



## Bioherbicidal Effects of Essential Oils Isolated from *Thymus fallax* F., *Mentha dumetorum* Schult. and *Origanum vulgare* L.

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The chemical composition of essential oil isolated by hydrodistillation from the ground part of *Mentha dumetorum* Schult. collected from Tokat province in 2010 and *Thymus fallax* Fisch. & Mey. and *Origanum vulgare* L. collected from Ordu province in 2009. The main components of *Thymus fallax* were thymol (41.48 %), *o*-cymene (26.75 %),  $\zeta$ -terpinen (15.84 %), terpinoline (2.11 %) that of *Origanum vulgare* were thymol (50.41 %), carvacrol (12.96 %), 2-bornene (11.28 %),  $\zeta$ -terpinen (8.80 %), *o*-cymene (6.68 %) and that of *Mentha dumetorum* were carvone (39.64 %), eucalyptol (14.34 %), dihydrocarvone (12.78 %), limonene (7.79 %). To determine herbicidal activities of the essential oils two layers of filter paper were placed petri dish (6 cm diameter) then seeds of *Avena sterilis* L., *Datura strumarium* L., *Cucumis sativus* L. and *Lactuca sativa* L. were homogeneously distributed on filter paper. Filter papers were thoroughly moistened using distilled water. Piece of filter paper was glued inner parts of each petri dish's lid. Four different concentrations (0, 3, 5 and 7  $\mu$ L/petri dish) of the essential oil were applied to the filter paper pieces. Then lid of each petri dish was closed immediately and sealed with parafilm. Petri dishes were incubated at 12 h dark-12 h light periods with an average temperature of 24 °C for 7 days. All of the essential oils tested inhibited seeds germination, roots and shoots growths of *A. sterilis*, *L. sativa*, *D. strumarium*, *C. sativus*. Consequently, it was determined that essential oils of the plants had bioherbicidal effects on seed germination and growths of some crops and weeds, but more detailed studies should be done under field conditions.

**Key Words:** Bioherbicidal, Essential oil, *Thymus fallax* Fisch. & Mey., *Mentha dumetorum* Schult., *Origanum vulgare* L.

### INTRODUCTION

Nowadays, researches have widely studied on the increase of food production needed for the fast increase of world population and of synthetic pesticides to reduce damage to the environment and human health. Unfortunately, substantial yield losses occur due to insects, plant diseases and weeds caused by fungi, bacteria and viruses<sup>1,2</sup>.

Synthetic chemicals are widely used in the control of plant diseases, pests and weeds. However, these chemicals may cause toxic residues in treated products<sup>3</sup>. As mentioned above, synthetic pesticides can also cause environmental pollution owing to their slow biological disruption<sup>4,5</sup>. In addition, other disadvantages of synthetic pesticide usage are the risk of developing resistance by microorganisms, weeds, insects and the high cost<sup>6-8</sup>. Another major problem in world agriculture are weeds caused losses in crop yield. Therefore, farmers have widely used herbicide. However, wide use of synthetic herbi-

cides can cause pollution in soil and groundwater, development of weed resistance<sup>7,9</sup> and also herbicides at high concentrations can increase the risk of toxic residues in agricultural products.

Therefore, researchers have searched for natural substances, having different and selective herbicidal mechanisms in comparison to their synthetic counterparts<sup>9-12</sup>.

In Turkey, aromatic plants widely distributed and there are rich and diversified floras. These plants have recognition for nutritional and medicinal characteristic. They are used in various industries such as cosmetics, perfumes, detergents, as well as in pharmacology and food flavoring. In the world, to these rapidly evolving traditional sectors, a new industrial development could be added in the plant protection field<sup>13,14</sup>.

The family Lamiaceae (Labiatae) is represented in Turkey by 46 genera and 571 species of which 44.2 % are endemic, with subspecies, varieties and hybrids all together 763 taxa exists in the flora of Turkey.

*Thymus*, *Origanum* and *Mentha* are well known genera in the Lamiaceae family<sup>15</sup>. These genera are generally used as traditional remedy to treat various ailments such as antimicrobial, insecticides, antifungal, herbicidal repellents, expectorant carminative and aromatic for whooping and convulsive coughs, digestive disorders and menstrual problems sedative, anesthetic, antiseptic, abortifacient, antirheumatic<sup>16-21</sup>. The objective of this study involve to determine bioherbicidal effect of essential oils on some plant species. The toxicities of essential oil vapors obtained from three plant species, *Thymus fallax*, *Origanum vulgare* and *Mentha dumetorum* to test plants (*Avena sterilis* L., *Datura strumarium* L., *Cucumis sativus* L. ve *Lactuca sativa* L.) were investigated.

