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Herbicidal Activity of Essential Oils on the Germination of Some Problem Weeds

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The herbicidal activities of volatile compounds of plant origin (sweet basil, *Ocimum basilicum* L.; common sage, *Salvia officinalis* L.; English lavender, *Lavandula angustifolia* Mill.; lemon balm, *Melissa officinalis* L. and common thyme *i.e.*, *Thymus vulgaris*) were studied against 3 weeds (common cocklebur, *Xanthium strumarium* L.; sterile wild oat, *Avena sterilis* L. and short spiked canarygrass, *Phalaris brachystachys* L.) in laboratory experiments. Chemical composition of the essential oils were determined by capillary gas chromatography (GC) and GC/MS. The essential oil composition varied with the species. Thymol, geranial and β-thujone were the main constituent of *T. vulgaris*, *M. officinalis* and *S. officinalis* oils, respectively. Linalool was the main constituent of *O. basilicum* and *L. angustifolia* oils. Each essential oil was applied at the concentrations of 2, 4, 8, 16 and 32 µL on the filter paper at the top of the Petri dishes to determine germination and growth bioassays. Inhibition rate of essential oils increased with the increasing concentrations. Essential oils of *T. vulgaris* had the highest inhibitory effect on the germination of *X. strumarium* and *A. sterilis*, on the other hand essential oil of *O. basilicum* had the highest inhibitory effects on the germination of *P. brachystachys*. Each essential oil suppressed seedling and root growth of the tested weeds. Essential oil of *O. basilicum*, *S. officinalis*, *L. angustifolia*, *M. officinalis* and *T. vulgaris* could be used as alternatives of herbicides to suppress germination of *X. strumarium*, *A. sterilis* and *Phalaris brachystachys* seeds in organic farming systems.

Key Words: Labiatae, Essential oil composition, Allelopathy, Weeds, Terpenes, Germination, Inhibitors.

INTRODUCTION

Overall, 3.2 billion tons of pesticides are used and $36 billion are spent to control pests in cultivated lands. Weeds are the major pests of the crop plants, therefore, herbicides are accounted for 50 % of the total pesticides used in the World. Over 75 % of herbicides are used in developed countries. Extensive use of herbicide causes human health and environmental problems. Herbicides are increasingly found in groundwater and surface waters due to their extensive use in

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agriculture, forests, pastures, lakes, parks, root-sides as well as home lawns. Most of the well known synthetic herbicides have been reported as possible human carcinogens in animal tests. Other problems associated with the extensive use of herbicides are either development of resistant weeds or changing the weed spectrum so that the susceptible annual weeds are killed while the more difficult perennial weeds invade the area.

Synthetic herbicides can not be used to control weeds in the organic farming systems. Hence, hand hoeing, inter-row tillage, mulching and ground covering are the major approaches to control weeds. However, these techniques are costly and may not adequately control weeds. Therefore, naturally occurring biologically active compounds from plants receive more attention in recent years as a rich source of potential weed-control agents. Essential oils represent a rich potential source of an alternative and environmentally acceptable weed control compounds. Plants in Labiatae family possess essential oils, which could be utilized for suppressing weeds. The herbicidal activities of essential oil of plant origin are well known[1-5]. Hydrocarbons, (terpens) and oxygenated compounds (esters, aldehydes, ketones, alcohols, phenols and oxides) are 2 distinct groups of chemical constituents of pure essential oils that can be a primary source of potential allelochemicals[6,7].

Understanding of the plant biochemistry, physiology and chemistry of natural products have shown that the allelochemicals may be used for weed control to overcome the above problems associated with the herbicides. The present study was aimed to investigate the allelopathic effects of essential oils of *Ocimum basilicum, Salvia officinalis, Lavandula angustifolia, Melissa officinalis* and *Thymus vulgaris* on the germination and seedling and root growth of *Xanthium strumarium, Avena sterilis* and *Phalaris brachystachys.*

EXPERIMENTAL

Seed collection: The fruits of *Xanthium strumarium* were collected from infested areas in the experimental farm of Mustafa Kemal University in September 2007. The fruits were shade dried in the laboratory at ambient temperature (20-25 ºC) for 30 d. The panicles of *Avena sterilis* and *Phalaris brachystachys* were collected from the infested areas in May 2007 and dried at room temperatures for 7 d. The panicles were shaken gently to make the mature seeds fall into the paper sampling bags. To break dormancy, imbibed seeds were stored for 21 d at 4 ºC in the dark[8].

