INVESTIGATION OF CHEMICAL COMPOSITION AND
PHYTOTOXIC EFFECTS OF ESSENTIAL OILS OBTAINED
FROM *SCHINUS MOLLE* LEAVES AND RESINS

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Abstract

Chemical composition of the essential oils of *Schinus molle* leaves and resins, collected from East Mediterranean region of Turkey were identified by gas chromatography/mass spectrometry (GC/MS) and phytotoxic effects of the oils at different concentrations (0.5, 1.0, 2.0, 4.0 and 8.0 µl/ml) were investigated on *Portulaca oleracea* L. and *Raphanus raphanistrum* subsp. *sativus* (L.) Domin. At the highest concentration, it was found that the essential oil of *S. molle* leaves and resins inhibited at rates of 57%, 67% and 70%, 80% the seed germination of purslane and radish, respectively, when compared to the control. Also, *S. molle* leaves essential oil at the same concentration decreased Radicle length (RL) and Plumule lengths (PL) of purslane and radish in the rates of 82.94, 78.09% and 94.36, 81.30%, while RL and PL of both plants tested were inhibited in the rates of 78.03, 68.99% and 88.91, 76.95% by *S. molle* resins essential oil. This study is the first report on *P. oleracea* L. and *R. raphanistrum* subsp. *sativus* (L.) Domin about phytotoxic effects of the essential oils of *Schinus molle* leaves and resins.

Key words: allelopathy, phytotoxicity, volatile compounds, resin, *Schinus molle*

Introduction. Pepper tree (*Schinus molle* L., yalancı karabiber in Turkish), belonging to the family Anacardiaceae, is frequently grown as an ornamental tree in the Mediterranean region, and originated from South America and its habitat

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ranged from southern Brazil to Chile and Mexico. All parts of this shrubby tree have high oil and essential oil content that produce a spicy scent.

Essential oils are complex mixture of a kind of monoterpenes and sesquiterpenes, and aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones and used commonly as flavourings for foods, candies and spices, as well as in perfume and cosmetic industries as pleasant aroma materials [1].

Earlier, many studies have been done on the essential oil of *S. molle* by different researchers from various regions [1–6]. *S. molle* has been proven to be a good source of essential compounds that could have several uses in folk medicine due to its antibacterial, antioxidant, antirheumatic, healing tuberculosis, bronchitis, cough, anti-inflammatory, antifungal, antiviral, topical antiseptic, anti-spasmodic, antitumoural, digestive and purgative diuretic, analgesic properties and in the food industry [1].

Allelopathy, a multidisciplinary science, is an important biological and ecological mechanism of chemical interactions between plants and allelopathic substances which are used in field crops to manage weeds, insect pests and diseases in the ecosystem [7]. Although allelopathy, which is a chemical-based communication between plants, has attracted a lot of attention from different researchers, it is still approached with suspicion. There are many studies on allelopathic effects, but there is no definite information about how these effects occur. Allelochemicals produced by different plants are known to affect cell division, differentiation, signal transduction, gene expression and permeability of cell membrane and wall structure, and can affect physiological functions such as seed germination, respiration, photosynthesis, ion uptake, enzyme activity, water status, transpiration, stomatal opening, hormone levels, and can also induce the production of reactive oxygen species [8].

Phytotoxic effects of some species such as *Pistacia vera*, *P. lentiscus*, *Cotinus coggygria* belonging to the family Anacardiaceae were investigated by different researchers on various seeds (*Raphanus sativus*, *Lepidium sativum*, *Lactuca sativa*, *Solanum lycopersicum*, *Lolium multiflorum*, *Portulaca oleracea*, *Silybum marianum*, *Sinapis arvensis*, *Trifolium campestre*, *Phalaris canariensis*) [9,10]. Therefore, this study is the first report on *P. oleracea* and *R. sativus* about phytotoxic effects of the essential oils of *Schinus molle* leaves and resins.

In order to develop an effective biopesticide from natural plant compounds with multiple mechanisms of action, it is important to analyze them appropriately and to get an idea about the physiological effects of these compounds. The purpose of this study was to investigate the volatile oil content and phytotoxic effect on two different seeds (*Portulaca oleracea* and *Raphanus raphanistrum* subsp. *sativus*) of essential oils obtained from leaves and resins of *S. molle* grown in Osmaniye province (East Mediterranean region) of Turkey.

