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Investigation of Pesticidal Activities of Essential Oil of *Eucalyptus camaldulensis* Dehnh

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Abstract: In this study, chemical compositions of the volatile oil extracted from *Eucalyptus camaldulensis* Dehnh. were analyzed by using GC and GC-MS. The oxygenated sesquiterpenes, monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated monoterpenes compositions were detected in the sample studied. Pesticidal effects of this oil were investigated on storage insect pests like *Rhizopertha dominica* F. (Col.: Bostrychidae), *Sitophilus granarius* L. (Col.: Curculionidae), *Tribolium confusum* Duv. (Col.: Tenebrionidae), *Callosobruchus maculatus* F. and *Acanthoscelides obtectus* Say. (Col.: Bruchidae). The essential oil was tested on some fungal pathogens and weeds. As fungal pathogens, *Verticillium dahliae* Kleb, *Fusarium oxyporum* Schl., *Phytium debaryanum* Auct. non R. Hesse, *Sclerotinia sclerotiorum* (Lib.) de Barry and *Rhizoctania solani* Kühn. were used while tests on the weeds were performed on *Convolvulus arvensis* L., *Melilotus officinalis* L. and *Amaranthus retroflexus* L. in invitro conditions. *E. camaldulensis* essential oil was found to be effective at 10 and 20 µL against all the tested insect pests. Our results also showed that growth of fungal mycelial as well as weed stems and roots were significantly affected by essential oil. In 10 and 20 µL, *V. dahliae*, *P. debaryanum*, *F. oxyporum* and *S. sclerotiorum* mycelial growth were inhibited in 7 days, while no effect was observed on *R. solani* mycelial growth in this duration. On the other hand, the applications of the oil to the weeds showed different results for each species examined. Although at 5, 10 and 20 µL concentrations of *E. camaldulensis* essential oil did not affect the root and stem growth of *C. arvensis*, the stem and root growth of *M. officinalis* and *A. retroflexus* were reduced by the tested essential oil at the same concentration and time. The research results suggest that *E. camaldulensis* essential oil might have potential to be used as a natural pesticide as well as fungicide.

Keywords: *Eucalyptus camaldulensis* Dehnh.; essential oil; pesticidal effect.

1. Introduction

In recent years, scientists have focused on the ways to increase the food production because of the fast growing demand related to the growth of the world population. Unfortunately, substantial yield losses in food products occur due to insects and plant diseases [1]. Despite the fact that there are several methods for pest control including mechanical, chemical and biological approaches [2]. The materials used in these techniques may leave cause toxic residues in treated crops. Synthetic pesticides in particular can cause serious environmental pollution owing to their slow biodegradation. Besides, it has been shown that the

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intensive use and long term use of pesticide has resulted in development of chemical resistance amongst insects, disease and weeds [3, 4]. Consequently, scientists around the globe have focused on finding new potential biological pesticides which will show different selective insecticidal mechanisms in comparison to synthetic chemicals [5-7]. Esential oils obtained from different plants (*Pimpinella anisum, E. camaldulensis* Dehnh., *Eucalyptus globulus* and *Satureja thymbra*) have been demonstrated to have different mortality rates on different pest such as *Tribolim confusum* Duv., *T. castaneum, Sitophilus oryzae, S. granarius* L., *C. maculatus* F. and *A. obtectus* [8-10].

It was recorded that the essential oil obtained from plants such as *E. camaldulensis, E. unigera* and *E. globulus* had toxic effects on fungi like *Colletotrichum gloeosporioides, Fusarium moniliforme, F. oxysporum, F. solani, Pythium* spp., *P. ultimum* and *R. solani* [11-14].

Another important problem is weeds in agricultural areas. Albeit an effective method to eliminate the undesirable herbs in the field, intensive use of synthetic herbicides can result in soil and groundwater contamination, and development of weed resistance. A great number of research has been conducted about *E. camaldulensis* essential oils to prevent germination of many weeds as *A. retroflexus, Chenopodium album, Cyperus rotundus* and *Solanum nigrum* in the cultivated sites [4, 15-20]. Adverse effects of essential oil of *E. camaldulensis* were determined on the germination and seedling growth of many species of weed including *Amaranthus hybrid, A. retroflexus, C. album, Cirsium arvense, Rumex crispus* and *Portulaca oleracea* [3, 21].