Five species of native plants in Labiatae family, *Ocimum basilicum, Salvia officinalis, Lavandula angustifolia, Melissa officinalis* and *Thymus vulgaris*, were grown in Telkalis Research Farm of Mustafa Kemal University, Hatay, Turkey in 2007. Essential oil was extracted by water distillation for 3 h from air-dried leaves of each individual species, using a Clevenger-type apparatus.

Gas chromatography (GC): The essential oils obtained from extraction were dried over anhydrous sodium sulfate (Merck, Buenos Aires, Argentina) and stored at 4 ºC in a refrigerator until analysis. The GC analyses were carried out using Hewlett-Packard 6890 GC with FID. A HP-5 MS capillary column (30 m × 0.25

mm i.d. 0.25 µm film thickness) was used. Helium was used as a carrier gas (1.4 mL/ min). The column was temperature programmed as follows: 5 min at 45 °C; then at 3 °C /min to 220 °C and held for 10 min. The injector and detector temperatures were to 220 and 250 °C, respectively. Injection was carried out automatic mode. Samples [0.5 µL of the oil solution in hexane (1:100)] were injected by the splitless technique into helium carrier gas. Peak areas and retention times were measured by electronic integration.

Gas chromatography mass spectrometry (GC/MS): GC/MS analyses of the essential oils were carried out on Hewlett Packard 5970A mass selective detector (MSD), directly coupled to a HP 6890 GC. The column, temperature programme and injection were performed as described above. Injection was carried out automatic mode. Library search was carried out using "Wiley Library, WILEY275, NBS75K, NIST98, FLAVOR". EI mass spectra were measured at 70 eV ionization voltage over the mass range 10-400u. Identification of the compounds was achieved by comparing retention times and mass spectra with those of the standards in the library[9,10].

Germination bioassay: *Xanthium strumarium* (20 seeds), *A. sterilis* (50 seeds) and *P. brachystachys* (50 seeds) seeds were surface sterilized with water:bleach solution (10:1) and were placed evenly on the filter paper in sterilized 9 cm petri dishes and 10 mL of distilled water was added in each petri dish. Essential oils were applied at the concentrations of 2, 4, 8, 16 and 32 µL on filter paper at the top of the petri dishes. No essential oil was added in the control petri dishes. Petri dishes of *X. strumarium* were placed in a growth chamber at 28/32 ºC for 12/12 h and dark/light period for 16/8 h. Other petri dishes were placed in a growth chamber at constant 15 ºC and dark/light period for 16/8 h. Treatments were arranged in a completely randomized design with 4 replications. After 2 weeks, number of germinated seeds were counted. Per cent germination inhibition was calculated as:

$$GI = [(CG-TG)/.CG] \times 100 \quad (1)$$

where, GI is the per cent germination inhibition (%); CG, germination rate in check treatment; TG, germination rate in extract treatment. Analysis of variance was performed for all data using a general linear model procedure[11]. Data from 2 experiments were pooled and mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level[11].

Growth bioassay: Seeds of *Xanthium strumarium, Avena sterilis* and *Phalaris brachystachys* were germinated on filter paper in growth chambers. Ten 2 cm long seedlings of each test weed were planted in a 90 mm Petri-dish filled with sterilized quartz sand. Essential oils were applied at the concentrations of 2, 4, 8, 16 and 32 µL on filter paper at the top of the Petri dishes. No essential oil was added in the control Petri dishes. Experiments were arranged in a completely randomized design with 4 replications. Petri dishes were, then, incubated in growth chambers, as described above. The shoot and root length of seedlings were measured on 7 d after the treatments. Per cent Growth Inhibition was calculated as:

$$GRI = [(LC-LT)/LC] \times 100 \quad (2)$$

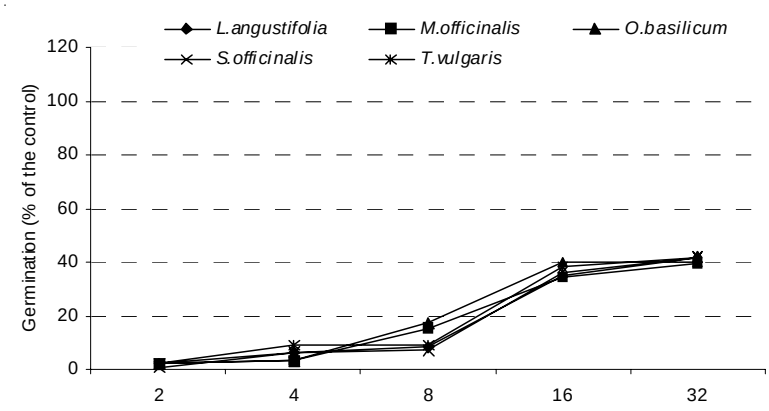
where, GRI is the per cent growth inhibition (%); LT, shoot or root length of treated weed seedling; LC, shoot and root length of untreated check weed seedling.

All experiments were conducted twice in a completely randomized design with four replications. Analysis of variance was performed for all data using a general linear model procedure[11]. Data from two experiments were pooled and mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level.

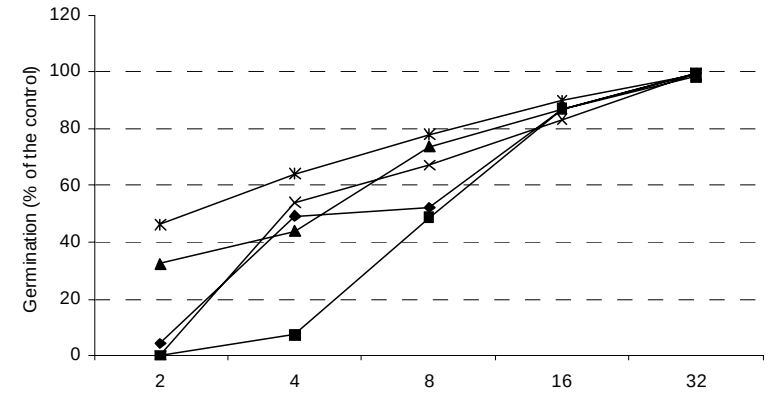
RESULTS AND DISCUSSION

Germination inhibition: Essential oils of *O. basilicum*, *S. officinalis*, *L. angustifolia, M. officinalis* and *T. vulgaris* inhibited *X. strumarium* seed germination at concentrations 2, 4, 8, 16 and 31 µL (Fig. 1). The lowest inhibition rate was observed at 2 µL, while the highest was obtained at 32 µL doses for essential oil of 5 Labiatae species. Germination inhibition rate of essential oils increased with the increasing concentrations. *Thymus vulgaris* essential oil was the most inhibitory on the germination of *X. strumarium* seeds at the concentration of 2, 4, 8 and 16 µL. When the concentration increased from 4 to 8 µL, the inhibition rate of *S. officinalis, T. vulgaris* and *L. angustifolia* did not much change, but the inhibition rate of *M. officinalis* and *O. basilicum* remarkably increased. At 16 µL the highest inhibition rate was obtained from *O. basilicum* followed by *T. vulgaris* and *S. officinalis*. However, at 32 µL concentration essential oils represented close inhibition rates. Essential oils of *L. angustifolia* and *M. officinalis*, when applied at 2 µL, were less toxic than *O. basilicum*, *S. officinalis* and *T. vulgaris*. Among the Labiatae species, the highest mean inhibitory effect on the germination of *X. strumarium* was obtained from *T. vulgaris* and the lowest was obtained from *M. officinalis* essential oil.