## EXPERIMENTAL

**Isolation of essential oils:** *Thymus fallax* and *Origanum vulgare* were collected from Ordu/Turkey in July, 2009 and were confirmed by Prof. Dr. Hamdi G. Kutbay, Department of Biology, Faculty of Science and Art, Ondokuz Mayıs University. *M. dumetorum* was harvested from Gaziosmanpasa University Agriculture Faculty test area in May, 2010. The essential oils were isolated from plant materials using water distillation technique via Neo-clevenger type apparatus. To extract volatile compounds, the plant materials were weighed (100 g) then 400 mL deionizer water was added and distillation process was continued for approximately 2 h. Essential oils were separated and dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and stored in dark bottles at 4 °C until use and analysis.

**Gas chromatographic-mass spectrometer analysis:** Gas chromatographic (GC) analyses were performed using a Perkin Elmer Clarus 500 Series GC system, in split mode, 50:1, equipped with a flame ionization detector (FID) and a mass spectrometer (MS) equipped BPX-5 apolar capillary column (30 m × 0.25 mm and 0.25 μm ID). Helium (1.0 mL min<sup>-1</sup>) was used as carrier gas. The injector temperature was set at 250 °C and the FID was operated at 250 °C. An initial column oven temperature of 50 °C was elevated to 220 °C at a rate of 8 °C/min and held for 5 min. The mass spectrometer conditions were as follows: transfer line temperature at 250 °C, ion source at 250 °C and the ionization energy at 70 eV. The standard components were available for the majority of the essential oil constituents and Kovats retention indices were determined for all the sample components using Van den Dool and Kratz equation according to homolog *n*-alkane series retention times. Two MS libraries were used to confirm the identities of the compounds: Wiley MS Library and NIST. Identification of oil components was accomplished based on comparison of their retention times with those of authentic standards (co-injection) and by comparison of their mass spectral fragmentation patterns. The relative peak area 103 % of compounds were calculated based on the FID data.

**Seed germination and seedling growth experiments:** The experiments were conducted in 60 mm diameter petri dishes containing two layers of filter paper. Depending on the species (*Avena sterilis* L., *Datura strumarium* L., *Cucumis sativus* L. and *Lactuca sativa* L.), 15-25 seeds were homogeneously placed in each petri dish and the petri dishes were watered using distilled water (5 mL petri dish). Since essential

oils have a low solubility in water, they were used in the gas phase. A given volume of each oil was put on a piece of filter paper that was glued to the inside cover of each petri dish<sup>12</sup>. The cover was closed and immediately sealed with parafilm. By using a micropipette doses of 0 (control), 2, 5, 10 and 15 μL petri dish<sup>-1</sup> were applied. Experiments were conducted in four replicates. Petri dishes were incubated at an average temperature of 24 °C to 2 weeks. Then at the end of incubation period, the number of germination seeds and seedling lengths were measured. The experiments were repeated twice. The experiments were replicated two.

**Statistical analysis:** The data were analyses using Analysis of Variance (ANOVA) test. The means of treatments were grouped on the basis of least significant difference (DUNCAN) at the 0.05 probability level. The software SPSS 13.0 was used to conduct all the statistical analysis.

## RESULTS AND DISCUSSION

**Chemicals composition of the oils:** The compositions of the volatile oils extracted by hydro distillation from the aerial part of the plants were reported in Table-1 together with the Kovats'Indices (KI) calculated for each compound, the percentage composition and the identification methods. About 24 (94.86 % of the total oil), 19 (98.26 % of the total oil) and 17 (97.51 % of the total oil) constituents were identified from *Mentha dumetorum*, *Thymus fallax* and *Origanum vulgare* essential oils, respectively. The volatile compounds of *M. dumetorum* were found rich in carvone (39.69 %), eucalyptol (14.34 %), dihydrocarvone (12.78 %) and limonene (7.79 %). *T. fallax* essential oils were rich in thymol (41.48 %), *o*-cymene (26.75 %), ζ-terpinen (15.84 %) and 2-isopropyl-1-methoxy-4-methyl benzene (5.10 %) while *O. vulgare* essential oil was rich in thymol (50.41 %), carvacrol (12.96 %), 2-bornene (11.28 %), ζ-terpinen (8.80 %) and *o*-cymene (6.68 %). GC-MS analysis of the oils showed that the abundance of oxygenated monoterpenes in the all plants, 76.35, 48.44 and 64.69 %, respectively for *M. dumetorum*, *T. fallax* and *O. vulgare*. The monoterpen contents were 10.2, 48.57 and 29.32 % for *M. dumetorum*, *T. fallax* and *O. vulgare*, respectively.

Previous researches showed that essential oils isolated from some *Mentha*, *Thymus* and *Origanum* species growing in different regions of the world were characterized by the high content of oxygenated monoterpenes<sup>22-29</sup>.

