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CHEMICAL COMPOSITION, ANTIOXIDANT, ANTIFUNGAL AND HERBICIDAL ACTIVITIES OF ESSENTIAL OILS FROM THREE THYMUS SPECIES

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Abstract: The current study aimed to research the chemical composition, antioxidant, herbicidal and antifungal effect three essential oils, as obtained from *Thymus canoviridis* Jalas., *Thymus eriocalyx* (Ronni.) Jalas. and *Thymus fallax* Fisch. et C.A. Mey. Antioxidant capacities of essential oils were determined by 1.1dipheny1-2-picrylhydrazyl (DPPH) method. The antifungal potential was tested *in-vitro* against *Fusarium equiseti* (Corda) Sacc., *Fusarium graminearum* Schwab., *Fusarium moniliforme* J. Sheld and *Fusarium oxysporum* Schlec. Bioherbicidal effect was studied *in-vivo* and *in-vitro* against weed seeds of *Amaranthus retroflexus* L., *Convolvulus arvensis* L. and *Chenopodium album* L. Essential oils were assayed in laboratory three concentrations (5, 10 and 20 µL/petri) and one (20 µL/pot) concentration in the greenhouse. The chemical composition of essential oils was analyzed by (GC) and (GC/MS). The major constituents were *p*-cymene, 1,8-cineole and γ-terpinene. As a result of the antioxidant study, it was determined that *Thymus* essential oils had remarkable antioxidant activity. On the other hand, oils decreased mycelial growth of pathogens at different rates due to increasing doses and inhibited 9.33-100% *in-vitro* conditions. In the bioherbicidal study assays showed that tested essential oils had inhibitory effects on the seed germination and seedling growth of weeds. The study concludes that *Thymus* essential oils might has the potential use as bioherbicide and biofungicide can constitute an alternative process of weed control and fungus.

Keywords: Antioxidant effect, Antifungal effect, Bioherbicidal effect, Essential oil, *Thymus*

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1. Introduction

Turkey due to take place at the intersection of different climates, plant species and is a very rich country in terms of diversity. It is one of the leading countries in the world market in the export of tea plants and spices, and Lamiaceae (Labiatae) family takes the first place among the plant species traded (Kocabas and Karaman 2001; Özkan, 2007). In addition, the Lamiaceae family includes medicinal and aromatic herbs with powerful antimicrobial and antioxidant properties (Goudjil et al., 2020). Lamiaceae is a plant family represented by 236 genera and 7280 species and is distributed worldwide, especially in the temperate zone. In Turkey Flora Lamiaceae family of 45 genera, 565 species represented by and a total of 735 taxa (Davis, 1970). The biological and pharmacological plant species of this family have been known for many years. The phytotherapy feature of these plants is mostly due to the essential oils they contain (Bozin et al., 2006). The essential oil yield of the Lamiaceae family is very high, and the important known species are *Thymbra*, *Thymus*, *Origanum*, *Satureja*, *Mentha*, *Teucrium*, *Ballota*, *Stachys*, *Salvia*, *Ajuga*, *Prunella*, *Melissa*, *Lamium*, *Sideritis* and *Marrubium*. *Thymus* is one of the 8 largest genera of Lamiaceae family in terms of number of species. The species belonging to the genus *Thymus* are known as "thyme" or "stone thyme" in our country (Tümen et al., 1998; Bagci et al., 2005). The leaf and flowering parts of the *Thymus* plant with high essential oil content are mostly used as herbal tea, tonic and flavoring (Zargari, 1990; Amin, 2005). In addition, *Thymus* essential oils are also used in medicine and pharmacology because of their antiseptic, antibacterial, antifungal, antispasmodic, antitussive, expectorant, analgesic properties (Cosentino et al., 1999; Hedlili et al., 2002; Kabouche et al., 2005; Rasooli et al., 2006). *Thymus* have been found to be approximately 270 terpenes and one or more of them have been reported to be dominant. Especially thymol, carvacrol, linalool, p-cymene, geraniol, borneol are the most important terpenes. *Thymus* species is the most important source of monoterpenoid phenols in the plant kingdom (Stahl-Biskup, 2002). Therefore, considering that the main components of essential oils of vegetable origin are terpenes, it can be said that they have a potential to be used in weed control and fungal diseases (Kordali et al., 2009; Üstüner et al., 2018). Studies have shown that