Inhibition rate of 5 essential oils on the germination of *A. sterilis* seeds were much more different at the lower concentrations, while the effects on the germination was much more less at the higher concentrations (Fig. 1). Essential oils of *M. officinalis* had no effect on the germination of *A. sterilis* at 2 µL concentration while its effect greatly increased after 4 µL. Essential oil of *L. angustifolia* had less inhibitory effect on the germination of *A. sterilis* seeds at 2 µL while the inhibitory effects increased at 4 µL and its effects increased after 8 µL. Essential oils of *S. officinalis* and *T. vulgaris* inhibited germination of *A. sterilis* seeds even at the lowest concentrations and their effect increased with the increasing concentrations. Inhibitory effects of tested essential oils on *A. sterilis* germination were similar at the higher concentrations. The inhibitory effects of the essential oils of *T. vulgaris, O. basilicum, S. officinalis, L. angustifolia* and *M. officinalis* on the germination of *A. sterilis* were similar to *X. strumarium*. Among the Labiatae species, *T. vulgaris* had the highest while the *M. officinalis* had the lowest inhibitory effects on the germination of *A. sterilis* seeds.



(A)



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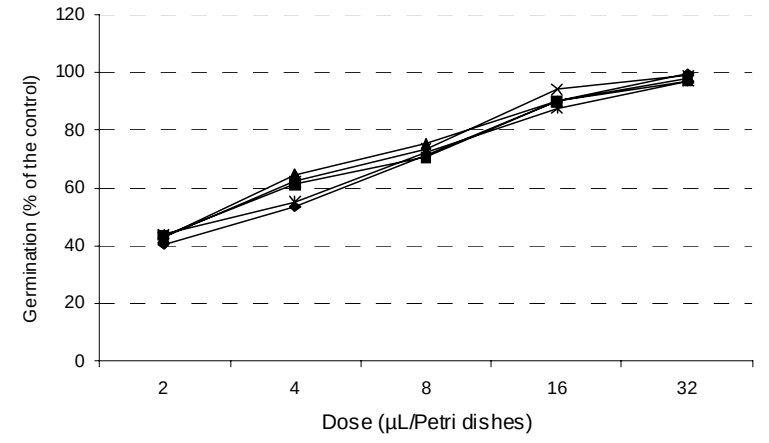

(C)

Fig. 1. Inhibitory effect of *O. basilicum*, *S. officinalis*, *L. angustifolia*, *M. officinalis* and *T. vulgaris* essential oils on the germination of (A) *X. strumarium*, (B) *A. sterilis* and (C) *P. brachystachys*

Essential oils of *O. basilicum, S. officinalis, L. angustifolia, M. officinalis* and *T. vulgaris* represented similar inhibitory effects on the germination of *Phalaris brachystachys* at the tested concentrations (Fig. 1). The inhibitory effects increased with the increasing essential oil concentrations. Almost complete inhibition was obtained at 32 µL for all tested essential oils. In contrast to *X. strumarium* and *A. sterilis, Phalaris brachystachys* seeds differently effected from the tested essential oils. *Ocimum basilicum* had the highest while *L. angustifolia* had the lowest inhibitory effects on the germination of *Phalaris brachystachys* seeds.

Seedling growth inhibition: The essential oils of tested Labiatae species had inhibitory effect on the seedling growth of *X. strumarium* after 2 µL concentration and the inhibition rate increased with the increasing essential oil concentrations. The inhibitory effects of *O. basilicum, S. officinalis, M. officinalis* and *T. vulgaris* had similar patterns of inhibition rates while the essential oil of *L. angustifolia* represented different pattern of inhibition on seedling growth of *X. strumarium* (Fig. 2). Seedling growth inhibition of *X. strumarium* was less affected than the germination inhibition.

The tested essential oils had similar pattern of inhibition rates on *A. sterilis*, except for *M. officinalis* essential oil (Fig. 2). Initially, the inhibitory effect of *M. officinalis* increased with the increasing concentrations but the increase was not much at 8, 16 and 32 µL.

Essential oils of *O. basilicum, S. officinalis, L. angustifolia* and *T. vulgaris* inhibited *Phalaris brachystachys* seedling growth at all tested concentrations (Fig. 2). However, essential oil of *M. officinalis* represented different inhibition pattern. Its inhibitory effect was not much till 16 µL.