**Bioherbicidal effects of the oils:** All the essential oils used in the experiments were highly phytotoxic on seed germination and seedling growth of the plants tested.

Dependent on the applied dose and essential oils and test plants, significant difference was observed on seed germination, root and shoot length in compared with control.

The results further revealed that in general inhibitory effects of the essential oils on seed germination and seedling growth increased with increase concentrations of essential oils. The highest inhibitory effect on seed germination and seedling growth obtained with essential oil of *M. dumetorum*. On the other hand, all concentrations of essential oils of *T. fallax*, *O. vulgare* and *M. dumetorum* completely prevented seed germination and seedling growth of *D. strumarium*, *A. sterilis* and *L. sativa* (Tables-2-5).

TABLE-1  
ESSENTIAL OIL CONTENTS OF *M. dumetorum* (MD), *T. fallax* (TF) AND *O. vulgare* (OV) PLANTS

RI*	Compounds	MD	TF	OV	Identification thechnique
953	$\alpha$ -Thujene	–**	0.95	tr***	MS, RI
965	Camphene	–	0.11	–	MS, RI
988	$\alpha$ -Pinene	0.41	0.45	0.73	MS, RI
990	$\beta$ -Thujene	0,23	–	–	MS, RI
999	$\beta$ -Pinene	1.05	0.95	0.54	MS, RI
1013	3-carene	0.20	0.68	–	MS, RI
1022	$\alpha$ -Phellandrane	0.52	0.22	tr	MS, RI
1033	Terpineolene	–	2.11	0.87	MS, RI
1043	<i>o</i> -Cymene	–	26.75	6.68	
1048	Linalaol formate	1.46	–	–	MS, RI
1067	3-Octanol	0.21	–	–	MS, RI
1074	$\zeta$ -Terpinen	–	15.84	8.80	MS, RI
1102	Limonene	7.79	0.36	0.42	Co-injection
1110	Eucalyptol	14.34	0.14	tr	Co-injection
1247	Thymol methyl ester	–	–	0.94	MS, RI
1257	2-Isopropyl-1-methoxy-4-methyl benzene	–	5.10	0.38	MS
1271	Borneol	1.31	0.11	tr	Co-injection
1275	4-terpineol	0.47	–	–	Co-injection
1294	Dihydrocarvone	12.78	–	–	MS
1316	Thymol	–	41.48	50.41	Co-injection, NMR
1322	Carvacrol	–	1.28	12.96	Co-injection, NMR
1339	Isopulegone	0.53	–	–	MS, RI
1348	Carvone	39.64	0.33	–	Co-injection
1365	2-Bornene	–	0.15	11.28	MS, RI
1379	Isobornyl acetate	0.32	–	–	MS, RI
1418	Dihydrocarveol	5.32	–	–	MS, RI
1481	$\alpha$ -Bourbonene	3.57	–	–	MS, RI
1501	Methyl-eugenol	0.21	–	–	Co-injection
1521	Caryophyllene	1.73	1.06	1.31	Co-injection
1525	$\alpha$ -Bisabolane	tr	0.20	2.19	MS, RI
1532	$\alpha$ -Cubebene	0.27	–	–	MS, RI
1548	Germacrene	0.50	tr	–	MS, RI
1572	Isoledene	0.55	–	–	MS
1596	Copaene	1.67	–	–	MS
–	Monoterpens	10.2	48.57	29.32	–
–	Oxygenated monoterpens	76.35	48.44	64.69	–
–	Sesqui terpenes	8.29	1.26	3.5	–
–	Total	94.86	98.27	97.51	–

RI: Retention index, tr: < 0,05 %, nd: not detected; MS: Mass spectrophotometer.

The results showed that, in particular, the oils have potent inhibitory effect on the seed germination and seedling growth of *D. atrumarium*, *A. sterilis* and *L. sativa* (Tables 2, 3 and 5). Essential oils did not affect the germination of *C. sativus*, whereas it significantly reduced the seedling growth of *C. sativus* (Table-4).

Recent researches showed that oxygenated monoterpenes and the essential oils, which are relatively rich in oxygenated monoterpenes possess strong inhibitory effects on seed germination and seedling growth of plants<sup>7,12,30-34</sup>. This study, thymol,

carvacrol, pinene, terpinene, borneol determined herbicidal properties<sup>7,30,32,35</sup> were found in *T. fallax*, *O. vulgare* and *M. dumetorum*. However, other major and/or minor component(s) in the essential oils of *T. fallax*, *O. vulgare* and *M. dumetorum* (Table-1) may give rise to the herbicidal effects and there are also possible synergistic and antagonistic interactions among the components.