essential oils obtained from plants in our country and in the world have the potential to prevent the growth of plant pathogenic fungi and bacteria. (Zambonelli et al., 1996; Bianchi et al., 1997; Wilson et al., 1997; Türküsay and Onoğur 1998; Ristic et al., 2000; Walter et al., 2001; Abou-Jawdah, 2002; Bouchra et al., 2003; Daferera et al., 2003; Bowers and Locke, 2004; Cakir et al., 2005; Soylu et al., 2005a; Soylu et al., 2005b; Soylu et al., 2006; Lee et al., 2007; Kotan et al., 2010). Some essential oils create an isolated area where the plants can grow easily by preventing the germination and growth of weed seeds (allelopathic effect). Effects of essential oils on germination and plant growth; They damage their intracellular structures, inhibit cell growth and development, slow down photosynthesis and respiration, and stop germination, seedling and plant growth by acting on oxygen uptake (Abrahim et al., 2000). The use of essential oils obtained from plants in the fight against plant diseases has started to attract the attention of researchers today. It is thought that essential oils penetrate through the cell wall of fungi and disrupt the structure of the cell wall, stop fungus growth and conidia production, and cause deformations in hyphae and create cytoplasmic currents (Chang et al., 2001; Ultee et al., 2002; Soylu et al., 2006).

In the present study, the purpose were to evaluate the antioxidant, herbicidal and fungicidal effect of the essential oil isolated from *Thymus canoviridis*, *T. eriocalyx* and *T. fallax* on some fungi and weeds. In addition, it is thought that this study conducted with different *Thymus* species will provide a source for antifungal and herbicidal studies and contribute to the literature.

2. Materials and Methods

2.1. Plant Material and Essential Oil Extraction

Weed seeds of *A. retroflexus*, *C. arvensis* and *C. album* were collected from Erzurum region of Turkey between June-September of 2015-2016. *Thymus canoviridis* Jalas. from Erzurum-Kırkdegirmenler (2122 m), *Thymus eriocalyx* (Ronniger) Jalas. from Igdir-Tuzluca (2028 m) and *Thymus fallax* Fisch. et C.A. Mey. from Igdır-Korhan (1899 m) were collected at flowering stage from the different localities of Turkey between June 2014 and August 2015. Plant herbariums have been deposited in the Department of Plant Protection, herbarium at Ataturk University in Erzurum, Turkey. The tested plants were identified by Prof. Dr. Vladimir I. DOROFEYEV Komarov Botanical Institute (Herbarium), Russian Academy of Sciences, St. Petersburg-Rusya; Prof. Dr. Ali KANDEMİR Erzincan Binali Yildirim University, Faculty of Arts and Sciences, Department of Biology Erzincan-Turkey; Prof. Dr. Tuncay DİRMENCİ Balıkesir University Necatibey, Faculty of Educatıon, Department of Biology Education, Balıkesir-Turkey and Prof. Dr. Meryem ŞENGÜL KÖSEOĞLU Atatürk University, Faculty of Science, Department of Biology, Erzurum-Turkey. Aerial parts of the plants were dried for 8 days in the shade and ground in a grinder (nearly 0.200-0.500 mm). The dried plant samples (500 g) were subjected to hydro distillation for 3-4 hours using a Clevenger-type apparatus. The oils were stored at 4 ^{o}C until used for antioxidant activity, herbicide and fungicide bioassays.

2.1.1. Antioxidant activity

DPPH free radical scavenging activity

The free radical scavenging activity of essential oils was determined using the method proposed by Doshi et al., (2015). Briefly, 50 µl of diluted (1:10 v/v) essential oil in methanol were added to 950 µl of the DPPH methanolic solution (60 µM, freshly prepared). The mixture was vortexed and maintained at room temperature for 30 min in the dark then the absorbance was measured at 517 nm against the corresponding blank. A mixture consisting of 50 µl methanol and 950 µl of DPPH solution was used as control. Each determination was carried out in triplicate, and results of the radical-scavenging activity were expressed as microgram Trolox equivalent per gram of essential oils (µg TE/g EO). The inhibition % values were calculated according to the Equation 1 shown below.

$$\text{Inhibition } \% = [(A_{DPPH}-A_{E.O})/A_{DPPH})*100] \quad (1)$$

2.2. Fungal Isolates and Antifungal Test

The plant pathogenic fungi; *Fusarium equiseti*, *F. graminearum*, *F. moniliforme* and *F. oxysporum* were obtained from the collection Mycology of Prof. Dr. Berna TUNALI (Ondokuz Mayıs University, Department of Plant Protection. Primarily, fungi were plated on potato dextrose agar (PDA, Oxoid, CM0139) mixed with P-aminobenzoic acid 10 mg/L (Sigma, A-9878). The cultures incubated at the darkness with 25 ± 2°C in the incubator for 7-10 days. The antifungal effects of essential oils evaluated by contact phase effects against mycelial growth of *Fusarium equiseti, F. graminearum, F. moniliforme* and *F. oxysporum*. From 7-10 days old cultures, 5 mm agar blocks containing hyphal tips from the colony margins cut with the fungal borer. And, the blocks transferred to PDA mixed with different concentrations of essential oils (5 µL (250 ppm), 10 µL (500 ppm) and 20 µL (1000 ppm), in each 20 mL PDA medium) from *Thymus canoviridis*, *T. eriocalyx* and *T. fallax*. To mix the essential oils in the medium 100 µL absolute ethanol (Sigma-Aldrich) in each 20 mL PDA was used. In controls, 100 µL absolute ethanol mixed with 20 mL PDA without essential oil. The 9x1.5 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.