Root growth inhibition: Root growth of *X. strumarium* was inhibited by the application of *O. basilicum, S. officinalis, L. angustifolia, M. officinalis* and *T. vulgaris* essential oils (Fig. 3). The inhibitory effect of *M. officinalis* and *O. basilicum* on *X. strumarium* were quite similar at all tested concentrations. The remaining essential oils represented similar impacts on the germination of *X. strumarium*.

Application of essential oils inhibited root growth of *A. sterilis* (Fig. 3). As expected, root growth inhibition increased with the increasing concentrations at 4, 8, 16 and 32 µL. The highest inhibition rates were obtained from *M. officinalis* essential oil.

Essential oils differently affected root growth of *P. brachystachys* (Fig. 3). Root growth inhibition started at 2 µL and increased with the increasing concentrations. Inhibition rate of tested essential oils were close at 2, 4 and 32 µL compared with 8 and 16 µL.

Active ingredient determination: More than 25 components were identified in each essential oil, but most of them constituted less than 1 % (data not given). Only 9 components detected in the essential oil of *O. basilicum* in concentrations greater than 2 % were 1,8-cineole, linalool, eugenol, β-elemene, α-bergamotene,

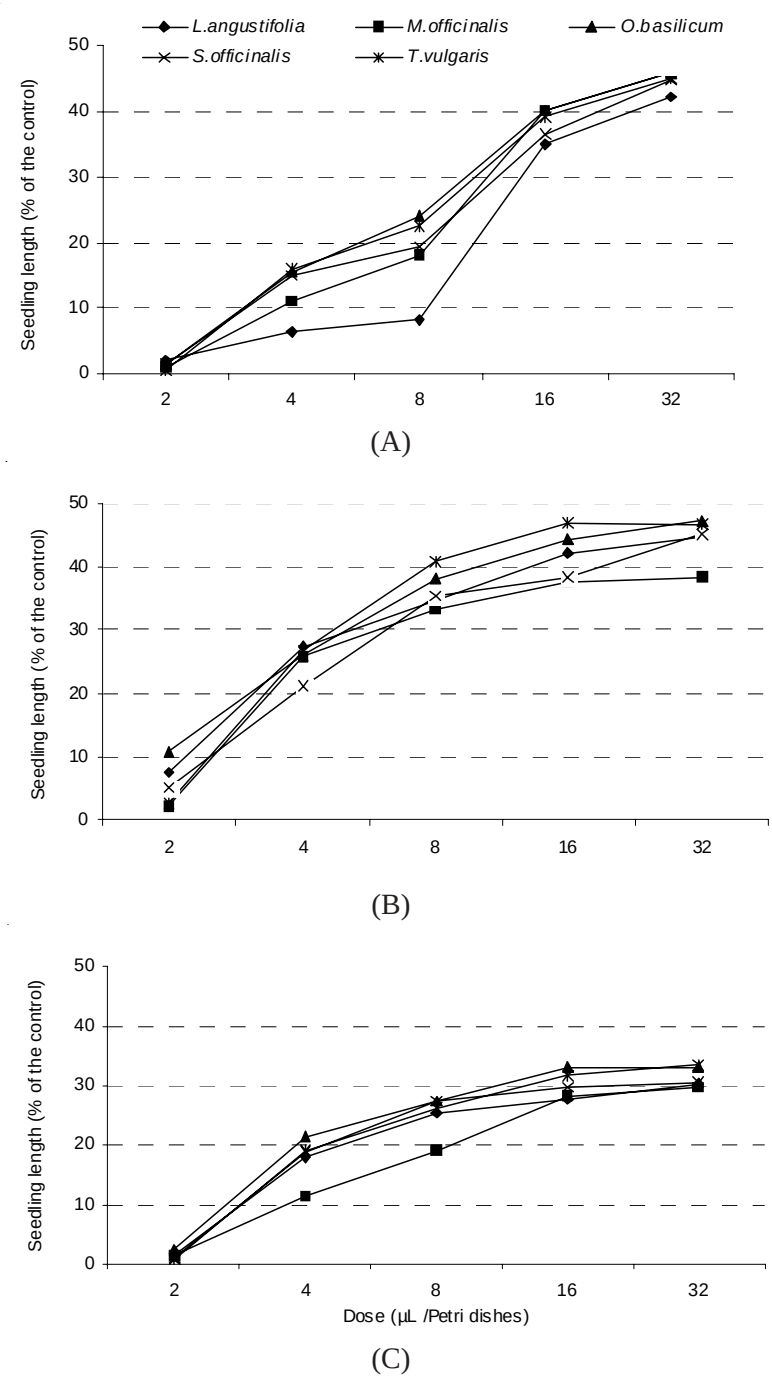

Fig. 2. Inhibitory effect of *O. basilicum, S. officinalis, L. angustifolia, M. officinalis* and *T. vulgaris* essential oils on the hypocothyle length of (A) *X. strumarium*, (B) *A. sterilis* and (C) *P. brachystachys*

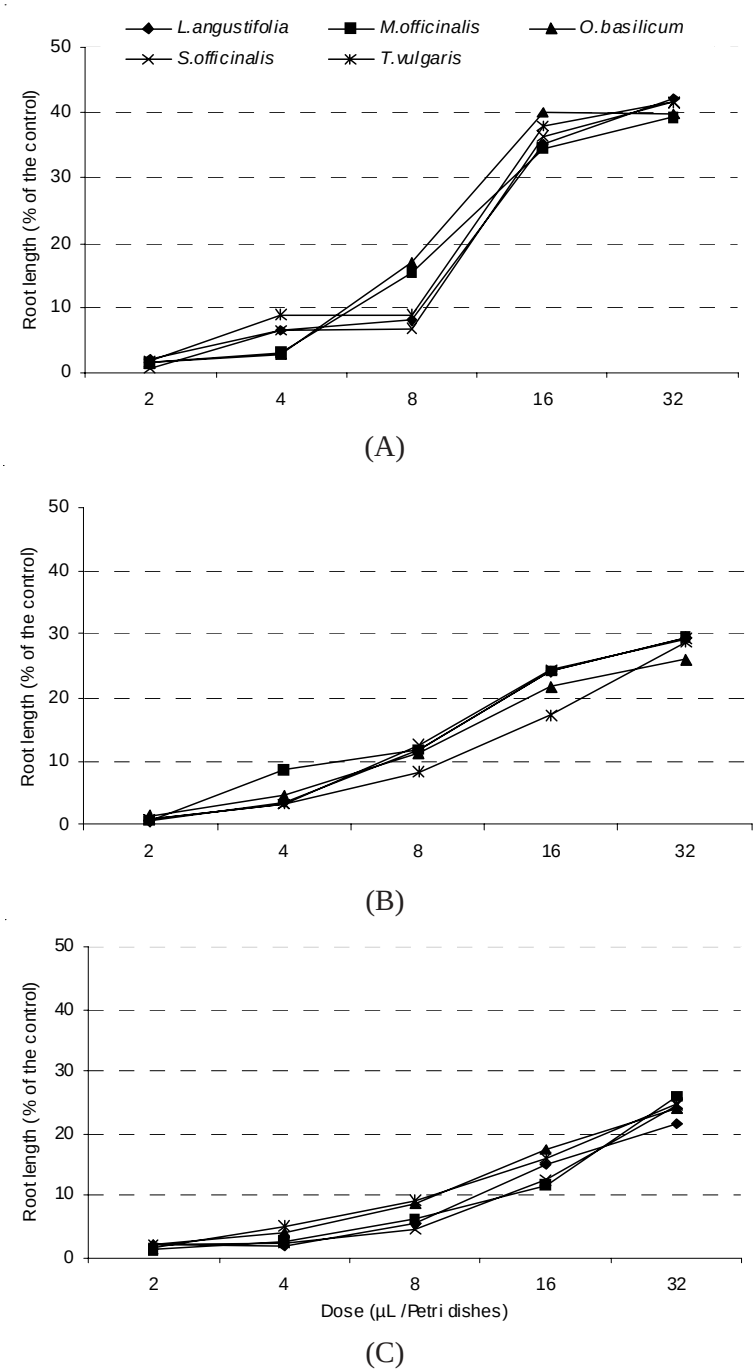

Fig. 3. Inhibitory effect of *O. basilicum, S. officinalis, L. angustifolia, M. officinalis* and *T. vulgaris* essential oils on the radicle length of (A) *X. strumarium*, (B) *A. sterilis* and (C) *P. brachystachys*