Our results showed that the oils of *T. fallax*, *O. vulgare* and *M. dumetorum* have herbicidal effects against two important weeds in cultivated areas. It is well known that phytotoxic

TABLE-2  
EFFECT OF ESSENTIAL OILS (*M. dumetorum* (MD), *T. fallax* (TF) AND *O. vulgare* (OV)] ON GERMINATION, ROOT AND SHOOT LENGHTS, DRY WEIGHT OF *Datura strumarium*

Treatments (E. oils) ( $\mu$ L petri dish <sup>-1</sup> )	Germination (%)			Root length (mm)			Shoot length (mm)			Dry weight (g)		
	TF	OV	MD	TF	OV	MD	TF	OV	MD	TF	OV	MD
0 (control)	43.3a	43.3a	43.3a	7.26a	7.26a	7.26a	5.18a	5.18a	5.18a	0.02a	0.02a	0.02a
2	3.3b	3.3b	3.3b	0.00b	0.93b	0.53b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b
5	0.00b	0.00b	3.30b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b
10	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b

\*Means in the same column by the same letter are not significantly different to the test of ANOVA ( $\alpha = 0.05$ ).

TABLE-3  
EFFECT OF ESSENTIAL OILS (*M. dumetorum* (MD), *T. fallax* (TF) AND *O. vulgare* (OV)]  
ON GERMINATION, ROOT AND SHOOT LENGTHS, DRY WEIGHT OF *Avena sterilis*

Treatments ( <i>E. oils</i> ) ( $\mu\text{L}$ petri dish <sup>-1</sup> )	Germination (%)			Root length (mm)			Shoot length (mm)			Dry weight (g)		
	TF	OV	MD	TF	OV	MD	TF	OV	MD	TF	OV	MD
Control	55.0a	55.0a	55.0a	39.1a	39.1a	39.1a	39.1a	39.1a	39.1a	0.031a	0.031a	0.031a
2	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b
5	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b
10	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b

\*Means in the same column by the same letter are not significantly different to the test of ANOVA ( $\alpha = 0.05$ ).

TABLE-4  
EFFECT OF ESSENTIAL OILS (*M. dumetorum* (MD), *T. fallax* (TF) AND *O. vulgare* (OV)]  
ON GERMINATION, ROOT AND SHOOT LENGTHS, DRY WEIGHT OF *Cucumis sativus*

Treatments ( <i>E. oils</i> ) ( $\mu\text{L}$ petri dish <sup>-1</sup> )	Germination (%)			Root length (mm)			Shoot length (mm)			Dry weight (g)		
	TF	OV	MD	TF	OV	MD	TF	OV	MD	TF	OV	MD
Control	100a	100a	100a	55.2a	55.2a	55.2a	11.34a	11.34a	11.34a	0.045a	0.045a	0.045a
2	100a	85a	80a	0.00b	0.00b	0.00b	4.44ab	0.00b	0.00b	0.010b	0.010b	0.010b
5	100a	80a	80a	0.00b	0.00b	0.00b	0.96b	0.00b	0.00b	0.00b	0.00b	0.00b
10	90a	80a	50a	0.00b	0.00b	0.00b	0.46b	0.00b	0.00b	0.00b	0.00b	0.00b

\*Means in the same column by the same letter are not significantly different to the test of ANOVA ( $\alpha = 0.05$ ).

TABLE-5  
EFFECT OF ESSENTIAL OILS (*M. dumetorum* (MD), *T. fallax* (TF) AND *O. vulgare* (OV)]  
ON GERMINATION, ROOT AND SHOOT LENGTHS, DRY WEIGHT OF *Lactuca sativa*

Treatments ( <i>E. oils</i> ) ( $\mu\text{L}$ petri dish <sup>-1</sup> )	Germination (%)			Root length (mm)			Shoot length (mm)			Dry weight (g)		
	TF	OV	MD	TF	OV	MD	TF	OV	MD	TF	OV	MD
Control	83.3a	83.3a	83.3a	40.5a	40.5a	40.5a	30.9a	30.9a	30.9a	0.010a	0.010a	0.010a
2	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00a	0.00a
5	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00a	0.00a
10	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00a	0.00a

\*Means in the same column by the same letter are not significantly different to the test of ANOVA ( $\alpha = 0.05$ ).

essential oils and monoterpene components in plant essential oils may cause anatomical and physiological changes in plant seedlings leading to accumulation of lipid globules in the cytoplasm, reduction in some organelles such as mitochondria, possibly due to inhibition of DNA synthesis or disruption of membranes surrounding mitochondria and nuclei<sup>21,29,40</sup>.

## Conclusion

Essential oils have strong inhibitory effects on germination and seedling growth of weeds. Therefore, essential oils are a potential source for the development of new bioherbicides.

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