2.3. Fungal 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. Mycelia Growth Inhibitory (MGI) values were obtained using the equation 2:

$$MGI\ (\%) = [(C-T)/C] \times 100 \quad (2)$$

where C and T represent mycelia growth diameter in control and treated Petri plates respectively.

2.4. *In-Vitro* Herbicidal Activity Experiments

Weed seeds were sterilizied with sodium hypochloride (15 %) for 10 minutes and then they were washed 3-7 times with sterile distilled water. The sterilized seeds (n=50) of *A. retroflexus, C. arvensis* and *C. album* were placed into petri dishes (9 cm diameter) with 2 layers of filter paper (Whatman No.1) (Kordali et al., 2007; Kordali et al., 2008; Üstüner et al., 2018). To determine the contact herbicidal effects of oils, the oils were dissolved in ethanol–steril water solution (10%, v/v) and adjusted into 5, 10 and 20 µL/mL final concentrations. The prepared solutions were transferred into petri dishes (8 mL Petri^{-1} dishes). The petri dishes were immediately closed and covered tightly with parafilm and petri dishes were incubated kept at 25±2 ºC in a growth room providing with 12 h of fluorescent light and humidity of 80%. At the end of 7-10 days, the number of germinated seeds percentages (%) was determined and their root and shoot lengths (mm) were measured by using ruler and calculated with the following Equation 3 and 4. Petri dishes containing 8 mL Ethanol–water solution (10%, v/v) was used as the negative control. In addition, Beststok 330 EC (330 g/L Pendimethalin) (5, 10 and 20 µL/mL) was used as positive control. All experiments were prepared in a completely randomized design with three replications.

$$Germination\ \% = \frac{Number\ of\ Germinated\ Seeds}{Number\ of\ Total\ Seeds} \text{X } 100 \quad (3)$$

$$Inhibition\ \% = \frac{C-T}{C} \text{X } 100 \quad (4)$$

C: % germination and seedling (root and radicle) length in control

T: % germination and seedling (root and radicle) length in control in treatment with essential oil

2.5. *In-Vivo* Herbicidal Activity Experiments

Post-emergence experiment were applied study the effect of *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils on 1-2-week-old *A. retroflexus*, *C. arvensis* and *C. album* plants of the weedy species under controlled greenhouse conditions. Firstly, pots (14 x 17 cm) were filled with 300 g soil (soil/potting soil 3:1, w/w) sterilized in the autoclave. Then, 50 seeds sterilized of the weeds were planted into the pots and kept under photoperiod conditions (20 ± 3 ºC, 12 h light and 12 h dark photoperiod) and relative humidity (60 ± 3%) in a growth room to allow germination and growth of the plant samples. The pots were irrigated with tap water when necessary. The number of germinated seeds of the respective weed samples in each pot was considered. Then, the oils was emulsified in 10 mL of Ethanol-water solution (10%, v/v) and was used for control treatment. The latest concentration of applications was 20 µL/mL. Prepared emulsions were sprayed equivalently with a glass spray bottle on the surface of all plants in each pot in the stage of 3-4 real leaves. The plants sprayed on Best Amin 500 SL (2,4-D Amin 500 g/L) (20 µL/mL for each pot) were used as a positive control. At the end of 24 and 48 hours the dead plants was recorded by counting. The experiments were performed in a completely randomized plan with four replications including controls. The phytotoxicity of the treatments was expressed as percent mean of dead plants (Kordali et al., 2008). The percentage of effect was calculated following the Equation 5:

$$Percentage\ of\ effect\ \% = \frac{\text{Dead Leaves}}{\text{Number of Total Leaves}} \text{X } 100 \quad (5)$$