germacrene-d, γ-cadinene, aromadendrene and β-cubebene (Table-1). Four components *i.e.,* linalool, *p*-cymene, γ-terpinene and thymol were detected for *T. vulgaris*. Three components *i.e.*, citronellal, z-citral and geranial were detected for *M. officinalis*. Ten components *i.e.*, 1,8-cineole, linalool, terpinene-4-ol, linalool oxide, borneol, nerol, α-terpineol, linallyl acetate, α-cadinole and nerol were detected for *L. angustifolia*. Eight components *i.e.*, 1,8-cineole, α-humulene, β-pinene, α-thujone, β-thujone, l-camphor, β-caryophyllene and viridiflorol were detected for *S. officinalis*. The active ingredients for herbicidal activity in *O. basilicum, T. vulgaris, M. officinalis, L. angustifolia* and *S. officinalis* were linalool, thymol, geranial, linalool and thujone, respectively.

Essential oils have long been recognized for their herbicidal activity. They have been shown to inhibit germination of seeds and growth of plants[12-14]. Seeds of *Xanthium strumarium, Avena sterilis* and *Phalaris brachystachys* were exposed to various concentrations of essential oils of *Ocimum basilicum, Salvia officinalis, Lavandula angustifolia, Melissa officinalis* and *Thymus vulgaris*. When the essential oils were applied at 2, 4, 8, 16 and 32 µL inhibition of *X. strumarium* seed started at 4 µL and increased with the increasing concentrations. The inhibition rate of tested essential oils on *X. strumarium* was 40 %. However at this concentration, maximum inhibition rate (100 %) was obtained for *A. sterilis* and *P. brachystachys* seeds.

As shown in Table-1, the essential oil of *O. basilicum, T. vulgaris, M. officinalis* and *L. angustifolia* and *S. officinalis* contained linalool (42.8 %), thymol (43.5 %), geranial (33.5 %), linalool (34.8 %) and thujone (29.3 %), respectively as major components, which showed potent herbicidal effects in the present study (Table-1). The herbicidal effects of the essential oils can be attributed to their major components. Similar results were reported by Dudai *et al.*[4], Angelini *et al.*[13], Tworkoski[15] and Kordali *et al.*[16].

Essential oils cause anatomical and physiological changes in plant seedlings that lead to accumulate lipid globules in the cytoplasm, reduce size of some cell organelles, possibly due to disruption of membranes, inhibition of DNA synthesis or alter membrane permeability[15,17-19]. The inhibitory effects of tested essential oils could be due to one or more of these factors. However, the exact mechanism of the inhibition caused by essential oils has not been determined.

Essential oils of *O. basilicum, T. vulgaris, M. officinalis, L. angustifolia* and *S. officinalis* inhibited seed germination, seedling and root growth of *X. strumarium, A. sterilis* and *P. brachystachys*. *Thymus vulgaris* essential oil had the highest germination inhibition on *X. strumarium* and *A. sterilis* whereas *O. basilicum* essential oil had the highest germination inhibition on *P. brachystachys* (Table-2). Differences in herbicidal activity of essential oils have been ascribed to differences in major active ingredients (linalool, thymol, geranial and, thujone). The present study confirms the findings of Onen *et al.*[12], Angelini *et al.*[13], Tworkoski[15] and Kordali *et al.*[16].