Materials and methods. Plant material. In July 2018, the fresh leaves (3 kg) and resins (500 g) of *Schinus molle* were collected in urban areas of Osmaniye

province (Fakıuşağı district, Osmaniye Korkut Ata University campus) in East Mediterranean region of Turkey (37°02'36.6"N, 36°13'22.2"E, 145 m altitude). Plant sample was botanically identified by a senior plant taxonomist, Dr. Fuat Bozok (voucher no: FBozok00715) and deposited in the Department of Biology at Osmaniye Korkut Ata University.

Essential oil extraction. Essential oils of fresh leaves (300 g) and resins (100 g) were hydrodistilled (10 times for leaves, 5 times for resins) for 30 min by using Clevenger apparatus adapted to a microwave oven (340 watt) and were stored at -20°C in a refrigerator. The yield (% w/w) of essential oil obtained from leaves and resins of *S. molle* was calculated on a fresh-weight basis.

GC/MS analysis. Gas chromatography/mass spectrometry (GC/MS, Agilent 7000 Series Triple Quad) with equipped capillary column (HP-5MS, 30 m length $\times$ 0.25 mm diameter $\times$ 0.25 μm film thickness, 5% phenyl methyl poly siloxane) and a flame ionization detector (FID Thermo Scientific Focus) were used to determine the essential oil contents of leaves and resins according to BOZOK [11]. Initial temperature was set at 50°C , followed by increasing to 240°C at $3^{\circ}\text{C}/\text{min}$. Carrier gas was helium. Mass spectra were measured at 70 eV.

The relative percentages (%) of the essential oil components were determined by GC peak areas. All compounds were confirmed by comparison retention time and mass spectra of authentic standards relative to C8-C32 n-alkanes. The authentic standards and mass spectrums from various libraries such as Wiley and NIST (Wiley Registry 12th Edition/NIST 2020 Mass Spectral Library) were used to identify and compare the essential oil compounds.

Phytotoxic effect. *Portulaca oleracea* (purslane) and *Raphanus raphanistrum* subsp. *sativus* (radish) seeds purchased from a local market were used to determine the phytotoxic effects of the essential oils according to Bozok [11]. Seeds were planted in petri dishes immediately after purchase. Since these seeds are widely available in all markets, they were chosen for use in experimental studies, due to the short germination time. Briefly, essential oils were dissolved in tween 80 (0.5%) and solutions at different concentrations (0.5, 1.0, 2.0, 4.0, 8.0 $\mu\text{l}/\text{ml}$) were prepared. These prepared solutions (10 ml) were added to petri dishes (90 mm in diameter) containing (Whatman No. 9) filter papers for each treatment. Thereafter, purslane and radish seeds (30 units) were placed in each petri dish. Petri dishes were sealed with parafilm to prevent contamination and moisture loss. The samples were incubated at 25°C for 7 days (the temperature was adjusted and kept constant by the air conditioner). At the end of the 7th day, germination percentages (%) of seeds tested were calculated and radicle length (RL) and plumule length (PL) were measured by a ruler (Fig. 1). All assays were carried out in triplicate in laboratories at the Department of Biology of the same university.

Statistical analysis. For the statistical analysis one-way analysis of variance (ANOVA) was used to estimate the significant difference. The differences between

Fig. 1. Allelopathic effects of essential oils obtained from *Schinus molle* leaves and resins on the seed germination, radicle and plumule length of *Portulaca oleracea* and *Raphanus sativus*. Different points represent means of independent bioassays. Mean with different letter in curves is significant at $p < 0.05$. C: Control; LEOR: Effect of leaves essential oil on radish; REOR: Effect of resins essential oil on radish; LEOP: Effect of leaves essential oil on purslane; REOP: Effect of resins essential oil on purslane; Leo: Leaves essential oil; Reo: Resin essential oil; RL: Radicle length; PL: Plumule lengths

samples were considered significant at $p < 0.001$ (Tukey HSD test). All statistical analyses were performed using SPSS 20 software package.

Results and discussion. Thirty-five and thirty-one components in the essential oils of *Schinus molle* leaves and resins were determined by GC-MS in the present study, respectively (Table 1). The essential oil yields (% w/w) of *S. molle* leaves and resins were 0.4% and 0.3%, respectively. There were differences in amounts and contents between leaves and resins essential oils of *S. molle*. In terms of major compounds, α -phellandrene (20.3%), D-limonene (16.4%), germacrene D (15.5%), bicylogermacrene (12.3%) in the leaves and germacrene B (20.2%), caryophyllene (18.3%), bicylogermacrene (9.2%) in the resin were determined.