2.6. GC-MS Analysis

The chemical constituents of the essential oils of *T. canoviridis*, *T. eriocalyx* and *T. fallax* were decided by Gas Chromatography-Mass Spectrometry (GC-MS). DB-1 fused silica non-polar capillary column (30 m × 0.25 mm I.D., film density 0.25 µm) was used for the analysis. Helium was used as carrier gas with 1.4 mL/min flux ratio. The ion source, injector and MS transfer route temperatures were 200, 220 and 290°C, respectively. The injection volume was 0.2 µL with a separate rate of 20:1. Ionization energy of EI–MS evaluations were taken at 70 eV. Mass area was from m/z 28 to 650 amu. Scan time was 0.5 s with 0.1 s interscan retardations. The oven heat was keep at 60°C for 5 min, then increased up to 240°C with 4°C/min rising and kept at this temperature for 10 min. Recognition of constituent of the essential oil was based on GC retention index and computer matching with the libraries of Wiley, NIST-2008 and TRLIB, as well as by comparison of the disintegration versions of the mass spectra. Quantitative data of the essential oils was acquired from the FID area rates. (Usanmaz Bozhüyük, 2020).

2.7. Statistical Analysis of Data

Study results was take estimating the statistical significance of differing treatments mean values against negative and positive control treatment using ANOVA and Duncan test at levels P<0.05 (Genç and Soysal, 2018). All assays were done SPSS program (version 17.0, SPPS Inc., Chicago, IL, USA) software package.

3. Results and Discussion

3.1. Yield and Chemical Composition of the *Thymus canoviridis*, *Thymus eriocalyx* and *Thymus fallax* Essential Oils

The essential oils obtained by hydrodistillation of dried *T. canoviridis*, *T. eriocalyx* and *T. fallax* were flowers and leaves yellow color and emitted a strong smell. The essential oil yields of *T. canoviridis*, *T. eriocalyx* and *T. fallax* were 0.70, 0.60 and 1.5% (w/v, dry weight basis), respectively. The essential oils compounds identified by GC–MS method are listed in Table 1. Based on GC-MS results, identified 100, 99.60 and 97.74% of the compounds present in the essential oils *T. canoviridis*, *T. eriocalyx* and *T. fallax*, respectively. The *Thymus* essential oils were characterized by the predominance of the monoterpene hydrocarbons class, among which *p*-cymene (23.06-32.97%), 1,8-cineole (11.34-25.34%) and γ-terpinene (14.40-24.79%) were the present. This class was followed by oxygenated monoterpenes and sesquiterpene hydrocarbons, while oxygenated sesquiterpenes were found in minor quantities.

Table 1. Chemical constituents (%) of the essential oils of *Thymus* species

No	Chemical constituent	*Thymus canoviridis*	*Thymus eriocalyx*	*Thymus fallax*
1	α-thujene	0.66	0.71	0.76
2	α-pinene	1.00	5.90	1.69
3	α-fenchene	0.49	0.67	0.59
4	Myrcene	1.05	1.93	0.80
5	α-terpinene	4.77	1.49	2.23
6	*p*-cymene	23.06	32.97	24.23
7	1,8-cineole	11.34	25.34	22.64
8	γ-terpinene	24.79	19.15	14.40
9	Camphor	-	1.07	1.36
10	Thymol	-	-	-
11	Thymol methyl ether	0.84	0.99	tr
12	Carvacrol methyl ether	1.97	0.80	1.55
13	Borneol acetate	0.58	0.52	1.55
14	α-terpineol acetate	17.00	2.62	3.27
15	β-bourbonene	-	-	1.03
16	β-caryophyllene	5.69	1.86	12.70
17	β-bisabolene	6.13	1.62	4.91
18	Methyl-α ionone	-	0.72	0.93
19	γ-cadinene	0.63	0.49	1.37
20	Caryophyllene oxide	-	0.75	1.73
Monoterpene hydrocarbons (%)		55.82	62.82	44.70
Oxygenated monoterpenes (%)		31.73	31.34	30.37
Sesquiterpene hydrocarbons (%)		12.45	4.69	20.94
Oxygenated sesquiterpenes (%)		-	0.75	1.73
Total (%)		100	99.60	97.74

Identification method: GC, identification based on the tR of authentic compounds on a SGE-BPX5 capillary column; MS, tentative identification based on computer matching of the mass spectra with those listed in the Wiley7N and TRLIB libraries and published data (Adams 2007). tr: Traces (< 0.10%).

Concerning the previously reported content of *Thymus canoviridis* (Azaz and Celen 2012), *Thymus eriocalyx* (Amiri, 2012) and *Thymus fallax* (Kucukbay et al., 2014) essential oils, it is point out that there were important quantitative differences suggesting that the environmental factors and genotypes strongly impact its chemical composition. For example, *p*-cymene (32.97%) was found to be the major constituent of *T. eriocalyx* essential oil in our research (Table 1). On the contrary, *p*-cymene component was found are very low concentration (4.1%), while the major component thymol was determined at 42.6% (Amiri 2012). *Thymus canoviridis* is very rich in thymol (Azaz and Celen 2012), *T. fallax* in thymol and *o*-cymene. Studies show that thymol is the main compound in almost all samples. It is accepted that the terpenes, thymol, p-cymene, 1.8-cineole and carvacrol are the major volatile components of *Thymus* species. Some studies have reported that thyme essential oil has high levels of phenolic substances, p-cymene, and γ-terpinene (Kabouche et al., 2005). The comparison between these results and the results of other reports showed differences, possibly due to plant types or areas, as well as harvest time, altitude, temperature.