TABLE-1
CHEMICAL COMPOSITION OF *Ocimum basilicum, Salvia officinalis, Lavandula angustifolia, Melissa officinalis* AND *Thymus vulgaris* ESSENTIAL OILS

Components	*O. basilicum*	*T. vulgaris*	*M. officinalis*	*L. angustifolia*	*S. officinalis*
1,8-Cineole	6.26	–	0.67	0.97	22.83
Linalool	42.83	1.42	0.67	34.87	–
Endobornylacetate	1.99	–	–	–	–
Eugenol	20.63	–	–	–	–
β-Elemene	2.74	–	–	–	–
α-Bergamotene	7.82	–	–	–	–
α-Humulene	0.76	–	–	–	2.82
Germacrene-D	2.59	–	–	–	–
β-Selinene	1.17	–	–	–	–
γ-Cadinene	2.93	–	–	–	–
Aromadendrene	2.28	–	–	–	–
β-Cubebene	8.00	–	–	–	–
Myrcene	–	0.82	0.28	0.43	1.96
α-Terpinene	–	0.91	–	–	0.15
p-Cymene	–	38.53	–	–	–
γ-Terpinene	–	8.22	0.31	–	0.26
Terpinene-4-ol	–	0.65	1.56	5.87	–
Thymol	–	43.46	–	–	–
trans-Caryophyllene	–	1.02	–	–	–
β-Pinene	–	–	–	–	6.63
α-Thujone	–	–	–	–	4.29
β-Thujone	–	–	–	–	25.05
L-Camphor	–	–	–	0.47	18.38
β-Caryophyllene	–	–	–	–	3.83
Viridiflorol	–	–	–	–	3.53
Limonene	–	–	0.68	1.24	–
cis-Ocimene	–	–	0.29	1.99	–
Linalool oxide	–	–	–	4.43	–
Borneol	–	–	0.15	2.29	–
Nerol	–	–	–	2.10	–
Cryptone	–	–	–	1.46	–
α-Terpineol	–	–	–	3.95	–
Linallyl acetate	–	–	–	13.32	–
α-Cadinole	–	–	–	4.04	–
1-Octen-3-ol	–	–	1.17	–	–
Citronellal	–	–	6.35	–	–
cis-Pinane	–	–	1.30	–	–
Pulegone	–	–	1.01	–	–
1-Octen-3-ol	–	–	1.17	–	–
z-Citral	–	–	28.80	–	–
Geranial	–	–	33.36	–	–
Carvacrol	–	–	1.45	–	–
Geranyle acetate	–	–	1.86	–	–
4-Octyne	–	–	1.41	–	–
Nerol	–	–	–	2.10	–

TABLE-2
EFFECTS OF *O. basilicum, T. vulgaris, M. officinalis, L. angustifolia* AND *S. officinalis* ESSENTIAL OILS ON GERMINATION, SHOOT AND ROOT GROWTH INHIBITION OF *X. strumarium, A. sterilis* AND *P. brachystachys*

Dose (µL/petri dishes)	*X. strumarium*			*A. sterilis*			*P. brachystachys*		
	GI	SI	RI	GI	SI	RI	GI	SI	RI
O. basilicum	58.6	25.4	20.3	67.1	33.3	13.0	76.6	23.6	11.3
T. vulgaris	61.7	24.6	19.8	75.5	32.8	11.7	71.3	22.3	11.4
M. officinalis	46.5	23.2	18.8	45.0	27.4	15.3	72.4	18.0	9.6
L. angustifolia	50.5	26.0	18.8	58.4	31.4	14.0	71.0	20.6	9.3
S. officinalis	49.6	23.5	18.4	60.8	29.1	14.1	74.3	21.5	9.2
LSD 0.5	11.32	N.S.*	N.S.	7.37	2.45	3.25	4.10	3.46	3.15

GI = Germination inhibition; SI = Shoot inhibition; RI = Root inhibition.
*N.S. = Not significant.

Development of natural herbicides would help to decrease human health and environmental problems. In this respect, natural pesticides and herbicides may be effective, selective, biodegradable and less toxic to the environment. *Ocimum basilicum, T. vulgaris, M. officinalis, L. angustifolia* and *S. officinalis* essential oils had significant herbicidal activity on *X. strumarium, A. sterilis* and *P. brachystachys*. Based on the present results, these oils could be suggested as alternative herbicides.

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