ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 12:6 (2018) 619-629

records of natural products

Herbicidal and Fungicidal Effects of *Cuminum cyminum, Mentha longifolia* and *Allium sativum* Essential Oils on Some Weeds and Fungi

Tamer Üstüner[1*], Saban Kordali[2] and Ayse Usanmaz Bozhüyük[3]

[1]*Plant Protection Department, Agriculture Faculty, University of Kahramanmaras Sutcu Imam, Kahramanmaras, Türkiye*
[2]*Plant Protection Department, Fethiye Agriculture Faculty, University of Mugla Sıtkı Kocman, Mugla, Türkiye*
[3]*Plant Protection Department, Agriculture Faculty, University of Igdır, Igdır, Türkiye*

(Received April 28, 2018; Revised June 8, 2018; Accepted June 10, 2018)

Abstract: In this study, chemical compositions of essential oil extracted from *Cuminum cyminum* L., *Mentha longifolia* L. and *Allium sativum* L. were analysed using GC and GC-MS methods. The herbicidal effects of the essential oils on seed germination, root and shoot growth of *Rumex crispus* L. and *Convolvulus arvensis* L. were investigated. Meanwhile their effects regarding the mycelial development in *Verticilium dahlia* Kleb. and *Fusarium oxysporum* Schl. were also evaluated. Only the highest three concentrations; 10, 15, 20 µg/cm^2 of *A. sativum* L. were 100% inhibited the seed germination, root and shoot growth of *R. crispus* L.; *C. cyminum* L. and *M. longifolia* L were affected at a rate of 100% by the four concentrations. All the three essential oils showed significant effect (100%) in the parameters examined against *C. arvensis* L. The essential oils obtained from *C. cyminum* L., *M. longifolia* L. and *A. sativum* L. were found to be 100% effective on *V. dahlia* Kleb. mycelium growth in all concentrations examined. However, *C. cyminum* L. and *M. longifolia* L. essential oils were found to be 30.0-36.6% effective at the concentration of 5 µg/cm^2 on mycelium development of *F. oxysporum* Schl.; and the other 3 concentrations totally inhibited the growth of the fungi. The essential oil of *A. sativum* L. was the most effective; and 100% inhibition of the mycelial growth of *F. oxysporum* was detected in all concentrations. The oils used in the study have the potential of being used as herbicides and fungicides.

Keywords: *Cuminum cyminum* L.; *Mentha longifolia* L.; *Allium sativum* L.; essential oil; weed; fungi.

1. Introduction

In recent years, researchers have focused on the ways to increase the food production because of the fast-growing demand related to the growth of the world population. Avoiding or mitigating crop losses due to plant diseases caused by pathogenic fungi, bacteria and weeds are one of the most important issues in plant production [1]. Synthetic pesticides are known to cause serious environmental pollution owing to their slow biodegradation. Consequently, scientists around the globe have focused on finding new potential biological pesticides which will show different selective pesticidal mechanisms in comparison to synthetic chemicals. Recently, there has been a growing interest for research concerning alternative pesticides and antimicrobial active compounds, including plant extracts and essential oils [2-7]. Odorous plants and their volatile compounds affect seed germination and plant growth, and therefore hold a great potential for

* Corresponding author: E-Mail: tamerustuner@ksu.edu.tr ; Phone: 03443002060; Fax: 03443002002

The article was published by ACG Publications
www.acgpubs.org/RNP © November-December 2018 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.80.18.04.106

agricultural purposes especially as plant growth regulators and bioherbicides [8]. Phytochemical analysis showed that *C. cyminum* L.,
M. longifolia L. and *A. sativum* L. contained alkaloid, anthraquinone, coumarin, flavonoid, glycoside, protein, resin, saponin, tannin, steroid and cardiac glycosides [9-11].

One of the most important problems is weed in agriculture. Although the use of synthetic herbicides in controlling weeds in agriculture is effective for some weeds, it can cause soil, water and environmental pollution. [12]. Because of this, biological control against weeds is important. In biological fight, there is no negative impact on the soil, water and environment. The phytotoxic effects of volatile oils of aromatic plants have increased the interest in exploring for potential weed management [13]. Studies have reported that the main allelopathic effects of essential oils on weeds slow the growth and development of weeds as well as inhibiting germination of their seeds [14,15]. Allelopathic effects of some essential oil plants such as *Mentha spicata* L., *Origanum onites* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Thymbra spicata* L. were investigated against some common weed species such as *Amaranthus retroflexus* L., *Rumex nepalensis* Spreng. and *Sinapis arvensis* L. that posed problems in the field and horticultural crops [16].

