

ASSESSMENT OF THE GROWTH INHIBITING EFFECT OF *SATUREJA* ESSENTIAL OILS ON DIFFERENT *FUSARIUM* SPECIES FROM WHEAT

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ABSTRACT

The antifungal effects of essential oils from *Satureja* species (*Satureja cilicica*, *S. cuneifolia*, *S. hortensis*, *S. montana*, *S. spicigera* and *S. thymbra*) tested for their efficacy against eight *Fusarium* species (*Fusarium avenaceum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. sambucinum*, *F. semitectum* and *F. solani*). The oils of *Satureja* species at three levels of concentrations (10, 20 and 30 µl of each 20 ml PDA medium) mixed with the medium and then, the mediums were inoculated with fungal isolates. To evaluate the efficacy of oils against the colony growth, the diameter of colonies measured every 24 hours and compared with their controls. The results showed that essential oils have antifungal activity at 0.5, 1, 2, and 5 µl/20mL concentrations and higher levels of oils (10, 20, and 30 µl/20 ml). Some fungal isolates (*Fusarium avenaceum*, *F. graminearum*, *F. oxysporum*, *F. sambucinum* and *F. solani*) could grow in the low oils concentration (0.5-5 µl/20 ml) especially when the fungal isolates treated with the *S. cuneifolia* oil. The higher concentrations of oil (10, 20, and 30 µl/20 ml) prevents the colony growth of *Fusarium* in the medium. The oils showed effective control of the plant pathogenic fungi growth in the medium with 100% inhibitory rates. According to the results of this experiment, the oil of *Satureja* species has the potential to inhibit the growth of *Fusarium* species.

KEYWORDS:

Fusarium, *Satureja*, essential oils, antifungal effects, summer savory

INTRODUCTION

Essential oils naturally occur in secondary metabolism of plants and have insecticidal and antimicrobial activities [1]. Essential oils have no side effect or they may have little side effect compared with the other chemicals those are used as chemical

control. Chemical compounds residue in the foods and their products can be the biggest reason of today's infectious disease among animals and human. Applying the higher concentrations of chemicals to control the postharvest diseases and pests are not the good way to control the pathogens because fruits and vegetables are consumed in a short time after harvest. Also, contamination of agro-food products with mycotoxins produced by plant pathogenic fungi are another unhealthy food supplying. Mycotoxins can cause both chronic or severe toxic effects and are responsible for repeated episodes of food poisoning both in livestock and humans.

Cereals are important crops for food safety and they infected by the different fungal pathogen that produces toxin in the grains before and after harvest. Each year several billion dollars of crop loss is done by *Fusarium* plant pathogen on different plants across the world. At the same time, *Fusarium* species are causal agents of diseases on the most important commercial and strategical crop plants such as wheat, corn, and potato [2,3]. *Fusarium* species as plant pathogenic fungi produce dangerous mycotoxin in their hosts such as Fumonisin (B1 and B2), and deoxynivalenol (DON) [4]. *Fusarium* species have the ability to cause the disease on a live host plant and grow on their husk or debris in the soil. So, there is opportunity to increase the population of the pathogen. According to the reasons mentioned above, some of the scientists try to find the bio-friendly and healthy compound to control the plant pathogenic fungi. One of the chemical compounds sourced from the secondary metabolism of plants is known as essential oils. There is some paper about the essential oil on pests and plant pathogens. For example, [5] studied the chemical composition of *Artemisia* essential oils and their inhibitory effects on *Fusarium oxysporum*, *F. sambucinum*, and *F. solani* and they found effective control of essential oils against fungal growth. Also, [6] tested the different *Artemisia* species essential oils on the 15 *Fusarium* species and found effective inhibitory rate against the plant pathogens growth. [7] evaluated the essential oils of *Hypericum linarioides* on six *Fusarium*

species (*F. acuminatum*, *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. sambucinum* and *F. solani*) but the results did not show significant inhibitory rates against the pathogen. [8] tested the efficacy of *Tanacetum aucheranum* and *T. chiliophyllum* var. *chiliophyllum* essential oils of 13 *Fusarium* species such as; *F. acuminatum*, *F. chlamydosporum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. incarnatum*, *F. nivale*, *F. oxysporum*, *F. proliferatum*, *F. sambucinum*, *F. scripi*, *F. semitectum*, *F. solani*, *F. tabacinum* and *F. verticillioides*. They found effective inhibitory against *Fusarium* species [8].