In previous studies, the essential oil of *S. molle* collected from various locations was investigated by different researchers. Epi- α -cadinol (27.3%), caryophyllene oxide (10.5%), γ -cadinene (9.1%) and δ -cadinene (14.2%), limonene (8.1%), sabinene (5.0%) as major compounds were determined in the essential oil of *S. molle* collected from Brazil at different times [12,13]. In other studies, it was found that α -phellandrene (25.9%), limonene (11.7%), β -myrcene (11.1%) and α -phellandrene (29.87%), β -phellandrene (21.08%), elemol (13.00%) and α -phellandrene (31.74%), limonene (16.82%), β -phellandrene (16.49%) were major components in the essential oil of *S. molle* collected from Portugal, Egypt and Turkey, respectively [1,14,15]. This study is similar to previous studies in terms of major compounds but differs in their amounts. These differences are thought to be due to geographical location and collection time, climate and analysis conditions.

Phytotoxic effects of essential oils in different concentrations from *S. molle* leaves and resins were tested on the seeds of *P. oleracea* and *R. sativus* and the germination, radicle and plumule length of these seeds were statistically affected depending on the concentration of the oils. At the highest concentration, it was found that the essential oil of *S. molle* leaves and resins inhibited at rates of 57% to 67% and 70% to 80% the seed germination of purslane and radish, respectively, when compared to the control. Also, *S. molle* leaves essential oil at the same concentration decreased RL and PL of purslane and radish in the rates of 83 to 78% and 94 to 81%, while RL and PL of both plants tested were inhibited in the rates of 78 to 69% and 88 to 77% by *S. molle* resins essential oil. As a result, both leaves and resins essential oils of *S. molle* inhibited RL of all tested species more than their PL.

In the study of ZAHED et al. [16] the essential oil content of *S. molle* fruit and leaves collected from Tunisia and its phytotoxic effect on only one species (wheat) was investigated and it was found that limonene+ β -phellandrene (35.9–65.4%), α -phellandrene (24.3–20.1%), myrcene (12.8–7.7%) and α -pinene (5.9–1.7%) were major components and the essential oil obtained from both fruit and leaves significantly inhibited the germination and radicle elongation of wheat. Another study found as major compounds α -pinene, limonene in the essential oil of *S. molle* leaves from Brazil and that the oil significantly reduced the germination,

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Essential oil compounds of *Schinus molle* leaves and resins collected from Osmaniye, Turkey