In the present study, we aimed at evaluating the pesticidal effect of the essential oil isolated from *E. camaldulensis* Dehnh. on some stored product pests, fungi and weeds.

2.Material and Methods

2.1. Plant Materials and Isolation of Essential Oils

E. camaldulensis leaves were collected from Tarsus region of Turkey between June and August of 2016. Tarsus located in the Latitude 36º'54'59.62" N and longitude 34º53'42.76" E, and its annual average temperature is 26.7ºC, while its altitude reaches about 23 m, the region also has clay-sandy soil. The samples collected from the region were sent to the herbarium laboratory, Department of Plant Protection, Faculty of Agriculture, Atatürk University, Erzurum, Turkey, where they were dried in shade and ground in a grinder. The dried samples (500 g) were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. The hydrodistillation of *E. camaldulensis* 1.5% (w/w) yielded the accumulations of its essential oil. Once obtained, the essential oil was stored at 4°C in a fridge for further tests.

2.2. GC and GC–MS Analysis

The analysis of the essential oil was performed with a Thermofinnigan Trace GC-FID and GC/Trace DSQ/A1300 (E.I. Quadrapole) equipped with a SGE-BPX5 MS fused silica capillary column (30 m×0.25 mm i.d., film thickness 0.25 μm). 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. diluted samples (1/100, v/v, in methylene chloride) of was injected in the splitless mode. Injector and MS transfer line temperatures were set at 220ºC and 290ºC, respectively (Table 1). The oven temperature was programmed to raise from 50ºC to 150ºC at 3°C/min, then to hold isothermal for 10 min. and finally raised to 250ºC at 10°C/min.

2.3. Insect Material

R. dominica, S. granarius, T. confusum, C. maculatus and *A. obtectus* adults were collected from private store houses in Erzurum/Turkey and kept on cowpea (the black-eyed pea), wheat grains, cracked grains, flour and kidney beans seeds depending on the species studied. The cultures were maintained in Department of Plant Protection, Faculty of Agriculture, Atatürk University, Erzurum, Turkey. In addition, the cowpea, wheat and kidney bean seeds were purchased from a local market and kept at -15 °C in a freezer in order to avoid any arthropod pests contamination prior to use for bioassay during two days. *C. maculatus* and *A. obtectus* adults were reared in 1-L jars containing cowpea and kidney bean seeds, whilst

R. dominica, S. granarius and *T. confusum* adults were reared in 1-L jars containing wheat grains, cracked grains and uninfected flour respectively. The cultures were maintained in the dark conditions in a growth chamber set at 25±2 °C and 65±5% rh. without exposure to any insecticide for several generations. Adult insects (three day-old) were used for the fumigant toxicity test. All experimental procedures were carried out under the same environmental conditions as mentioned above.

Table 1. Chemical composition of essential oil of *E. camaldulensis* Dehnh.

RI[a]	Components	(%)	Identification methods
924	α-Thujene	0.31	GC, GC-MS
932	α-Pinene	2.20	GC, GC-MS
1002	α-Phellandrene	0.51	GC, GC-MS
1020	*p*-Cymene	23.95	GC, GC-MS
1026	1,8-Cineole	32.85	GC, GC-MS
1054	γ-Terpinene	0.64	GC, GC-MS
1183	Cryptone	6.79	GC, GC-MS
1238	Cuminaldehyde	2.65	GC, GC-MS
1412	β-Caryophyllene	7.63	GC, GC-MS
1439	Aromadendrene	1.74	GC, GC-MS
1458	Alloaromadendrene	6.05	GC, GC-MS
1496	Viridiflorene	1.53	GC, GC-MS
1500	Bicyclogermacrene	5.65	GC, GC-MS
1582	Caryophylleneoxide	4.03	GC, GC-MS
Monoterpene hydrocarbons (%)		27.61	
Oxygenated monoterpenes (%)		42.29	
Sesquiterpene hydrocarbons (%)		22.60	
Oxygenated sesquiterpenes(%)		4.03	
Total (%)		96.53	