The essential oil of *Origanum acutidens* and their components carvacrol, thymol and p-cymene evaluated on eight *Fusarium* species. They found a high-level of inhibitory of fungal colony growth in the medium [9]. Also, [10] conducted a research to evaluate the *Salvia hydrangea* oils inhibitory against 14 *Fusarium* species. The oils of *S. hydrangea* had effective control against the pathogens. Also, the antifungal efficacy of *Achillea gypsicola* and *A. biebersteinii* oils and their n-hexane extracts evaluated on seven *Fusarium* species [9]. In their experiment, the oils and extraction could decrease and prevent the isolates growth in medium [9]. [11] found ginger (*Zingiber officinale* Roscoe) essential oil could control the growth of *F. verticillioides* and decrease fumonisin production.

As well, the oils of *Thymus vulgaris*, *Melissa officinalis*, *Cinnamomum zeylanicum*, *Mentha piperita*, *Salvia officinalis* and *Coriandrum sativum* prevent the mycotoxin produce in wheat seeds [12]. Also, the herbal plant's oils cause to decrease the amount of toxin in the seeds [12]. Moreover, the oil and extracts of *Eucalyptus grandis*, *E. microcorys* and *E. robusta* evaluated against the *F. solani* [13]. According to the research, 0.5% of oils were able to inhibit the growth of fungus [13]. Besides, the antifungal activity of *Thymus vulgaris*, *Satureja hortensis*, *Anethum graveolens*, *Mentha sativa* and *Capsicum annum* essential oils tested against the *F. graminearum* [14]. Results of the experiment showed that the growth of fungal isolate and zearalenone production stopped by the oils [14]. Likewise, terpinen-4-ol, eugenol, carvone, 1,8-cineole, and thymol showed a high level of six *Fusarium* species growth prevention [15]. Genus *Satureja* is an annual, aromatic and medical plant belonging to the Lamiaceae family, which is spread in Mediterranean region especially in Turkey. The aim of this study was to evaluate the efficacy of six *Satureja* species oils on the colony growth of eight *Fusarium* species.

MATERIALS AND METHODS

Plant Materials. The aerial parts of *Satureja* species (*Satureja cilicica* P. H. Davis, *Satureja cuneifolia* Ten., *Satureja hortensis* L., *Satureja montana* L., *Satureja spicigera* (C. Koch) Boiss. and *Satureja thymbra* L.), were collected from the different region of Turkey during the period of August and September 2017. The plant materials were dried at room temperature 25 °C and dark side.

Isolation of essential oils. Air-dried plant materials were ground with a grinder and the essential oils were extracted by steam distillation boiling technique at 3-6 hours by using Clevenger-type apparatus (EM5000/CE), based on European Pharmacopoeia method (1997). The oils were separated from the water and stored in test tubes at 4°C. The oil yields of *S. cilicica*, *S. cuneifolia*, *S. hortensis*, *S. montana*, *S. spicigera* and *S. thymbra* were %1.20, 1.5, 2.3, 1.28, 1.56 and 1.17 (w/w, dry weight basis), respectively.