3.2. Antioxidant Activity of *Thymus canoviridis, Thymus eriocalyx* and *Thymus fallax* Essential Oils

Antioxidant activity of *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils was measured by DPPH scavenging assay, while BHA, BHT, Trolox and α-Tokoferol was used as controls. Compared to standard BHA, BHT, Trolox and α-Tokoferol; it was determined that as the doses of essential oils increased, the inhibition values (IC_{50}) also increased (Table 2).

Table 2. Antioxidant activities of the *Thymus* essential oils tested as compared to standard BHA, BHT, Trolox and α-Tokoferol

Essential Oils and Standards	% Inhibition Values (IC_{50}) Concentrations		
	0.5 µg/mL	1 µg/mL	1.5 µg/mL
BHA	56.437	61.468	78.719
BHT	61.552	78.899	79.661
Trolox	91.534	93.578	94.350
α-Tokoferol	76.014	73.211	77.589
T. canoviridis	57.672	76.880	86.629
T. eriocalyx	58.201	79.266	85.876
T. fallax	77.072	79.633	83.428

The scavenging effects on the DPPH radical expressed as IC_{50} value was the highest for concentration of 1.5 µg/mL in *T. canoviridis* essential oil (86.629%) followed by *T. eriocalyx* (85.876%) and *T. fallax* essential oils (83.428%), showing a radical scavenging activity highest important than that the standarts BHA (IC_{50} 78.719%), BHT (IC_{50} 79.661%) and α-Tokoferol (77.589%). But, *Thymus* essential oils determination a antioxidative activity clearly less important than that the standart Trolox (IC_{50} 94.350%) (Table 2).

In the study conducted with *Thymus capitatus* essential oil and its 2 major components; antioxidant properties, determined by 2,2-diphenylpicrylhydrazyl assay, revealed that IC_{50} values were 119.403 and 105 µg/mL for oil, thymol and carvacrol respectively and especially carvacrol active compounds with strong antioxidativity (Džamić et al. 2015). Besides, thymol and carvacrol, the main component of *Thymus* and other thyme essential oils, are potent antioxidants and their use could be beneficial in the antioxidative conservation (Faleiro *et al*. 2005; Hazzit et al. 2006). It is hard to property the antioxidant activity to one or few active compounds of total essential oils, since both minor and major constituents could perform significant addition to the essential oil activity (Wang et al., 2008). Because, in our study, the high proportion of *p*-cymene and 1,8-cineole compounds in *Thymus* essential oils may suggest that they increase antioxidant activity. In general, it can be said that plant essential oils and especially *Thymus* essential oils have antioxidant capacity.

Table 3. Antifungal activities of *Thymus* essential oils against *F. equiseti*, *F. graminearum*, *F. moniliforme* and *F.oxysporum* fungi

Essential oils	5 µL/petri Growth Inhibition (mm)(%)	10 µL/petri Growth Inhibition (mm) (%)	20 µL/petri Growth Inhibition (mm) (%)	P. Control (10 µL/petri) Growth Inhibition (mm) (%)	N. Control Growth (mm)
			Fusarium equiseti		
T. canoviridis	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	33.7 ± 0.40 b 36	52.7 ± 0.92 c
T. eriocalyx	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	33.7 ± 0.40 b 36	52.7 ± 0.92 c
T. fallax	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	33.7 ± 0.40 b 36	52.7 ± 0.92 c
			Fusarium graminearum		
T. canoviridis	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	1.71 ± 0.10 ab 96.5	49.2 ± 1.0 g
T. eriocalyx	9.33 ± 1.01 d 81	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	1.71 ± 0.10 ab 96.5	49.2 ± 1.0 g
T. fallax	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	1.71 ± 0.10 ab 96.5	49.2 ± 1.0 g
			Fusarium moniliforme		
T. canoviridis	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	17.7 ± 1.48 d 68.7	56.7 ± 1.50 f
T. eriocalyx	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	17.7 ± 1.48 d 68.7	56.7 ± 1.50 f
T. fallax	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	17.7 ± 1.48 d 68.7	56.7 ± 1.50 f
			Fusarium oxysporum		
T. canoviridis	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	41.5 ± 0.60 c
T. eriocalyx	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	41.5 ± 0.60 c
T. fallax	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	41.5 ± 0.60 c

*The differences between the averages containing different letters in each column are statistically significant. (P≤0.05).