The *Verticillium* and *Fusarium* species have fairly wide host ranges. These species are soil-borne fungi and they can even survive for extended periods in the absence of a host plant by producing resilient resting structures. The application of fungicides to eradicate these species should be phased out because of the increasing attention to environmental and human health and the development of fungicide resistance [17, 18]. Consequently, alternative control strategies would be useful in reducing health hazards, environmental damage and the pollution potential [19]. Biofungicides may be an attractive alternative method to be used against these species. The antifungal assays showed that *Origanum acutidens* oil, carvacrol and thymol completely inhibited mycelial growth of 17 phytopathogenic fungi; and their antifungal effects were higher than commercial fungicide, benomyl [6]. Besides, *C. cyminum* L. essential oils possessed antifungal activity against *Botrytis cinerea, Rhizopus stolonifer* and *Aspergillus niger* such that the incorporation of 750 µl/L from *C. cyminum* L. oils to PDA medium completely inhibited the growth of these three species [20]. Essential oil extracted from the leaves of *Mentha arvensis* (43.45% menthol) was evaluated *in vitro;* and it was found to completely inhibit the growth of two pathogenic filamentous fungi, *A. niger* and *A. flavus* [21].

In the present study, the purpose was to evaluate the pesticidal effect of the essential oil isolated from *C. cyminum* L.*, M. longifolia* L. and *A. sativum* L. on some fungi and weeds.

2. Materials and Methods

2.1. The Plant Materials and Isolation of Essential Oils

The seeds of *C. cyminum* L. (Apiaceae) and the bulbs of *A. sativum* L. (Amaryllidaceae) were taken from the market. The leaves of *M. longifolia* L. (Lamiaceae) at flowering stage were collected from Erzurum region of Turkey between June and August of 2016. The plant materials were identified by Ass.Prof.Dr. Y. Ziya Kocabas from the University of Kahramanmaras, Turkoglu Vocational School (Kahramanmaras, Turkey). Herbarium number of specie is *M. longifolia* L (YZK 2190). Erzurum is located on the Latitude 39º 57' 23" N and longitude 41°10' 12" E, its annual average temperature is 9.4 ºC while its altitude reaches about 1900 m.

The seeds of *C. cyminum* L were dried at 70°C, grinded to powders and placed in a desiccator prior to use. Garlic (*A. sativum* L.) bulbs were finely chopped and dried in shade. The bulbs were finely sliced and the leaves of *M. longifolia* L. were dried and powdered in a grinder. The dried samples (500 g) were subjected to hydrodistillation for 4 hours using a Clevenger-type apparatus. The hydrodistillation of aromatic plants yielded the accumulations of its essential oil at a rate of 1.5% (w/w). The oils were dried over anhydrous Na_2SO_4 and stored under N_2 in a sealed vial at 4°C until used for herbicidal and fungicidal effect bioassays.

Table 1. Chemical composition of essential oil of *C. cyminum* L. and *M. longifolia* L.