[a]Retention index relative to n-alkanes on SGE-BPX5 capillary column; GC: identification based on retention times of authentic compounds on SGE-BPX5 capillary column; MS, RI: tentatively identified based on computer matching of the masss pectra of peaks with Wiley 7N and TRLIB libraries and published data, and comparison of retention index of the compounds compared with published data [22-24].

2.4. Bioassays

In order to test the toxicities of *E. camaldulensis* oil, at 5, 10 and 20 µL of essential oil were impregnated into Whatman no. 1 filter paper, which was stuck onto the inner top of the petri dishes where the insects would be placed. This prevented direct contact between the oils and the adult insects. Thirty-three adults of *R. dominica, S. granarius, C. maculatus, A. obtectus* and *T. confusum* were placed onto filter paper containing adequate amounts of wheat grains, cracked grains, uninfected flour, cowpea seeds and kidney bean seeds. The petri dishes were covered with a lid and transferred to an incubator, and then kept under standard conditions at 25±2 °C, 65±5 rh. and in the darkness for two days. Mortalities of the adults were counted on the 1st, 2nd, 3rd, 4th and the 5th days. For each species, another petri dish treated with only sterile water was used as control. Each assay was repeated three times for each concentration and exposure time combination, and insecticidal activities of the *E. camaldulensis* oil were expressed as percent mean mortalities of the adult insects.

2.5.Fungi Material

The plant pathogenic fungi were obtained from the culture collection at Atatürk University. All fungi cultures were maintained on potato dextrose agar (PDA) and stored at 4^{o}C. The fungal species used in the experiments were *V. dahliae, F. oxyporum, P. debaryanum, S. sclerotiorum* and *R. solani.* Antifungal

activity was studied by using a contact assay (in vitro), which produces hyphal growth inhibition [4]. 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 mg/Petri dish) 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. Extension diameter (mm) of hyphae from centers to the sides of the dishes and stored at 4°C. The diameter of the fungal species used in the dishes were measured at 24-h intervals for 7 days. Mean of growth measurements were 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 negative control. In addition, PDA plates treated with captan wp (20.0 mg/Petri dish) 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 equations:

$$GI\ (\%) = (C\text{-}T/C)x100$$

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

2.6.Weed Material and Seedling Growth Experiments

The seeds of *C. arvensis***,** *M. officinalis* and *A. retroflexus* were collected in the Erzurum region (Turkey) in October 2015. 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 dish (9 cm diameter) placed on the bottom two layers of filter paper (10 µL/Petri dishes). Afterwards, 50 seeds of *C. arvensis*, *M. officinalis* and *A. retroflexus* were placed on the filter paper [7, 25]. Petri dishes were closed with an adhesive tape to prevent escaping of volatile compounds and were kept at 23±2ºC on a growth chamber supply with 12 h of fluorescent light and humidity of 80% [26]. 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.

2.7. Statistical Analysis

In order to determine whether there is a statistically significant difference 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

3.1. The Insecticidal Effects of Essential Oil

When the toxic effects of *E. camaldulensis* essential oil concentrations and duration were evaluated, the difference between the treated and untreated samples were found to be statistically significant in most cases (Dose $F_{3,40}$= 1801.61; P<0.0001, Day $F_{4,40}$= 17.18; P<0.0001). The mortality rates of *R. dominica* adults were found as 60.6% at 5 µL, at 25±2 °C on the 5th day, while it was measured as 93.9% at 10 µL and 100% at 20 µL on the 1st day (Table 2). The differences of the applications were found statistically significant.

Table 2. The effect of eucalyptus volatile oil concentrations and treatment durations on the death rate of *R. dominica* adults.