3.3. Antifungal Activity of *Thymus canoviridis, Thymus eriocalyx and Thymus fallax* Essential Oils

The effectiveness of *T. canoviridis, T. eriocalyx* and *T. fallax* essential oils on plant pathogens *F. equiseti*, *F. graminearum*, *F. moniliforme* and *F. oxysporum* fungi was investigated *in-vitro.*

Antifungal activity in bioassay, our results showed that this essential oils strong fungicidal effect potential at different concentrations (5, 10 and 20 µL/petri). A rise in essential oils concentration increased inhibition of pathogens after 7 days of incubation. Compared to controls, 5, 10, and 20 µL/petri concentrations essential oils of *T. canoviridis, T. eriocalyx and T. fallax* from inhibited *F. equiseti* mycelium growth by 100%. It has been observed that positive control has 36% inhibition rate in the mycelium growth of the pathogen *F. equiseti*, but its effect rate is lower than *Thymus* essential oils. In contrast to the *F. equiseti* pathogen, 5 µL/petri concentration essential oil of *T. eriocalyx* showed lower than antifungal effects against the *F. graminearum* pathogen. But, *T. eriocalyx* (10 and 20 µL/petri), *T. canoviridis* and *T. fallax* (5, 10 and 20 µL/petri) of concentration inhibited the pathogen by 100%. Looking at the table, the fungicidal effects of the *Thymus* essential oils on *F. graminearum* pathogen mycelium growths are mostly higher than commercial fungicide, Captan 500 FL (10 µL/petri) (Table 3). *Fusarium moniliforme* and *Fusarium oxysporum* mycelium growth was completely inhibited at 5, 10 and 20 µL/petri concentration by 100%. Similarly, in the positive control the *F. moniliforme* drastically inhibited (68.7 %) the mycelium growth, while mycelium growth was completely (100%) inhibited at *F. oxysporum* (Table 3). The highest fungicidal effect was observed at a concentration of 20 µL/petri in all applied concentrations and *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils pathogen by 100% inhibited. When the antifungal activities of essential oils were compared among themselves, the least micellar growth diameters was observed in *T. eriocalyx* essential oil at concentrations of 5 µL/petri. Biological activity of *Thymus* essential oils and extracts on different microorganism, pathogens, weed and test plants have also been reported in other studies (Rasooli and Mirmostafa 2002; Mossa, 2019). In a study with *Satureja* one of the important thyme species, the antifungal effects of essential oils from 10, 20 and 30 µL/petri concentration *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.) tested for their antifungal effect against eight *Fusarium* species (*Fusarium avenaceaum, F. culmorum, F. equiseti, F. graminearum, F. oxysporum, F. sambucinum, F. semitectum* and *F. solani*). The higher concentrations of oil (10, 20, and 30 µl/20 ml) prevents the colony growth of *Fusarium* in the medium and *Satureja* oils showed effective control of the plant pathogenic fungi growth in the medium with 100% inhibitory rates (Usanmaz bozhuyuk et al., 2019). In our study; it was determined that 5, 10 and 20 µL/petri doses of *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils formed 100% inhibition zone in *F. equiseti*, *F. graminearum* and *F. oxysporum* fungi. The studies seem to be similar to each other. The results further reveal the toxicity of *Thymus* essential oils against fungicides.

3.4. Herbicidal activity of *Thymus canoviridis, Thymus eriocalyx and Thymus fallax Essential Oils*

The seed germination, root and shoot growth of *A. retroflexus*, *C. arvensis* and *C. album* plants were strong touched by *T. canoviridis, T. eriocalyx* and *T. fallax* essential oils. The effects differed depending on the concentration of essential oils and the test weed seed, and the herbicidal effect increased with the increased concentration. The increase in *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils concentration decreased the seeds germination and seedlings root and shoot growth of *A. retroflexus*, *C. arvensis* and *C. album* than negative and positive control. Compared to negative and positive control, the application of essential oils at 5, 10 and 20 µL/petri completely (100%) inhibited seed germination and seedlings root and shoot growth of two weeds *A. retroflexus* and *C. album* (Table 4).

Thymus canoviridis at 5 µL/petri concentration decreased the germination of *C. arvensis* seeds by 80.2 than control respectively, while germination was completely (100 %) stopped at 10 and 20 µL/petri concentration (Table 4). At 5 µl/petri concentration, the root and shoot growth was inhibited strongly by 89.6 % and 85.3 in order of, whereas, the root growth was completely inhibited at 10 and 20 µL/petri concentration.