			***C. cyminum* L.**	***M .longifolia* L.**
RI[a]	**RI[b]**	**Compounds**	**Composition %**	**Composition %**
922	908-931[a,b,c]	α-thujene	0.30	-
931	912-939[a,b,c]	α pinene	29.20	
954	954-971[a,c,e,f]	sabinene	0.60	0.30
973	967-979[b,c,d,f]	β-pinene	9.05	0.70
988	981-990[a,b,c,f]	myrcene	0.60	t
990	990-996[d,e,f]	3-octanol	-	0.10
1018	1013-1017[b,c,d,f]	α-terpinene	t	t
1025	1013-1025[a,b,c,d]	*p*-cymene	0.30	t
1029	1025-1029[a,b,c,d,e,f]	limonene	21.70	0.90
1051	1051-1059[a,b,c,e]	γ- terpinene	0.60	0.63
1030	1030-1032[b,c,e,f]	1,8-cineole	18.10	0.10
1082	1082-1090 [a,f]	terpinolene	0.30	t
1093	1089-1110,[a,b,d,e,f]	linalool	t	0.20
1153	1153-1155[d,e]	menthone	-	7.03
1169	1169-1183[a,c,d,e,f]	terpinene-4-ol	0.50	0.60
1182	1182-1188[c,f]	*p*-cymen-8-ol	0.40	0.40
1180	1180-1189[a,c,d]	α-terpineole	3.17	0.40
1248	1248[a]	linalyl acetate	4.80	-
1262	1256-1262[d,f]	trans-piperitone epoxide	-	48.70
1290	1290[c]	p-ceymen-7-ol	4.60	-
1294	1290-1294[c,d,f]	thymol	2.80	1.40
1343	1342-1343[f]	piperitenone	-	0.40
1373	1368-1373[d,e,f]	piperitenone oxide	-	21.20
1425	1419-1425[f]	(*E*)-caryophyllene	-	2.30
1436	1432-1436[d,f]	β-copaene	-	0.60
1456	1456-1489[b,f]	germacrene D	0.10	9.80
1500	1500-1504[d,f]	bicyclogermacrene	-	0.40
1506	1480-1506[d,e,f]	α-muurolene	-	0.30
1529	1513-1529[d,e,f]	γ-cadinene	-	0.20
	Monoterpene hydrocarbons		40.35	2.50
	Sesquiterpene hydrocarbons		37.70	77.56
	Oxygenated sesquiterpenes		-	14.10
	Benzenoids		9.57	1.80
	Aliphatic compounds		9.50	0.70
		Total identified (%)	**97.12**	**96.66**

[a]RI: Retention index, The retention indices were determined on TRB Wax capillary column in reference to *n*-alkanes; [b]LRI : Retention index of literature; a) [23], b) [24], c) [25], d) [26], e) [27] and f) [28]; t: trace (<0.05%).

2.2. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas chromatography-Mass spectrometry (GC/MS) was used for the identification of the components in aromatic plants essential oil. GC/MS instrument (GC/MS-QP 2010 Plus Shimadzu, Japan) fitted with a TRB Wax capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness) was used for qualitative determination. Essential oils were diluted 1/10 in n-hexane (v/v) prior to the analysis. For GC-MS detection, an electron impact ionization system with ionization energy of 70 eV was used. Carrier gas was

Helium at a flow rate of 1.0 µL/min. Injector and MS transfer line temperatures were set at 220ºC and 290ºC, respectively (Tables 1 and 2). The oven temperature was programmed to raise from 50ºC to 150ºC at 3°C/min, then to hold isothermal for 10 min, and was finally raised to 250ºC at 10°C/min. The components were identified based on the comparison of their relative retention time (RI) and mass spectra with the standards of Wiley7N, TRLIB library data of the GC-MS system and literature data [22].

Table 2. Chemical composition of essential oil of *A. sativum* L. accordig to GC-MS

RI[a]	RI[b]	Compounds	Composition%
739	739-767[g,h]	dimethyl disulfide	0.40
741	741-776[g,h]	2-methyl-4-pentenal	t
743	743[g]	2-methylene-4-pentenal	0.40
801	801-802[g,h]	hexanal	t
842	842[g]	1,2-dithiolane	0.50
854	848-855[g,h]	diallyl sulfide	6.59
870	867-870[g,h]	allyl propyl sulfide	t
916	915-916[g,h]	allyl methyl disulfide	4.40
928	928[g]	methyl (*Z*)-1-propenyl disulfide	0.40
936	936[g]	methyl (*E*)-1-propenyl disulfide	0.60
953	953[g]	1,2-dithiolene	0.30
968	955-968[g,ı]	dimethyl trisulfide	2.00
1080	1079-1084[g,h,ı]	diallyl disulfide	20.80
1100	1100[g]	allyl (E)-1-propenyl disulfide	5.20
1131	1131-1138[g,h]	allyl methyl trisulfide	19.20
1181	1181-1183[ı]	2-vinyl-1.3-dithiane	3.90
1301	1301-1305[g]	diallyl trisulfide	33.40
1313	1313[g]	allyl propyl trisulfide	0.20
1325	1325[g]	allyl (E) -1-propenyl trisulfide	-
1540	1538-1555[g]	diallyl tetrasulfide	1.50
	Monoterpene hydrocarbons		12.79
	Sesqiterpene hydrocarbons		74.60
	Oxygenated monoterpenes		6.70
	Oxygenated sesquiterpenes		4.70
	Others		1.00
	Total identified (%)		**99.79**

[a]RI: Retention index, The retention indices were determined on TRB Wax capillary column in reference to *n*-alkanes; [b]RI : Retention index of literature; g) [29], h) [30] and ı) [31]; t: trace (<0.05%).