	Mortality rates of *R. dominica*±Standard error					
Concentrations	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**P and F**
5 µL	30.3±4.6 Cb	43.4±3.6 Cab	48.5±6.3 Ca	54.5±7.6 Ca	60.6±4.6 Ca	$F_{4,10}$=4.37 P<0.05
10 µL	80.8±2.7 Bc	86.9±2.7 Bbc	89.9±1.0 Bab	91.9±1.0 Bab	93.9±0 Ba	$F_{4,10}$=9.28 P<0.01
20 µL	100±0 A	100±0 A	100±0 A	100±0 A	100±0 A	$F_{4,10}$= - P= -
Control	0±0 Dc	0±0 Dc	0±0 Dc	2±0 Db	5±1 Da	$F_{4,10}$= 12.67 P<0.001
P and F	$F_{3,8}$=483.9 P<0.0001	$F_{3,8}$=634.4 P<0.0001	$F_{3,8}$=430.5 P<0.0001	$F_{3,8}$=165.7 P<0.0001	$F_{3,8}$=491.0 P<0.0001	

The mortality rates were recorded 100% at 5 µL concentrations on the 5th day, at 10 µL on the 3rd day and at 20 µL on the 2nd day for *S. granarius* adults at 25±2 °C (Dose $F_{3,40}$= 917.36; P<0.0001, Day $F_{4,40}$=29.36; P<0.0001). The differences between the applications were determined as statistically significant (Table 3).

Table 3. The effect of eucalyptus volatile oil concentrations and treatment durations on the death rate of *S. granarius* adults.

	Mortality rates of *S. granarius*±Standard error					
Concentrations	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**P and F**
5 µL	74.7±5.0 Cd	81.8±4.6 Bcd	89.9±3.6 Bbc	97±1.7 Bab	100±0 Aa	$F_{4,10}$=12.51 P<0.001
10 µL	88.8±4.0 Bb	92.9±3.6 Bb	100±0 Aa	100±0 Aa	100±0 Aa	$F_{4,10}$=6.58 P<0.01
20 µL	98.9±1.0 Aa	100±0 Aa	100±0 Aa	100±0 Aa	100±0 Aa	$F_{4,10}$= 1 P= 0.4516
Control	0±0 Dd	4.0±1.0 Cc	6.0±1.7 Cbc	8±1.0 Cab	11.1±1.0 Ba	$F_{4,10}$= 31.57 P<0.0001
P and F	$F_{3,8}$=137.7 P<0.0001	$F_{3,8}$=85.02 P<0.0001	$F_{3,8}$=256.54 P<0.0001	$F_{3,8}$=266.34 P<0.0001	$F_{3,8}$=5611.48 P<0.0001	

The elimination of *T. confusum* adults, on the other hand, was determined to be 84.8% at 5 µL, 95.9% at 10 µL, and 100% at 20 µL on the 5th day of the treatment (Dose $F_{3,40}$=917.36; P<0.0001, Day $F_{4,40}$=29.36; P<0.0001). The differences between the applications were determined as statistically significant (Table 4).

Table 4. The effect of eucalyptus volatile oil concentrations and treatment durations on the death rate of *T. confusum* adults.

	Mortality rates of *T. confusum*±Standard error					
Concentrations	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**P and F**
5 µL	17.2±3.6 Ce	28.3±2.0 Cd	53.5±4.4 Bc	67.7±3.6 Cb	84.8±1.7 Ca	$F_{4,10}$= 66.97 P<0.0001
10 µL	40.4±1.0 Bd	50.5±2.0 Bd	68.7±3.5 Bc	84.8±3.5 Bb	95.9±2.7 Ba	$F_{4,10}$= 36.38 P<0.0001
20 µL	76.8±4.4 Ab	81.8±3.5 Ab	98.9±1.0 Aa	98.9±1.0 Aa	100±0 Aa	$F_{4,10}$= 14.20 P<0.0001
Control	0±0 Db	1±1 Db	2±1 Cab	2±1 Dab	5.0±1.0 Da	$F_{4,10}$= 3.20 P=0.0619
P and F	$F_{3,8}$= 155.99 P<0.0001	$F_{3,8}$= 126.5 P<0.0001	$F_{3,8}$= 75.28 P<0.0001	$F_{3,8}$= 131.35 P<0.0001	$F_{3,8}$= 160.16 P<0.0001	