No	Compounds	RT	KI ^a	KI ^b	Identification	%	
						SML	SMR
1	α -Pinene	6.3	932	917	KI, MS	3.3	0.3
2	Camphene	6.8	946	934	KI, MS	trace	–
3	Sabinene	7.6	969	935	KI, MS	0.2	–
4	β -Pinene	7.7	974	938	KI, MS	trace	–
5	β -Myrcene	8.2	1000	953	KI, MS	4.6	trace
6	α-Phellandrene	8.6	1002	996	KI, MS	20.3	1.4
7	o-Cymene	9.4	1022	1021	KI, MS	2.9	0.5
8	D-Limonene	9.5	1027	1022	KI, MS	16.4	1.0
9	Eucalyptol	9.6	1031	1031	KI, MS	0.3	–
10	trans- β -Ocimene	10.3	1050	1050	KI, MS	trace	–
11	γ -Terpinene	10.7	1054	1062	KI, MS	trace	–
12	Linalool	12.4	1095	1100	KI, MS	0.3	–
13	γ -Elemene	22.4	1434	1342	KI, MS	0.6	0.4
14	α -Cubebene	22.9	1345	1351	KI, MS	trace	0.6
15	α -Copaene	24.0	1374	1354	KI, MS	0.3	1.2
16	β -Bourbonene	24.4	1387	1384	KI, MS	trace	0.5
17	β -Cubebene	24.6	1387	1389	KI, MS	trace	0.3
18	α -Gurjunene	25.3	1409	1409	KI, MS	1.1	trace
19	Caryophyllene	25.7	1409	1419	KI, MS	2.2	18.3
20	Aromandendrene	26.5	1450	1440	KI, MS	0.1	1.4
21	Thujopsene-I3	26.9	1441	1446	KI, MS	–	7.5
22	α -Humulene	27.1	1452	1452	KI, MS	0.7	1.3
23	Alloaromadendrene	27.3	1450	1457	KI, MS	0.4	0.3
24	γ -Muurolene	28.0	1477	1474	KI, MS	0.2	2.3
25	Germacrene D	28.2	1472	1480	KI, MS	15.5	8.9
26	β -Selinene	28.3	1489	1483	KI, MS	–	1.8
27	Bicyclogermacrene	28.8	1488	1492	KI, MS	12.3	9.2
28	α -Muurolene	29.0	1500	1497	KI, MS	0.3	0.6
29	α -Cadinene	29.5	1537	1511	KI, MS	0.5	1.6
30	β -Cadinene	29.9	1513	1515	KI, MS	1.7	3.5
31	Selina-3,7(11)-diene	30.5	1545	1542	KI, MS	–	0.6
32	Elemol	30.8	1548	1547	KI, MS	1.2	0.3
33	Germacrene B	31.1	1559	1557	KI, MS	1.2	20.2
34	Palustrol	31.5	1567	1567	KI, MS	0.1	–
35	Spathulenol (+)	31.9	1577	1576	KI, MS	–	0.6
36	Caryophyllene oxide	32.1	1582	1578	KI, MS	trace	0.6
37	Viridiflorol	32.4	1592	1600	KI, MS	0.2	0.2
38	Ledol	32.8	1602	1607	KI, MS	trace	–
39	α -Cadinol	34.7	1652	1660	KI, MS	trace	–
40	epi- γ -Eudesmol	39.4	1630	1662	KI, MS	–	0.1
41	Nerolidol, acetate	39.6	1676	1687	KI, MS	–	0.2
Monoterpene hydrocarbons (nos. 1–8, 10, 11)						48.0	3.2
Oxygenated monoterpenes (nos. 9, 12, 18, 35, 41)						1.4	0.8
Sesquiterpene hydrocarbons (nos. 13–17, 19–34, 37)						37.5	81.2
Oxygenated sesquiterpenes (nos. 36, 38–40)						0.2	0.7
Total						87.1	85.9

RT: Retention time, KI^a: Kovats Indices (KI^a indicates the results of Adams [¹⁹]), KI^b: Kovats Indices (KI^b indicates the results of the present study), MS: Mass Spectra, SML: *S. molle* leaves, SMR: *S. molle* resin. Note: Major compounds are shown as bold. Trace: Less than 0.01, (–): Not identified

radicle, plumule length of lettuce and onion [13]. Also, SAAD and ABDELGALEIL [15] researched the phytotoxic effect on the germination of *Silybum marianum* seeds by essential oil at different concentrations (0–3000 mg/ml) of *S. molle* in Egypt and determined that the oil at 3000 mg/ml inhibited in rate of 40% the seed germination.

Thus, in fact the present study is the first report on *P. oleracea* and *R. sativus* about the phytotoxic effects of the essential oils of *S. molle* leaves and resins. Results of the present study are similar to studies on allelopathic effects by essential oils of different plant species [11,17,18].

Conclusions. To the best of our knowledge, this is the first report on *P. oleracea* and *R. sativus* about phytotoxic effects of *S. molle* collected from the East Mediterranean region of Turkey. From this study, it could be concluded that the essential oil of *S. molle* has strong allelopathy against *P. oleracea* and *R. sativus* and can be a source for developing new herbicidal products. However, further studies are required to evaluate and confirm the herbicidal effect of this essential oil in real field conditions.