2.3. Fungal Material

The plant pathogenic fungi were obtained from the culture collection at Atatürk University. These fungi were identified by Prof.Dr. Saban Kordali from the University of Atatürk, Department of Plant Protection (Erzurum, Turkey). All fungus cultures were maintained on potato dextrose agar (PDA) and stored at 4ºC. The fungal species used in the experiments were *V. dahliae* Kleb. (SK 510) and *F. oxyporum* Schl. (SK 511). Antifungal activity was studied by using a contact assay (*in vitro*), which produces hyphal growth inhibition. Briefly, potato dextrose agar (PDA) plates were prepared in 9 cm diameter glass petri dishes. The essential oil was dissolved in dimethyl sulfoxide (DMSO) (Merck) at different concentrations (1%, v/v) (0.25, 0.5 and 1.0 mg/mL concentration) and required amounts of the solutions (20.0 µg/cm^2) were added to each of the PDA plates containing 20 mL of agar at 50°C. A disc (5 mm diameter) of the fungal species was cut from 1 week old cultures on PDA plates and then the mycelial surface of the disc was placed upside down on the centre of a dish with fungal species in contact with growth medium on the dish. Then, the plates were incubated in the dark at 25±2°C. The diameters of the fungal species used in the dishes were measured at 24-hour intervals for 7 days. Mean of growth measurements was calculated from

four replicates of each of the fungal species. PDA plates containing DMSO±water solution (1%, v/v), without essential oil solution were used as negatory control. In addition, PDA plates treated with captan wp (20.0 μg/cm^2) were used as positive control. Mycelial growth inhibition (GI) was calculated as a percentage from the difference between growth of treated and control mycelium using the following equation:

$$GI\ (\%) = \frac{C - T}{C} X\ 100$$

Here, C is the mean of the hyphal extension (mm) of negative controls, and T is the mean of the hyphal extension (mm) of the plates treated with the tested compounds.

2.4. Weed Material and Seedling Growth Experiments

The seeds of *R. crispus* L. and *C. arvensis* L. were gathered in the Erzurum region (Turkey) in October 2016. The weed materials were identified by Ass.Prof.Dr. Y. Ziya Kocabas from the University of Kahramanmaras, Turkoglu Vocational School (Kahramanmaras, Turkey). Herbarium numbers of species are *R. crispus* L. (YZK 2188) and *C. arvensis* L. (YZK 2189) Empty and undeveloped seeds were discarded by floating in tap water. To avoid possible inhibition caused by toxins from fungi or bacteria, the seeds were surface sterilized with 15% sodium hypochlorite for 20 min. and then rinsed with abundant distilled water. Trifluralin (Mega-Tref 48 EC) was used as a positive control. To determine the contact herbicidal effect of the oil, the oil was dissolved in DMSO-water solution (10%, v/v). The emulsions were transferred to Petri dishes (9 cm diameter) with two layers of filter paper placed on the bottom. Afterwards, 50 seeds of *R. crispus* L. and *C. arvensis* L. were placed on the filter paper [33]. Petri dishes were closed with an adhesive tape to prevent escaping of the volatile compounds and were kept at 23±2ºC on a growth chamber supply with 12 h of fluorescent light and humidity of 80% [34]. After 10 days, the number of germinated seeds was determined and stem and root lengths were measured. Germination was measured as the percentage of seeds from which a radicle emerges. The treatments were arranged in a completely randomized design with three replications including controls.

2.5. Statistical Analysis

In order to determine whether there were statistically significant differences among the obtained results for antifungal and herbicidal activity assays, variance analyses were carried out using SPSS 20 software package. Differences between means were tested by Duncan test and values with $p \leq 0.05$ were considered significantly different.