Fungal isolates and antifungal test. The plant pathogenic fungi; *Fusarium avenaceum* (Fr.) Sacc., *F. culmorum* (Wm. G. Sm.) Sacc., *F. graminearum* Schwabe, *F. sambucinum* Fuckel. and *F. solani* (Mart.) Sacc. were obtained from the culture collection of Mycology of Ataturk University (Faculty of Agronomy, Department of Plant Protection) and *F. equiseti* (Corda) Sacc., *F. oxysporum* Schldt. and *F. semitectum* Berk. & Ravenel. culture collection of Assoc. Prof. Dr. Berna TUNALI (Plant Protection Department of Agriculture Faculty of Ondokuz Mayıs University). First, fungi were plated on potato dextrose agar (PDA, Oxoid, CM0139) mixed with P-Aminobenzoic Acid 10 mgL⁻¹ (Sigma, A-9878). The cultures incubated at the darkness with 25°C in the incubator for seven days. The antifungal effects of essential oils evaluated by contact phase effects against mycelial growth of *Fusarium* species. Contact phase effect of essential oils tested by the poisoned food technique. From seven days old cultures, 5 mm agar blocks containing hyphal tips from the colony margins cut with the cork borer. And, the blocks transferred to PDA mixed with different concentrations of essential oils (0.5 µl (25 ppm), 1 µl (50 ppm), 2 µl (100 ppm), 5 µl (250 ppm), 10 µl (500 ppm), 20 µl (1000 ppm), and 30 µl (1500 ppm) in each 20 ml PDA medium) from different *Satureja* species. To mix the essential oils in the medium 200µl absolute ethanol (Sigma-Aldrich) in each 20 mL PDA was used. In controls, 200µl absolute ethanol mixed with 20 mL PDA without essential oil. The 9 cm plastic Petri dishes selected for the experiment. For each concentration, three replicate plates used. After each 24 hours, the colony diameter of treatments and control measured. The measuring of

colony diameter continued until the colony growth reaches to the sides of the petri dish in controls.

Inhibitory test. The mean growth of the pathogen determined by measuring the colony diameter in two directions. The growth of fungi isolates in oil treated Petri dishes compared with the control plates. To indicate the fungal hyphae growth, the initial fungal discs diameter (5 mm) subtracted from the final colony diameter of each treatment and control (Table 2). The Mycelial Growth Inhibitory (MGI) values were obtained using the formula “MGI (%) = [(c-t)/c] × 100”, where c and t represent mycelial growth diameter in control and treated Petri plates respectively.

Statically analysis. All experiments conducted twice to confirm the results. Because there was no significant difference between the two repeats for any of the treatments, the data of two experiments combined for final analyses. Results analyzed using a statistical analysis package SPSS 17.0 software at various significance levels with emphasis on one-way ANOVA and Duncan test. Statistically significant differences were considered at P<0.001 levels. The used experimental design was on a randomized basis with three Petri dishes for each isolate.

RESULTS AND DISCUSSION

The growth prevention of six essential oils against the eight *Fusarium* species represented in Table 2 and Figure 1. The oil components of all *Satureja* species; *S. cilicica*, *S. cuneifolia*, *S. hortensis*, *S. montana*, *S. spicigera* and *S. thymbra* represented in Table 1. The oils inhibitory efficacy tested against eight *Fusarium* species. As well, six oils minimum inhibitory concentration (MIC) by 25 ppm (0.5 µl), 50 ppm (1 µl), 100 ppm (2 µl) against the eight *Fusarium* species used. *Fusarium* species were able to grow in lower concentrations of

Satureja essential oils 0.5, 1, 2, and 5 µl (Figure 1). Among these oil concentrations, *Fusarium* species showed a bigger colony diameter in *S. cuneifolia* than other *Satureja* species oils. The oil of *S. cuneifolia* showed lesser growth inhibition against the *Fusarium* species. According to the GC-MS analysis of *Satureja* oils of species, the oil of *S. cuneifolia* has a lesser amount of Thymol (0.5%) than other species. Thymol amount of *S. cilicica*, *S. hortensis*, *S. montana*, *S. spicigera*, and *S. thymbra* were 22.7%, 43.4%, 15.4%, 35.1%, 33.8%, respectively (Table 1). According to the results of the experiment, the minimum inhibitory concentration (MIC) of *S. cuneifolia* was 10 µl (500 ppm) for different *Fusarium* species. But the MIC level of other *Satureja* oil showed between 2 and 5 µl (Figure 1).

