/Xpp	35.96	19.78	17.7	13.68	8.88	8.36	**15.63**
145/Xpp	30.62	29.14	12.88	8.08	7.36	7.1	**15.63**
256/Xpp	43.18	33.4	9.74	8.6	7.6	-	**31.25**
305/Xpp	31.58	31.42	12.6	10.9	9.38	8.36	**15.63**
406/Xpp	34.92	24.32	9.2	9.48	8.02	6.64	**15.63**
442/Xpp	47.02	25.08	11.48	-	-	-	**125**
436/Xpp	37.22	33.06	10.6	8.54	7.9	7.16	**15.63**
538/Xpp	51.88	38.42	11.24	9.56	8.28	7.7	**15.63**

*; Inhibition zone value (r/mm)

The MIC value of *Satureja cilicica* essential oil on Xcf, one of the bean pathogens, was found between 15.63 and 62.5 µl. It has been noted that the oil inhibits the development of Xpp pathogens by forming a zone at a concentration of 31.25-500 µl (Table 7).

Table 7. MIC values of *Satureja cilicica* essential oil on Xpp and Xcf strains

	Satureja cilicica						
	500 µl	**250 µl**	**125 µl**	**62.5 µl**	**31.25 µl**	**15.63 µl**	**MIC**
253/fus	28.00*	19.9	15.18	9.74	7.22	-	**31.25**
254/fus	24.04	15.6	8.56	7.46	8.38	-	**62.5**
264/fus	-	-	-	-	-	-	**15.63**
265/fus	38.28	9.54	8.52	8.24	8.24	-	**31.25**
266/fus	15.82	11	9.22	7.7	7.36	-	**31.25**
275/fus	45.44	11.22	9.38	8.82	7.26	-	**31.25**
490/fus	11.46	8.48	8.86	8.7	8.06	7.82	**15.63**
491/fus	31.42	11.04	8.36	7.92	-	-	**62.5**
495/fus	29.98	10.18	8.98	8.6	8.4	-	**31.25**

Table 7. MIC values of *Satureja cilicica* essential oil on Xpp and Xcf strains (continue)

	Satureja cilicica						
	500 µl	**250 µl**	**125 µl**	**62.5 µl**	**31.25 µl**	**15.63 µl**	**MIC**
498/fus	-	-	-	-	-	-	**15.63**
120/Xpp	47.36	37.44	35.1	21.02	14.24	14.12	**15.63**
124/Xpp	17.16	-	-	-	-	-	**500**
135/Xpp	19.64	11.4	8.74	-	-	-	**125**
145/Xpp	10.46	7.82	-	-	-	-	**250**
256/Xpp	13.98	9.02	8.26	7.20	-	-	**62.5**
305/Xpp	50.08	34.78	31.8	-	-	-	**125**
406/Xpp	32.76	13.3	-	-	-	-	**250**
442/Xpp	27.72	22.1	-	-	-	-	**250**
436/Xpp	24.32	13.08	8.62	7.40	6.58	-	**31.25**

*; Inhibition zone value (r/mm)

The Effects of Essential Oil Applications on Seed Germination and the Number of Infected Cotyledons *in vitro*

The disinfected seeds were first treated with mixed bacterial strains, then treated with essential oils (contact effect) and transferred to petri dishes containing NA. In the petri trials, it was observed that the seeds were failed to germinate due to the phytotoxic effect of the essential oils on treated seed. For this reason, the seeds were exposed to the volatile effect of essential oil by adding 10 µl of essential oil in the blotter papers fixed on the lid of the petri dishes. It was determined that the essential oils of *S. cuneifolia* and *S. spicigera* did not adversely affect the seed germination, and the same number of seeds germinated with the control group. *S. hortensis*, *S. thymbra* and *S. cilicica* essential oils were found to cause a little decrease in seed germination compared to control. No bacterial disease developments were observed on pathogen inoculated seeds and treated with *S. cuneifolia* and *S. spicigera* essential oils. In pathogen applications, infected cotyledon emergence was recorded as 100% for Xpp and Xcf (Tables 8 and 9).