On the other hand, 100% of *C. maculatus* adults was determined to be killed by 5 and 10 µL of the oil on the 3rd day, and by 20 µL on the 1st day of the experiment (Dose $F_{3,40}$= 1417.91; P<0.0001, Day $F_{4,40}$= 29.05; P<0.0001). The differences between the applications were found statistically significant (Table 5).

Table 5. The effect of eucalyptus volatile oil concentrations and treatment durations on the death rate of *C. maculatus* adults.

	Mortality rates of *C. maculatus*±Standard error					
Concentrations	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**P and F**
5 µL	86.9±2.7 Bc	93.4±3.5 Abc	100±0 Aab	100±0 Aa	100±0 Aa	$F_{4,10}$= 7.82 P<0.01
10 µL	91.9±2.7 Bb	99±1.0 Aa	100±0 Aa	100±0 Aa	100±0 Aa	$F_{4,10}$= 11.59 P<0.001
20 µL	100±0 A	100±0 A	100±0 A	100±0 A	100±0 A	$F_{4,10}$= - P= -
Control	1±1 Cb	5±1 Bc	8±1.0 Bab	10.1±1 Bab	14.1±1 Ba	$F_{4,10}$= 15.98 P<0.001
P and F	$F_{3,8}$= 226.96 P<0.0001	$F_{3,8}$= 107.02 P<0.0001	$F_{3,8}$= 424.83 P<0.0001	$F_{3,8}$= 5762.3 P<0.0001	$F_{3,8}$= 6472.6 P<0.0001	

Lastly, 5 µL of the oil caused the elimination of 100% of *A. obtectus* adults on the 2nd day at 25±2 °C while at 10 and 20 µL, all insects were dead on the 1st day (Dose $F_{3,40}$= 3515.92; P<0.0001, Day $F_{4,40}$= 10.49; P<0.0001) according to control. The differences between the applications were determined as statistically significant (Table 6).

Table 6. The effect of eucalyptus volatile oil concentrations and treatment durations on the death rate of *A. obtectus* adults.

	Mortality rates of *A. obtectus*±Standard error					
Concentrations	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**P and F**
5 µL	98±2.0 Aa	100±0 Aa	100±0 Aa	100±0 Aa	100±0 Aa	$F_{4,10}$= 1 P=0.4516
10 µL	100±0 A	100±0 A	100±0 A	100±0 A	100±0 A	$F_{4,10}$= - P= -
20 µL	100±0 A	100±0 A	100±0 A	100±0 A	100±0 A	$F_{4,10}$= - P= -
Control	2±1 Bd	7±1 Bc	10.1±1 Bbc	14.1±1 Bba	17.2±1 Ba	$F_{4,10}$= 16.45 P<0.001
P and F	$F_{3,8}$=198.62 P<0.0001	$F_{3,8}$=4614.9 P<0.0001	$F_{3,8}$= 5762.3 P<0.0001	$F_{3,8}$= 6472.6 P<0.0001	$F_{3,8}$=7110.8 P<0.0001	

3.2. The Fungicidal Effects of Essential Oil

The effect of eucalyptus essential oil whose effect was investigated at various concentrations (5, 10 and 20 µL) and days on fungal mycelial growth, was found being ineffective on mycelial growth of *V. dahliae* at 5 µL concentration up to the 3rd day even though it was found to be effective from the first day to the last (1-7) at 10 and 20 µL (Dose $F_{4,70}$= 759.95; p<0.0001, Day $F_{6,70}$=78.42; p <0.0001) (Table 7).