3. Results and Discussion

The major constituents detected in the seed oil from *C. cyminum* L. were α-pinene (29.20%), limonene (21.70), 1,8-cineole (18.10), β-pinene (9.05) and linalyl acetate (4.8). Also, in the leaf oil of *M. longifolia* L., we identified trans-piperidone epoxide (48.70%), piperidone oxide (21.20), germacrene D (9.80), and menthone (7.03), respectively. Additionally, we also identified the essential oils of *A. sativum* L. identified diallyl trisulfide (33.40%), diallyl disulfide (20.80), allyl methyl trisulfide (19.20), allyl (E)-1-propenyl disulfide (5.20) and 2-vinyl-1.3-dithiane (3.90). These volatile oil compounds and ratios were found to be similar to previous studies in this regard [22-32].

3.1. The Herbicidal Effects of Essential Oil

The influences of different concentrates of volatile oils obtained from these odorous plants (*C. cyminum* L., *M. longifolia* L. and *A. sativum* L.) were determined separately on *R. crispus* L. and *C. arvensis* L. seed germination, root and shoot growth. The Trifluralin active herbicide used as a positive control was determined for seed germination of weeds tested (Table 3). Essential oils and herbicide were used on seed germination, root and shoot growth of weeds (5, 10, 15 and 20 μg/cm^2).

Table 3. Herbicidal effects of *C. cyminum* L., *M. longifolia* L. and *A. sativum* L. essential oils on *R. crispus* L. seed germination, root and shoot growth.

Essential oils	C	Germination(%)	Germination inhibition rate (%)	Root growth (mm)	Root growth inhibition rate (%)	Shoot growth(mm)	Shoot growth inhibition rate (%)
Cuminum cyminum L.	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	15	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
Mentha longifolia L.	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	15	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
Allium sativum L.	5	21.6 ± 1.08 b	77.5	0.65 ± 1.11 a	96.6	1.60 ± 2.46 b	92.5
	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	15	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
P. control (Trifluralin)	5	100 ± 0.0 d	-3.84	1.66 ± 0.48 c	91.45	3.42 ± 0.92 c	84.08
	10	100 ± 0.0 d	-3.84	1.54 ± 0.63 bc	92.07	3.10 ± 0.74 c	85.57
	15	100 ± 0.0 d	-3.84	1.49 ± 0.50 bc	92.32	2.60 ± 0.88 c	87.90
	20	100 ± 0.0 d	-3.84	1.14 ± 0.23 b	94.12	2.92 ± 1.07 c	86.41
N. control (Sterile water+Ethanol)	-	96.3 ± 2.10 c	-	19.42 ± 7.81 d	-	21.49 ± 5.70 d	-

*The differences between the averages with different letters in each column are statistically significant (P≤0.05). C: Concentration, P: Positive, N: Negative

It has been determined that *C. cyminum* L. and *M. longifolia* L. inhibits 100% of root and shoot growth of *R. crispus* L. seeds at 4 different concentrations 5, 10, 15 and 20 μg/cm^2. The Trifluralin active herbicide was determined to be ineffective for seed germination of weeds tested (Figure S1). It was found that *A. sativum* L. volatile oil inhibited seed germination, root and shoot growth of *R. crispus* L. 100% at the concentrations of 10, 15 and 20 μg/cm^2 (Figures S2, S3). The volatile oil of *C. cyminum* L. completely inhibited *C. arvensis* L. seed germination, root and shoot growth at 5, 10, 15 and 20 μg/cm^2 (Table 4).

Table 4. Herbicidal effects of *C. cyminum* L., *M. longifolia* L. and *A. sativum* L. essential oils on *C. arvensis* L. seed germination, root and shoot growth.