Synthetic fungicides are widely used in the control of plant diseases. These chemicals have toxic residues in treated crops and cause to environmentally [1, 17]. In this respect, evaluation of the new control agents may be shown healthier fungal growth prevention compounds. So, there is an increasing interest in finding alternative fungicides with more safety and lesser risk to human health and the environment. The products of plant secondary metabolism such as essential oils and aromatic compounds are usually some natural antifungal agents have the potential to control the growth of the phytopathogenic pathogen on the crops [18]. Thus, *Satureja* species is more likely to be interested as an environmental friendly fungicide against the *Fusarium* species.

In the present study, *Satureja* species revealed that the major compounds were Carvacrol, *p*-Cymene, Thymol, and γ -Terpinene. Previous studies also showed that the essential oil isolated from different species of *Satureja* are described by a high content of Thymol and Carvacrol and [16] reported the chemical composition of the essential oil in aerial parts of *Satureja hortensis* L. collected from the Erzurum, Turkey. The main constituents of the essential oil are Thymol (72.18%), *p*-Cymene (9.74%), γ -Terpinene (7.61%) and Carvacrol

TABLE 1
***Satureja* species essential oils compounds and their percent**

Plant species	Essential oils compounds (%)	Literature
<i>S. cilicica</i>	Thymol (68.91%), <i>p</i> -Cymene (7.79%), Borneol (2.95%), Linalool (1.83%)	[16]
<i>S. cuneifolia</i>	γ -Amorphene (35.47%), Germacrene-D (17.63%), 6,9-Guaiadiene (11.67%), Spathulenol (3.56%)	[16]
<i>S. hortensis</i>	Thymol (72.18%), <i>p</i> -Cymene (9.74%), (γ)-Terpinene (7.61%), Carvacrol (7.29%)	[16]
<i>S. spicigera</i>	Carvacrol (90.25%), <i>p</i> -Cymene (4.12%), (γ)-Terpinene (2.58%), β -Bisabolene (1.38%)	[16]
<i>S. thymbra</i>	Carvacrol (57.13%), <i>p</i> -Cymene (21.95%), Thymol (7.98%), (γ)-Terpinene (4.40%)	[16]
<i>S. montana</i>	Carvacrol (71.31%), (γ)-Terpinene (11.87%), <i>p</i> -Cymene (6.06%), β -Caryophyllene (4.70%)	[16]