REFERENCES

- [1] ERYIGIT T., B. YILDIRIM, K. EKICI, M. CIRKA (2017) Chemical composition, antimicrobial and antioxidant properties of *Schinus molle* L. essential oil from Turkey, J. Essential Oil Bearing Plants, **20**, 570–577.
- [2] BASER K. H. C., M. KÜRKÇÜOĞLU, B. DEMİRÇAKMAK, N. ÜLKER, S. H. BEIS (1997) Composition of the Essential Oil of *Schinus molle* L. Grown in Turkey, J. Essent. Oil Res., **9**, 693–696.
- [3] ABRHA L. H., C. R. UNNITHAN (2014) Chemical composition and antibacterial activity of essential oil of *Schinus molle*, Int. J. Pharm. Biol. Sci. Res. Dev., **02**(01), 9–12.
- [4] BERNHARD R. A., R. WROLSTAD (1963) The essential oil of *Schinus molle*: the terpene hydrocarbon fraction, J. Food Sci., **28**, 59.
- [5] CAVALCANTI A. D. S., M. D. S. ALVESB, L. C. P. D. SILVA, D. D. S. PATROCÍNIO et al. (2015) Volatiles composition and extraction kinetics from *Schinus terebinthifolius* and *Schinus molle* leaves and fruit, Rev. Bras. Farmacogn., **25** 356–362.
- [6] CECILIA D., S. QUESADA, O. BRENES, G. AGUILAR, F. J. CICCIO (2008) Chemical composition of *Schinus molle* essential oil and its cytotoxic activity on tumour cell lines, Nat. Prod. Res., **22**(17), 1521–1534.
- [7] MACÍAS F. A., F. J. R. MEJÍAS, J. M. G. MOLINILLO (2019) Recent advances in allelopathy for weed control: from knowledge to applications, Pest. Manag. Sci., **75**(9), 2413–2436.
- [8] WEIR T. L., P. SANG-WOOK, V. M. JORGE (2004) Biochemical and physiological mechanisms mediated by allelochemicals, Curr. Opin. Plant Biol., **7**, 472–479.
- [9] ULUKANLI Z., S. KARABÖRKLÜ, F. BOZOK, M. ÇENET, B. ÖZTÜRK et al. (2014) Antimicrobial, insecticidal and phytotoxic activities of *Cotinus coggygria* Scop. essential oil (Anacardiaceae), Nat. Prod. Res., 1–8.

- [¹⁰] ISMAIL A., H. LAMIA, H. MOHSEN, J. BASSEM (2012) Chemical composition and herbicidal effects of *Pistacia lentiscus* L. essential oil against weeds, Int. J. Med. Arom. Plants., **2**(4), 558–565.
- [¹¹] BOZOK F. (2018) Herbicidal activity of *Nepeta flavida* essential oil, J. Essent. Oil-Bear. Plants, **21**, 1687–1693.
- [¹²] SIMIONATTO E., M. O. CHAGAS, M. T. L. P. PERES, S. C. HESS, C. B. SILVA et al. (2011) Chemical composition and biological activities of leaves essential oil from *Schinus molle* (Anacardiaceae), J. Essent. Oil-Bear. Plants, **14**, 590–599.
- [¹³] PAWLOWSKI Â., E. KALTCHUK-SANTOS, E. B. CARAMÃO, G. L. G. SOARES, M. C. BRASIL et al. (2013) Chemical composition of *Schinus lentiscifolius* March. essential oil and its phytotoxic and cytotoxic effects on lettuce and onion, S. Afr. J. Bot., **88**, 198–203.
- [¹⁴] MARTINS M. R., S. ARANTES, F. CANDEIAS, M. T. TINOCO, J. CRUZ-MORAIS (2014) Antioxidant, antimicrobial and toxicological properties of *Schinus molle* L. essential oils, J. Ethnopharmacol., **151**, 485–492.
- [¹⁵] SAAD M. M. G., S. A. M. ABDELGALEIL (2014) Allelopathic potential of essential oils isolated from aromatic plants on *Silybum marianum* L., Glo. Adv. Res. J. Agric. Sci., **3**(9), 289–297.
- [¹⁶] ZAHED N., K. HOSNI, N. B. BRAHIM, M. KALLEL, H. SEBEI (2010) Allelopathic effect of *Schinus molle* essential oils on wheat germination, Acta Physiol. Plant., **32**, 1221–1227.
- [¹⁷] MANCINI E., N. A. ARNOLD, V. DE FEO, C. FORMISANO, D. RIGANO et al. (2009) Phytotoxic effects of essential oils of *Nepeta curviflora* Boiss. and *Nepeta nuda* L. subsp. *albiflora* growing wild in Lebanon, Journal of Plant Interactions, **4**, 253–259.
- [¹⁸] BOZOK F., M. CENET, G. SEZER, Z. ULUKANLI (2017) Essential oil and bioherbicidal potential of the aerial parts of *Nepeta nuda* subsp. *albiflora* (Lamiaceae), J. Essent. Oil-Bear. Plants, **20**, 148–154.
- [¹⁹] ADAMS R. P. (2009) Identification of essential oils components by Gas Chromatography/Quadrupole Mass Spectroscopy, Allured Publ., Carol Stream, IL.

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