Essential oils	C	Germination (%)	Germination Inhibition rate (%)	Root Growth (mm)	Root Growth Inhibition rate (%)	Shoot Growth (mm)	Shoot Growth Inhibition rate (%)
Cuminum cyminum L.	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	15	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
Mentha longifolia L.	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	15	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
Allium sativum L.	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	15	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
P. control (Trifluralin)	5	93.0 ± 0.0 c	3.62	1.75 ± 0.54 c	64.7	3.50 ± 0.84 d	80.5
	10	90.0 ± 0.0 b	6.73	1.37 ± 0.43 b	72.3	2.73 ± 0.76 c	84.8
	15	87.1 ± 0.0 b	9.74	1.23 ± 0.40 b	75.2	2.00 ± 0.60 b	88.8
	20	88.8 ± 0.0 b	7.97	1.18 ± 0.38 b	76.2	1.66 ± 0.63 b	90.7
N. control (Sterile water+Ethanol)	-	96.5 ± 1.44 d	-	4.96 ± 2.60 d	-	18.00 ± 6.79 e	-

*The differences between the averages with different letters in each column are statistically significant (P≤0.05). C: Concentration, P: Positive, N: Negative

It has been determined that *M. longifolia* L. fully eradicated *C. arvensis* L. seed germination, root and shoot growth at the concentrations of 5, 10, 15 and 20 µg/cm^2 (Figure S4). It has been seen that *A. sativum* L. volatile oil inhibited *C. arvensis* L. seed germination, root and shoot growth 100% at the concentrations of 5, 10, 15 and 20 µg/cm^2 (Figures S5, S6).

3.2. The Fungicidal Effects of Essential Oil

The effects of essential oils obtained from the aromatic plants (*C. cyminum* L., *M. longifolia* L. and *A. sativum* L.) on the growth of *V. dahlia* Kleb. and *F. oxysporum* Schl. mycelium were determined separately. Meanwhile, the effect of Captan active ingredient fungicide was used as a positive control. Essential oils from *C. cyminum* L.*, M. longifolia* L. and *A. sativum* L. inhibited *V. dahlia* Kleb. mycelial growth 100% at 4 different concentrations (5, 10, 15 and 20 µg/cm^2). Essential oils obtained from *C. cyminum* L. and *M. longifolia* L. inhibited 30.0-36.6% *F. oxysporum* Schl. mycelial growth at 5 µg/cm^2 concentration and 100% at the other 3 concentrations. Captan activa fungicide used as a positive control inhibited 100% germination of fungi tested at 10 µg/cm^2 (Table 5, Figure S7).

Table 5. Fungicidal effect of evaporating oils obtained from *C. cyminum* L.*, M. longifolia* L. and *A. sativum* L. on *V. dahlia* Kleb. mycelial growth.

	Verticillium dahliae Kleb.										
Essential oil	**5 µL/petri**		**10 µL/petri**		**15µL/petri**		**20 µL/petri**		**P. control** (Captan)		**N. control**
	Growth inhibition		**Growth inhibition**		**Growth inhibition**		**Growth inhibition**		**Growth inhibition**		**Growth**
	(mm)	**(%)**	**(mm)**	**(%)**	**(mm)**	**(%)**	**(mm)**	**(%)**	**(mm)**	**(%)**	**(mm)**
Cuminum cyminum L.	0,0 ± 0,0 a	100	0,0±0,0 a	100	0,0±0,0 a	100	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	43,2 ± 1,10 b
Mentha longifolia L.	0,0 ± 0,0 a	100	0,0±0,0 a	100	0,0±0,0 a	100	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	43,2 ± 1,10 b
Allium sativum L.	0,0 ± 0,0 a	100	0,0±0,0 a	100	0,0±0,0 a	100	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	43,2 ± 1,10 b

*The differences between the averages with different letters in each column are statistically significant (P≤0.05). C: Concentration, P: Positive, N: Negative

The effect of the evaporating oils acquired from *C. cyminum* L.*, M. longifolia* L. was found to inhibit 30.0-36.6% *F. oxysporum* Schl. *mycelium* growth at 5 µg/cm^2 concentration, while 100% was inhibited in the other 3 concentrations. The effect of volatile oils obtained from *A. sativum* L. was 100% *F. oxysporum* Schl. mycelium development at the concentrations of 5, 10, 15 and 20 µg/cm^2 (Table 6, Figure S8).

Table 6. Antifungal effect of *F. oxysporum* Schl. *mycelial* growth of volatile oils obtained from *C. cyminum* L.*, M. longifolia* L. and *A. sativum* L.