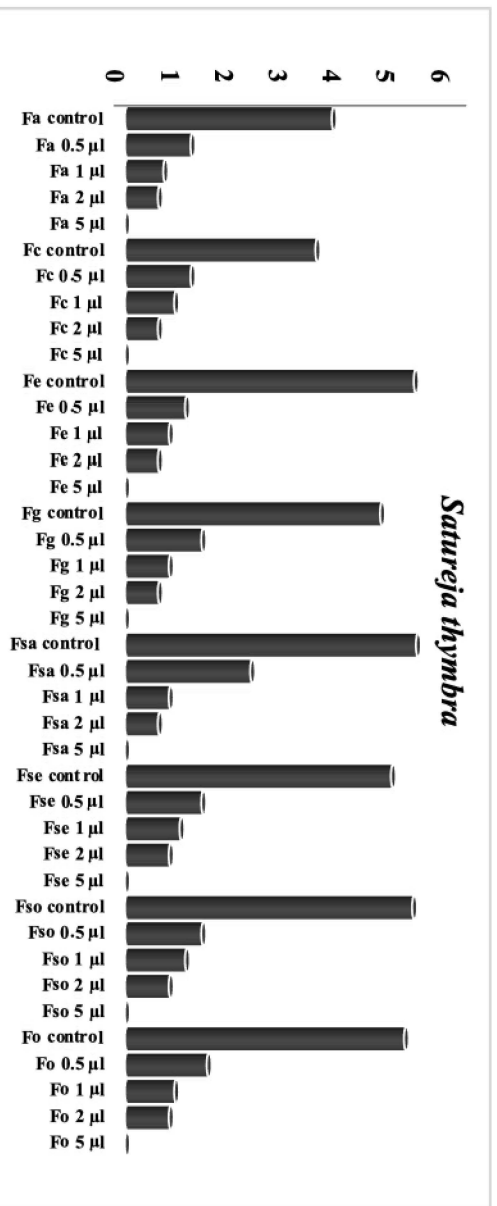
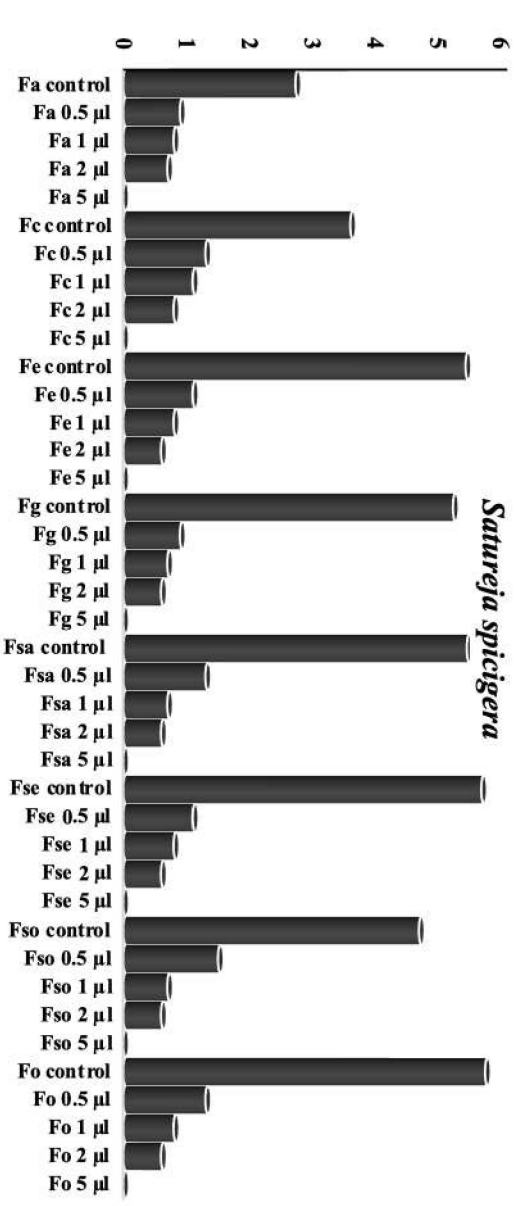
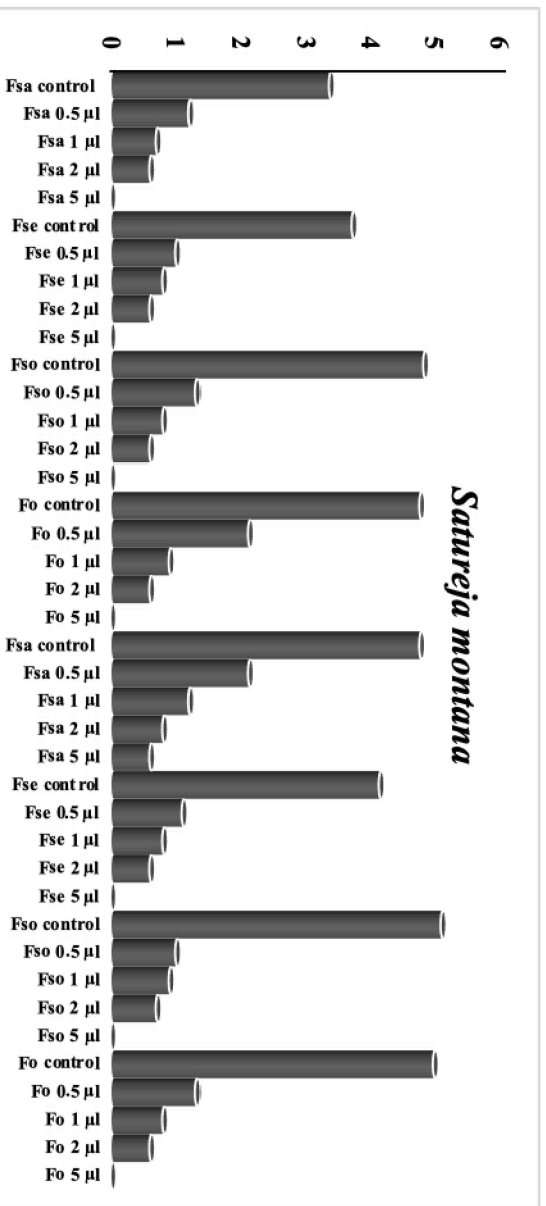


FIGURE 1
Antifungal activities of essential oils of *Satureja* species at three different concentration (1, 2, and 5 µl/20 mL medium).