Table 7. The effect of eucalyptus essential oil concentrations and treatment durations on mycelial growth of *V. dahliae*

	***Verticillium dahliae* Kleb.**							
Conc.	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**7th day**	P and F
5 µL	0.7±0.2 Cd	1.4±.2 Bdc	1.9±0.3 Adc	1.9±0.3 Bdc	2.7±0.3 Bc	4.6±0.5 Bb	6.9±1.2 Ba	$F_{6,14}$= 17.17 P<0.0001
10 µL	0.5±0 Ca	0.5±0 Da	0.5±0 Ca	0.5±0 Da	0.5±0 Ca	0.5±0 Ca	0.5±0 Ca	$F_{6,14}$= - P= -
20 µL	0.5±0 Ca	0.5±0 Da	0.5±0 Ca	0.5±0 Da	0.5±0 Ca	0.5±0 Ca	0.5±0 Ca	$F_{6,14}$= - P= -
Control	2.3±0.01 Af	3.75±0.02 Ae	5±0.05 Ad	6.5±0.01 Ac	7.9±0.03 Ab	9±0 Aa	9±0 Aa	$F_{6,14}$= 8206.5 P<0.0001
Positive control	1.0±0.01 Ba	1.0±0.01 Ca	1.0±0.01 Ba	1.0±0.01 Da	1.0±0.01 Ca	1.0±0.01 Ca	1.0±0.01 Ca	$F_{6,14}$= 1.03 P= 0.4488
P and F	$F_{4,10}$=55.40 P<0.0001	$F_{4,10}$=237.47 P<0.0001	$F_{4,10}$=234.37 P<0.0001	$F_{4,10}$=8633.8 P<0.0001	$F_{4,10}$=632.52 P<0.0001	$F_{4,10}$=286.3 P<0.0001	$F_{4,10}$=60.8 P<0.0001	

ANOVA was applied to the data, and the differences between the mean values were given at the 5% significance level according to DUNCAN test.

Positive control was also found to be effective in 7 days. Eucalyptus essential oil was found to be effective on mycelial growth of *P. debaryanum* at 5, 10 and 20 µL in the first 5 days but it was found ineffective at 5 µL, on the 6th and 7th days according to control (Dose $F_{4,40}$=420.88; p<0.0001, Day $F6_{,40}$=182.41; p<0.0001). The difference was significant at 10 and 20 µL concentration in 7 days according to control (Table 8).

Table 8. The effect of eucalyptus essential oil concentrations and treatment durations on mycelial growth of *P. debaryanum*.

	***Phytium debaryanum* Auct. non R. Hesse**							
Conc.	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**7th day**	P and F
5 µL	2.4±0.07 Bf	3.5±0.02 Be	4.2±0.2 Bd	6.4±0.2 Bc	7.4±0.3 Bb	8.3±0.3 Aa	9±0 Aa	$F_{6,14}$= 128.93 P<0.0001
10 µL	1.5±0.08 Df	2.05±0.18 Cf	2.7±0.2 De	3.7±0.3 Cd	4.9±0.2 Cc	5.9±0.2 Bb	7.1±0.2 Ba	$F_{6,14}$= 113.96 P<0.0001
20 µL	1.2±0.08 Ec	1.5±0.18 Dc	1.9±0.3 Ecb	2.3±0.4 Dbac	2.7±0.6 Dbac	3.3±0.8 Cba	3.9±0.8 Ca	$F_{6,14}$= 3.57 P<0.05
Control	4.5±0.08 Ad	6.5±0.05 Ac	7.7±0.14 Ab	9±0 Aa	9±0 Aa	9±0 Aa	9±0 Aa	$F_{6,14}$= 661.73 P<0.0001
Positive control	1.8±0.04 Ce	2.6±0.1 Cd	3.5±.1 Cc	4.1±.1 Cb	4.4±0.1 Cb	4.9±0.1 Ba	4.9±.1 Ca	$F_{6,14}$= 86.52 P<0.0001
P and F	$F_{4,10}$= 304.29 P<0.0001	$F_{4,10}$= 147.53 P<0.0001	$F_{4,10}$= 117.11 P<0.0001	$F_{4,10}$= 117.83 P<0.0001	$F_{4,10}$= 60.24 P<0.0001	$F_{4,10}$= 38.84 P<0.0001	$F_{4,10}$=38.47 P<0.0001	

ANOVA was applied to the data, and the differences between the mean values were given at the 5% significance level according to DUNCAN test.