	Fusarium oxysporum Schl.										
Essential oil	5 µL/petri		10 µL/petri		15µL/petri		20 µL/petri		P. control (Captan)		N. control
	Growth inhibition		Growth inhibition		Growth inhibition		Growth inhibition		growth inhibition		Growth
	(mm)	(%)	(mm)	(%)	(mm)	(%)	(mm)	(%)	(mm)	(%)	(mm)
Cuminum cyminum L.	30.0 ± 1.98 b	26,4	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	40,8 ± 1,00 d
Mentha longifolia L.	36,6 ± 2,21 c	10,2	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	40,8 ± 1,00 d
Allium sativum L.	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	0,0 ± 0,0 a	100	40,8 ± 1,00 d

*The differences between the averages with different letters in each column are statistically significant (P≤0.05). C: Concentration, P: Positive, N: Negative

Synthetic pesticides have caused many serious economic and environmental problems due to their broad-spectrum toxicity. Therefore *C. cyminum* L.*, M. longifolia* L. and *A. sativum* L. essential oil compounds were investigated as natural alternative to be used against to weeds and pathogenic fungi at different concentrations for 7th days, *in vitro*. The tested essential oils (*C. cyminum* L.*, M. longifolia* L. and *A. sativum* L.) were found to be 100% effective against *R. crispus* L. used at 10, 15 and 20 µg/cm^2 concentrations on 7th day. These three volatile oils were found to inhibit seed 100% germination, root and shoot growth of *C. arvensis* L. at a rate of 100% at 4 concentrations (5, 10, 15 and 20 µg/cm^2). Trifluralin,

which was used as a positive control, was found not to prevent the weed germination at all doses and its effect was statistically insignificant.

In other studies conducted in Turkey and worldwide, it was determined that the rates of the essential oil compounds vary according to the plants and extraction methods. The fungicidal effects of the essential oils obtained at various rates from different plants varied on weeds. It was reported that the essential oils like the α-pinene, limonene, 1,8-cineole, β-pinene, linalyl acetate, trans-piperidone epoxide, piperidone oxide, germacrene D, diallyl trisulfide, diallyl disulfide, allyl methyl trisulfide, which are obtained from Amaryllidaceae, Anacardiaceae, Apiaceae, Asteraceae, Lamiaceae and Myrtaceae families, prevented the germination of weeds. The killing effects of essential oils on germination, growth of roots and offshoots of weeds were found to be different. They were found to be influential on many weeds like *A. retroflexus*, *C. album, C. arvensis, C. juncea, Lolium rigidum, R. crispus*, *Trifolium repens, P. angulata, S. arvensis, Setaria viridis* and *Phalaris minor*. It was reported that these herbicidal effects varied between 1-100% [35-45].

These (*C. cyminum* L., *M. longifolia* L.and *A. sativum* L.)volatile oils were found to be 100% effective in 3 concentrations(10, 15 and 20µg/cm^2) on *F. oxysporum* Schl. mycelial growth, while 4 concentrations (5, 10, 15 and 20 µg/cm^2) were effective on *V. dahlia* Kleb. Additionally,Captan activa fungicide, which was used as a positive control, inhibited the germination of fungi tested at a rate of 100% at the concentration 10 µg/cm^2.

In a previous investigation, the mycelial growth of most fungi used in the study was affected by the essential oil, which shows the potential of this oil and its inhibitory effect against some important pathogenic fungi. Similarly, it was also found that these essential oils were effective on fungal pathogens at various rates. It was also reported that the fungicidal effects varied between 1-100% on pathogens like *B. cinerea, F. avenaceum*, *Fusarium culmorum*, *F. oxysporum* ,*Fusarium solani*, *F. subglutinans, Pythium ultimum, Rhizoctonia cerealis, R. solani* and *V. dahlia* [23, 45-53].

4. Conclusion

There are many reports supporting the results of our work concerning with the development of new alternative pesticides, such as fungal pathogens and weeds, toxic natural products including plant essential oils, extracts and secondary metabolites for pest control in agriculture.

These results suggest that *C. cyminum* L., *M. longifolia* L. and *A. sativum* L. essential oil might have the potential to be used as natural herbicide as well as fungicides.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

ORCID ID
Tamer Üstüner: 0000-0003-3584-4249
Şaban Kordali: 0000-0001-5669-5831
Ayşe Usanmaz Bozhüyük: 0000-0003-2450-6850

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