TABLE 2
Antifungal activities of essential oils of *Satureja* species at three different concentration (10, 20, and 30 µl/20 mL medium) against eight species of *Fusarium* (T: treated; I%: Inhibitory Percentage)

Fungus		<i>S. cilicica</i>	<i>S. cuneifolia</i>	<i>S. hortensis</i>	<i>S. montana</i>	<i>S. spicigera</i>	<i>S. thymbra</i>	
10 µl/20 ml	<i>F. avenaceum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. culmorum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. equiseti</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. graminearum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. oxysporum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. sambunicum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. semitectum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. solani</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
20 µl/20 ml	<i>F. avenaceum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. culmorum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. equiseti</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. graminearum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. oxysporum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. sambunicum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. semitectum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. solani</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
30 µl/20 ml	<i>F. avenaceum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. culmorum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. equiseti</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. graminearum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. oxysporum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. sambunicum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. semitectum</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
	<i>F. solani</i>	T	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		I%	100%	100%	100%	100%	100%	100%
Control	<i>F. avenaceum</i>	T	3.47±0.13	2.67±0.29	3.60±0.46	3.35±0.20	2.73±0.30	3.80±0.32
	<i>F. culmorum</i>	T	3.5±0.23	3.70±0.12	3.2±0.23	3.70±0.12	3.60±0.21	3.5±0.13
	<i>F. equiseti</i>	T	4.81±0.07	5.54±0.03	5.35±0.18	4.81±0.07	5.43±0.05	5.31±0.09
	<i>F. graminearum</i>	T	5.35±0.11	5.16±0.11	5.18±0.03	4.75±0.14	5.23±0.02	4.69±0.17
	<i>F. oxysporum</i>	T	4.58±0.11	5.27±0.21	4.31±0.63	4.96±0.22	5.74±0.05	5.13±0.14
	<i>F. sambunicum</i>	T	4.98±0.45	5.23±0.43	5.33±0.19	4.75±0.13	5.45±0.08	5.35±0.21
	<i>F. semitectum</i>	T	4.70±0.20	4.96±0.33	5.14±0.41	4.12±0.20	5.68±0.03	4.89±0.12
	<i>F. solani</i>	T	5.04±0.08	4.70±0.22	3.95±0.05	5.08±0.04	4.69±0.01	5.28±0.04

(7.29%). The main components of *S. spicigera* (C. Koch) Boiss of Anatolia region in Turkey were tested and indicated Carvacrol (90.25%), *p*-Cymene (4.12%), (γ)-Terpinene (2.58%) and β -Bisabolene (1.38%) respectively [16]. Similar results were obtained in *S. spicigera* in Iran, in which the oil obtained from aerial parts of plants was character-

ized by high *p*-Cymene and Thymol contents [19]. The variation of essential oils compounds among the herbal plant species depends on the environmental conditions such as; climate, location, seasonal factors, and developmental stages [20]. Generally, volatile compounds of herbal plants essential oils have the potential to control the plant pathogen-

ic fungi and pests [1]. The antifungal activity of extracts and essential oils of *Satureja* species against the different fungal pathogens were previously reported [21, 22, 23].

In conclusion, based on findings of the present study, all isolates have the potential to prevent the growth of fungal isolates of *Fusarium*. The essential oils components diversity and their concentration are effective on their actions against the pathogens. In this context, it is worthwhile to consider the components of essential oils and their acts on the different plant pathogens. The isolation and evaluation of each compound on pathogens could be subject to evaluate.

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