The result also showed that the effect of eucalyptus essential oil was significantly on mycelial growth of *F. oxyporum* at 5, 10 and 20 µL concentration in 7 days compared to the control (Dose $F4_{,40}$= 1735.06; p<0.0001, Day $F6_{,40}$= 546.34; p<0.0001) (Table 9).

Table 9. The effect of eucalyptus essential oil concentrations and treatment durations on mycelial growth of *F. oxyporum*

	***Fusarium oxyporum* Schl.**							
Conc.	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**7th day**	P and F
5 µL	1.6±0.07 Bf	2.2±0.07 Be	2.5±0.08 Be	3.3±0.11 Bd	3.8±0.15 Bc	4.5±0.14 Bb	5.1±0.18 Ba	$F_{6,14}$=102.45 P<0.0001
10 µL	1.4±0.02 Cg	1.8±0.1 Cf	2.2±0.07 Ce	2.6±0.1 Cd	2.9±.06 Cc	3.4±0.03 Cb	3.75±0.1 Ca	$F_{6,14}$=122.23 P<0.0001
20 µL	1.1±0.05 De	1.4±0.06 Dd	1.8±0.09 Dc	2.0±0.1 Dcb	2.3±0.1 Db	2.6±.1 Da	2.8±0.15 Da	$F_{6,14}$=33.28 P<0.0001
Control	2.3±0.01 Ag	3.3±0.01 Af	4.1±0.1 Ae	5.2±0.04 Ad	5.9±0.0 Ac	7.1±0.07 Ab	8.2±0.05 Aa	$F_{6,14}$=356.39 P<0.0001
Positive control	0.5±0 Ac	0.5±0 Ec	1.0±0.01 Eb	1.1±0.04 Eb	1.3±0.08 Ea	1.3±0.08 Ea	1.3±0.08 Ea	$F_{6,14}$=41.18 P<0.0001
P and F	$F_{4,10}$=220.76 P<0.0001	$F_{4,10}$=158.53 P<0.0001	$F_{4,10}$=166.53 P<0.0001	$F_{4,10}$=266.41 P<0.0001	$F_{4,10}$=171.28 P<0.0001	$F_{4,10}$=490.92 P<0.0001	$F_{4,10}$=438.83 P<0.0001	

ANOVA was applied to the data, and the differences between the mean values were given at the 5% significance level according to DUNCAN test.

The effect of eucalyptus essential oil was significant on mycelial growth of *S. sclerotiorum* at 10 and 20 µL concentrations in first 4 days (Dose $F4_{,40}$= 3298.17; p<0.0001, Day $F6_{,40}$= 1152.53; p<0.0001), whereas it was insignificant at 5 µL in 5-7 days (Table 10). But it was found significant at 20 µL in 1-7 days.

Table 10. The effect of eucalyptus essential oil concentrations and treatment durations on mycelial growth of *S. sclerotiorum*