Table 8. The effects of essential oils belonging to *Satureja* species on the numbers of germinated seeds and Xpp infected cotyledons

Applications	Number of Infected Cotyledons	Number of Germinated Plants
Xpp* + *S. cuneifolia***	0.0 ± 0.0 a	10.0 ± 0.0 f
Xpp + *S. spicigera*	0.0* ± 0.0 a**	9.50 ± 0.0 e
Xpp + *S. thymbra*	1.0 ± 0.0 b	7.75 ± 0.35 c
Xpp + *S. hortensis*	1.0 ± 0.0 b	7.0 ± 0.0 b
Xpp + *S. cilicia*	1.0 ± 0.0 b	8.75 ± 0.35 d
S. cuneifolia	0.0 ± 0.0 a	10.0 ± 0.0 f
S. spicigera	0.0 ± 0.0 a	10.0 ± 0.0 f
S. thymbra	0.0 ± 0.0 a	7.0 ± 0.0 b
S. hortensis	0.0 ± 0.0 a	6.0 ± 0.0 a
S. cilicia	0.0 ± 0.0 a	8.0 ± 0.0 c
Negative Control	0.0 ± 0.0 a	10.0 ± 0.0 f
Positive Control (Mix of Xpp strains)	9.0 ± 0.0 c	9.0 ± 0.0 d

*Values are the averages of 3 replicates, **There are no statistically significant differences between values expressed with the same letters according to Duncan's Multiple Range Test (p≤0.05), *** Xpp inoculum was prepared as mixture of Xpp strains

Table 9. The effects of essential oils belonging to *Satureja* species on the numbers of germinated seeds and infected cotyledons

Applications	Number of Infected Cotyledons	Number of Germinated Plants
Xcf *+ *S. cuneifolia***	0.0* ± 0.0 a**	10.0 ± 0.0 d
Xcf + *S. spicigera*	0.0 ± 0.0 a	10.0 ± 0.0 d
Xcf + *S. thymbra*	1.0 ± 0.0 b	7.0 ± 0.0 b
Xcf + *S. hortensis*	2.0 ± 0.0 c	6.0 ± 0.0 a
Xcf + *S. cilicia*	2.0 ± 0.0 c	8.0 ± 0.0 c
S. cuneifolia	0.0 ± 0.0 a	10.0 ± 0.0 d
S. spicigera	0.0 ± 0.0 a	10.0 ± 0.0 d
S. thymbra	0.0 ± 0.0 a	7.0 ± 0.0 b
S. hortensis	0.0 ± 0.0 a	6.0 ± 0.0 a
S. cilicia	0.0 ± 0.0 a	8.0 ± 0.0 c
Negative Control	0.0 ± 0.0 a	10.0 ± 0.0 d
Positive Control (Mix of Xcf strains)	8.0 ± 0.0 d	8.0 ± 0.0 c

*Values are the averages of 3 replicates, **There are no statistically significant differences between values expressed with the same letters according to Duncan's Multiple Range Test (p≤0.05), *** Xcf inoculum was prepared as mixture of Xcf strains

The Effects of Essential Oils on Common Leaf Blight Disease *in vivo*

Symptoms caused by pathogens in pot trials were also evaluated according to a disease scale of 1-5. No disease development was observed in negative control (sterile water- sprayed) in all treatments and only plants treated with essential oil of five *Satureja* species. In pathogen inoculated control treatments, disease severity value caused by Xpp strain was 3.25, while the disease severity values on Xpp + *S. thymbra* treatment was 2.0, Xpp + *S. hortensis* treatment was 1.25, and Xcp + *S. cilicia* treatment was 2.25. No disease was detected in treated with *S. cuneifolia* and *S. spicigera* plants which were infected with Xpp (Table 10).