In the seeds *C. abum*; *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils and positive control at 5, 10 and 20 µL/petri concentration inhibited the seed germination, root and shoot growth were completely (100%) inhibited (Table 4). In a study, the 5 and 10 µL/petri dose of *T. fallax* essential oil completely inhibited germination, root and shoot growth of *Avena sterilis*, *Cucumis sativus* and *Lactuca sativa* seeds (Yilar et al., 2013). The essential oils of *T. fallax*, *O. vulgare* and *M. dumetorum* completely inhibited the seed germination and seedling growth of *A. theophrasti* at a 15 µL dosage (Onaran et al. 2014). They reported that thymol and carvacrol, the main compounds of thyme, completely prevented the germination of

Amaranthus retroflexus, *Chenopodium album* and *Rumex crispus* seeds (Kordali et al., 2008). In this study, herbicidal effects of *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils have been demonstrated. The *Thymus* essential oils decreased the germination, root and shoot growth of *A. retroflexus, C. arvensis* and *C. album* at higher doses than negative and positive control.

3.5. Herbicidal activity of *Thymus canoviridis, Thymus eriocalyx and Thymus fallax* Essential Oils Under Greenhouse Conditions

In the pot studies in which essential oils are practical in post-emergent conditions are limited. Therefore, *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils was sprayed on 1-2-week-old weed plants.

Table 4. Herbicidal effects of *Thymus* essential oils on germination, root and shoot growth on *A. retroflexus*, *C. arvensis* and *C. album* seeds.

Essential Oils	C	Germination (%)	Germination Inhibation (%)	Root Growth (mm)	Root Growth Inhibation (%)	Shoot Growth (mm)	Shoot Growth Inhibation (%)
				Amaranthus retroflexus			
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. canoviridis	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. eriocalyx	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. fallax	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	94.0±0.00 c	2.69	1.76±0.54 c	64.6	3.60±0.85 a	80.0
P. Control	10	90.0±0.00 b	6.83	1.38±0.43 b	72.2	2.74±0.76 a	84.8
	20	88.0±0.00 b	8.90	1.18±0.38 b	76.3	1.66±0.63 a	90.7
N. Control	-	96.6±1.44 d	-	4.98±2.65 d	-	18.04±6.82 b	-
				Convolvulus arvensis			
	5	15.3±0.54 b	80.20	2.20±3.26 a	89.6	6.25±8.74 b	85.3
T. canoviridis	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. eriocalyx	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. fallax	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	56.0±0.00 e	27.55	1.64±0.94 a	91.57	5.76±2.82 b	86.52
P. Control	10	54.0±0.00 d	30.14	1.50±0.99 a	92.29	4.38±2.42 ab	89.74
	20	52.0±0.00 c	32.72	1.22±0.82 a	93.73	3.82±2.22 ab	91.06
N. Control	-	77.3±2.88 f	-	19.46±9.44 b	-	42.73±16.9 c	-
				Chenopodium album			
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. canoviridis	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. eriocalyx	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. fallax	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
P. Control	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
N. Control	-	95.3±0.54 b	-	18.1±4.63 b	-	20.7±4.01 b	-

Differences between the averages containing different letters in each column are statistically significant (P≤0.05), C= concentration.

Table 5. Phytotoxic effects of *Thymus canoviridis*, *Thymus eriocalyx* and *Thymus fallax* essential oils against weed seedlings growth in greenhouse conditions

Applications	Phytotoxic effect (% Mean death)	
	24. hour	48. hour
	Amaranthus retroflexus L.	
Essential oils		
T. canoviridis	24.1 ± 2.20 c	70.0 ± 3.81 c
T. eriocalyx	35.8 ± 0.83 d	60.0 ± 1.44 b
T. fallax	34.1 ± 3.00 d	57.5 ± 1.44 b
Controls		
Positive Control	9.16 ± 0.83 b	90.8 ± 0.83 d
Negative Control	0.0±0.0 a	0.0±0.0 a
	Convolvulus arvensis L.	
	Essential oils	
T. canoviridis	5.00 ± 1.44 b	22.5 ± 1.44 b
T. eriocalyx	20.8 ± 0.83 d	29.1 ± 0.83 c
T. fallax	30.0 ± 1.44 e	46.6 ± 0.83 d
Controls		
Positive Control	12.5 ± 1.44 c	87.5 ± 1.44 e
Negative Control	0.0±0.0 a	0.0±0.0 a
Chenopodium album L.		
Essential oils		
T. canoviridis	25.8 ± 0.83 c	67.5 ± 3.81 cd
T. eriocalyx	25.0 ± 2.88 c	64.1 ± 3.0 c
T. fallax	30.8 ± 1.66 d	55.0 ± 1.44 b
Controls		
Positive Control	12.5 ± 1.44 b	87.5 ± 1.44 e
Negative Control	3.33 ± 1.66 a	6.66 ± 0.83 a

Differences between the averages containing different letters in each column are statistically significant. (P≤0.05).