Table 10. The effects of essential oils belonging to *Satureja* species on the numbers of germinated seeds and disease severity caused by mixed Xpp strain

Applications	Disease Severity Index	Number of Germinated Plants
Xpp* + S. cuneifolia**	1.0* ± 0.0 a**	8.75 ± 0.0 c
Xpp + S. spicigera	1.0 ± 0.0 a	9.50 ± 0.0 d
Xpp + S. thymbra	2.0 ± 0.0 c	7.75 ± 0.35 b
Xpp + S. hortensis	1.25 ± 0.0 b	8.0 ± 0.0 b
Xpp + S. cilicia	2.25 ± 0.0 d	8.75 ± 0.35 c
S. cuneifolia	1.0 ± 0.0 a	9.0 ± 0.0 c
S. spicigera	1.0 ± 0.0 a	9.0 ± 0.0 c
S. thymbra	1.0 ± 0.0 a	7.0 ± 0.0 a
S. hortensis	1.0 ± 0.0 a	9.0 ± 0.0 c
S. cilicia	1.0 ± 0.0 a	7.0 ± 0.0 a
Negative Control	1.0 ± 0.0 a	8.0 ± 0.0 b
Positive Control (Mix of Xpp strains)	3.25 ± 0.0 e	8.0 ± 0.0 b

*Values are the averages of 3 replicates
**There are no statistically significant differences between values expressed with the same letters according to Duncan's Multiple Range Test ($p \leq 0.05$)
***Xpp inoculum was prepared as mixture of Xpp strains

While the disease severity caused by Xcf treated plant was found to be 4.25 in the experiment, it was determined that essential oils of *S. cuneifolia*, *S. hortensis* and *S. spicigera* prevented the development of the disease completely and the disease had not been observed in the plants treatments. The disease severity values of Xcf + *S. thymbra* and *S. cilica* were determined as 2.75 and 1.75, respectively (Table 11).

Table 11. The effects of essential oils belonging to *Satureja* species on the numbers of germinated seeds and disease severity caused by mixed Xcf strain

Applications	Disease Severity Index	Number of Germinated Plants
Xcf *+ S. cuneifolia**	1.0* ± 0.0 a**	8.75 ± 0.0 d
Xcf + S. spicigera	1.0 ± 0.0 a	9.13 ± 0.17 e
Xcf + S. thymbra	2.75 ± 0.0 c	7.75 ± 0.35 b
Xcf + S. hortensis	1.00 ± 0.0 b	7.0 ± 0.0 a
Xcf + S. cilicia	1.75 ± 0.0 b	8.75 ± 0.35 d
S. cuneifolia	1.0 ± 0.0 a	9.0 ± 0.0 de
S. spicigera	1.0 ± 0.0 a	9.0 ± 0.0 de
S. thymbra	1.0 ± 0.0 a	7.0 ± 0.0 a
S. hortensis	1.0 ± 0.0 a	9.0 ± 0.0 de
S. cilicia	1.0 ± 0.0 a	7.0 ± 0.0 a
Negative Control	1.0 ± 0.0 a	8.0 ± 0.0 c
Positive Control (Mix of Xcf strains)	4.25 ± 0.0 d	7.0 ± 0.0 a

*Values are the averages of 3 replicates
**There are no statistically significant differences between values expressed with the same letters according to Duncan's Multiple Range Test ($p \leq 0.05$)
*** Xcf inoculum was prepared as mixture of Xcf strains

It has been determined in many previously completed studies that the essential oils or extracts of many plant species included in the genus *Origanum, Thymus, Salvia, Satureja* and *Artemisia* show antimicrobial activity against fungal and bacterial disease agents (Pradhanang et al., 2003; Baydar et al., 2004; Skočibušić et al., 2006; Kokoskova and Pavela 2005; Sefidkon et al., 2007; Soylu et al., 2009; Soylu et al., 2010; Mengulluoglu and Soylu, 2012; Sureshjani et al., 2013; Alexa et al., 2018; Bozkurt et al., 2020; Kara et al., 2020).