Thymus essential oils and commercial herbicide, Best Amin 500 SL (500 g/L 2,4 Amin salt) also tested for their phytotoxic effects against 3-4 leaf stage seedlings of pots on the weeds at greenhouse condition and the results showed that the oils caused mortality rate of 5.0-35.8% at 24 h and 22.5-70.0% at 48 h after the treatment as compared with negative and positive controls (Table 3). In the laboratuvary parallel with petri experiments, *Thymus* essential oils exhibited similar her effects against *A. retroflexus*, *C. arvensis* and *C. album* in the pots greenhouse conditions. In the plants phytotoxic effects of essential oils increase depending on the exposure time. In particular, the phytotoxic effect of essential oils was higher than the commercial herbicide at 24 hours. Furthermore, between *Thymus* essential oils, the most phytotoxic effect with 35.8 % mean death in 24 hour wasfound to be *T. eriocalyx* oil against *A. retroflexus.* Again, the *A. retroflexus* the highest phytotoxic effect was determined in *T. canoviridis* essential oil with 70.0% seedlings death in 48 hour. In negative control, while there was no death in *A. retroflexus* and *C. arvensis* seedlings; in *C. album* seedlings, 3.33% death at 24th hour and 6.66% at 48th hour were determined. In addition; commercial herbicide Best Amin 500 SL (500 g/L 2,4 Amin salt) showed the highest phytotoxic effect at 24 h with 12.5% death rate in *C. arvensis* and *C. album* seedlings. The highest phytotoxic effect was detected at 48 th hour with 90.8% mortality in *A. retroflexus* seedlings. In a study conducted under greenhouse conditions; *T. capitata* essential oil was sprayed on *Portulaca oleracea*, *Avena fatua* and *Echinochloa crus-galli* seedlings and showed bioherbicidal effects at different rates depending on the hours (Verdeguer et al., 2020). In another study; *Nepeta meyeri* essential oil it showed a phytotoxic effect between 28.7-42.7% after 24 hour in *Amaranthus retroflexus*, *Chenopodium album*, *Cirsium arvense* and *Sinapsis arvensis* seedlings; between 53.3-64.0% at the end of 48 hour (Kordali et al., 2015). At the same time, it can be said that this may be related to the plant leaf structure and the cuticle layer. In the pot work done in the greenhouse; peppermint and caraway essential oils in *Chenopodium album* and *Avena fatua* seedlings with herbicidal effects were determined (Synowiec and Drozdek, 2016). In the study conducted with twenty-five different essential oils; the essential oils were applied to shoots of common lambsquarters, common ragweed, and johnsongrass in the greenhouse; shoot death occurred within 1 h to 1 d after application. Cinnamon essential oil was found to have high herbicide activity (Tworkoski, 2002). Generally, it can be said that the phytotoxic effect increases as the hour and day increase in seedling death.

4. Conclusion

Thymus canoviridis, *T. eriocalyx* and *T. fallax* essential oils showed antifungal effects on plant pathogens *F. equiseti*, *F. graminearum*, *F. moniliforme* and *F. oxysporum* pathogens and herbicidal effects on *A. retroflexus*, *C. arvensis* and *C. album* weeds in conditions in pre-emerge (*in-vitro*) and post-emergence (*in-vivo*). According to the results of the present study; *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils have strong antifungal, antioxidant and bioherbicidal effects. Therefore, essential oils are a potential source for the development of new bioherbicides and biopesticides. More studies are needed with different doses and volumes of essential oils. However, studies should be carried out not only in petri dishes and greenhouses, but also in the field stage. However, with more trials compared to synthetic pesticides, further studies need to be addressed to show essential oils form of effect, cost-effectiveness, safety and phytotoxicity against the plants as potential pesticides and herbicides.

Author Contributions

Concept: A.U.B. (50%) and Ş.K. (50%), Design: A.U.B. (50%) and Ş.K. (50%), Supervision: A.U.B. (50%) and Ş.K. (50%), Data collection and/or processing: A.U.B. (50%) and Ş.K. (50%)„ Data analysis and/or interpretation: A.U.B. (50%) and Ş.K. (50%), Literature search: A.U.B. (50%) and Ş.K. (50%), Writing: A.U.B. (50%) and Ş.K. (50%), Critical review: A.U.B. (50%) and Ş.K. (50%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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