Sökmen et al. (2004) found that the vegetable oil obtained from *Origanum acutidens* showed antimicrobial effects against 77% of 35 bacteria species and 67% of 18 fungus species. Sahin et al. (2004) reported that the vegetable oil obtained from the *Origanum vulgare* spp. *vulgare* plant collected from the Eastern Anatolia region showed antimicrobial effects against many bacteria and fungi species that are important in the food industry and medicine (infectious diseases), and that this plant can be used in the food industry and pharmacology industry as a natural preservative. Kızıl and Uyar (2006) determined that *Thymus kotschyanus, Satureja hortensis, Origanum onites* and *Thymbra spicata* species are effective against some fungal and bacterial species that cause disease in plants. Karaman et al. (2001) stated that the vegetable oil obtained from the *Thymus revolutus* plant and its components showed varying degrees of antimicrobial effect against 11 bacteria and 4 fungus species in different concentrations and even some concentrations prevent the growth of microorganisms more effectively than different antibiotics used commonly as standard options. Antibacterial properties of essential oils derived from several plant species such as thyme (*Thymbra spicata* L. subsp. *spicata* and *Thymus serpyllum* L.), origanum (*Origanum majorana* L.), mint (*Mentha spicata* L.), fennel (*Foeniculum vulgare*), lavender (*Lavandula stoechas* L. subsp. *stoechas*), lemon balm (*Melissa officinalis* L.), rosemary (*Rosmarinus officinalis* L.) and basil (*Ocimum basilicum* L.) were investigated and significant antibacterial activivites reported against seedborne plant pathogenic bacterium, *Acidovorax avenae* subsp. *citrulli*, *Clavibacter michiganensis* subsp. *michiganensis*. *Pseudomonas syringae* pv. *tomato*, gall forming bacterial disease agents such as *Rhizobium radiobacter, Pseudomonas savastanoi* pv. *savastanoi and P. savastanoi* pv. *nerii* disease agents (Soylu et al., 2009; Mengulluoglu and Soylu, 2012; Bozkurt et al., 2020). Gormez et al. (2016) evaluated the antibacterial effect of *Origanum rotundifolium* essential oil against 20 plant pathogens and found that it showed a significant effect. Božik et al. (2017) reported that the essential oils obtained from 4 aromatic plants (*Cinnamomum zeylanicum*, *Thymus vulgaris*, *Origanum vulgare* and *Syzygium aromaticum*) exhibit antibacterial activity against plant pathogens *Pectobacterium* spp. and *Pseudomonas* spp. species with the most effective result determined in cinnamon essential oil.

In antimicrobial studies of essential oils, different levels of activity of the same plant species against the same organism have been reported by several researchers. It is stated that this difference may be stemming from the variety of the plant, its genetic structure, the plant component used, the time of its harvest, the ecological conditions in the growing environment, the methods of obtaining essential oil, the characteristics of the microorganisms tested, the content of the nutrient medium, pH and temperature. The literature review shows that the results obtained in this study are in line with the results of other researchers. All the data obtained as a result of the study indicate that the essential oils of the *Satureja* species have the potential to be used as an antimicrobial in the control of plant bacterial diseases.

CONCLUSION

Plants produce phytochemicals that protect them from various environmental stresses. Most of the phytochemicals have antimicrobial activity and affect membrane lipids by interacting with the pathogen membrane. Therefore, the potential of essential oils obtained from plants to be used instead of pesticides is quite high.

In this study, it was determined that essential oils belonging to *Satureja* species significantly prevent the development of Xpp and Xcf strains *in vitro,* while essential oils of especially *S. cuneifolia* and *S. spicigera* plants prevent disease development 100% *in vivo* experiments. The fact that the essential oils of these plant species are highly effective against bacteria shows that they can be used as an environmentally-friendly application as part of the integrated control of bacterial pathogens. However, detailed research should be done on the practical use of essential oils, their mechanism of action, their effectiveness in different conditions, and their toxicity, and efforts should be made to expand the range of such applications.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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