	***Sclerotinia sclerotiorum* (Lib.) de Barry**							
Conc.	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**7th day**	P and F
5 µL	0.5±0 Be	1.4±0.06 Bd	3.8±1.3 Bc	6.8±0.2 Bb	9±0 Aa	9±0 Aa	9±0 Aa	$F_{6,14}$= 1819.6 P<0.0001
10 µL	0.5±0 Be	1.0±0.01 Ce	1.3±0.1 Ce	3.0±0.2 Cd	5.2±0.4 Bc	7.5±0.4 Bb	9±0 Aa	$F_{6,14}$= 194.51 P<0.0001
20 µL	0.5±0 Be	0.5±0 De	0.5±0 De	1.0±0.03 Dd	1.6±0.08 Cc	2.4±0.1 Cb	3.6±0.1 Ba	$F_{6,14}$= 273.47 P<0.0001
Control	2.9±0.2 Ad	5.4±0.2 Ac	7.9±0.2 Ab	9±0 Aa	9±0 Aa	9±0 Aa	9±0 Aa	$F_{6,14}$= 353.27 P<0.0001
Positive control	0.5±0 Ba	0.5±0 Da	0.5±0 Da	0.5±0 Ea	0.5±0 Da	0.5±0 Da	0.5±0 Ca	$F_{6,14}$= - P= -
P and F	$F_{4,10}$= 167.51 P<0.0001	$F_{4,10}$= 539.04 P<0.0001	$F_{4,10}$= 601.63 P<0.0001	$F_{4,10}$= 973.95 P<0.0001	$F_{4,10}$= 384.18 P<0.0001	$F_{4,10}$= 486.33 P<0.0001	$F_{4,10}$=4464.86 P<0.0001	

ANOVA was applied to the data, and the differences between the mean values were given at the 5% significance level according to DUNCAN test.

The result showed that eucalyptus essential oil was found to be ineffective on mycelial growth of *R. solani* at 5, 10 and 20 µL concentrations in 7 days (Dose $F_{3,40}$= 23.24; p<0.0001, Day $F_{4,40}$= 7.70; p<0.0001) (Table 11).

Table 11. The effect of eucalyptus essential oil concentrations and treatment durations on mycelial growth of *R. solani.*

	***Rhizoctania solani* Kühn**							
	1st day	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**7th day**	P and F
5 µL	1.3±0.1 Bd	1.5±0.08 Cdc	1.6±0.1 Cdcb	1.7±0.1 Babcd	1.9±0.2 Babc	2.1±0.3 Bab	3.3±0.1 ABa	$F_{6,14}$= 3.77 P<0.05
10 µL	1.7±0.2 Bb	2.1±0.4 BCab	2.1±0.3 BCab	2.4±0.3 ABab	2.6±0.2 ABa	2.8±0.2 ABa	2.8±0.1 ABa	$F_{6,14}$= 3.02 P<0.05
20 µL	1.6±0.07 Bb	1.85±0.20 BCab	2.1±0.2 BCab	2.3±0.3 Bab	2.55±0.3 ABa	2.55±0.3 ABa	2.55±0.3 ABa	$F_{6,14}$= 2.50 P=0.0744
Control	2±0.05 ABe	2.4±0.05 ABd	2.6±0.07 ABcd	2.7±0.08 ABc	2.8±0.08 ABbc	3±0.07 ABab	3.1±0.1 ABa	$F_{6,14}$= 22.42 P<0.0001
Positive control	2.70.6 Aa	2.9±0.3 Aa	3.2±0.5 Aa	3.3±0.5 Aa	3.4±0.5 Aa	3.4±0.5 Aa	3.4±0.5 Aa	$F_{6,14}$= 0.33 P=0.9096
P and F	$F_{4,10}$= 3.45 P=0.0512	$F_{4,10}$= 5.67 P<0.05	$F_{4,10}$= 4.27 P<0.005	$F_{4,10}$= 3.83 P<0.05	$F_{4,10}$=3.15 P=0.0642	$F_{4,10}$= 2.58 P=0.1018	$F_{4,10}$=2.14 P=0.1507	

ANOVA was applied to the data, and the differences between the mean values were given at the 5% significance level according to DUNCAN test.

73.3. The Herbicidal Effects of Essential Oil

Eucalyptus essential oil ineffective on root growth of *C. arvensis* at 5, 10 and 20 µL concentrations in 7 days (Table 12), while it was effective on root growth of *M. officinalis* and *A. retroflexus* at the same concentrations in 7 days (Dose: $F_{4,2235}$=260.22 P<0.0001).

Table 12. The effect of eucalyptus essential oil concentrations and treatment durations on root growth of *C. arvensis, M. officinalis* and *A.retroflexus*

Root (cm)	***C. arvensis***	***M. officinalis***	***A.retroflexus***	**P and F**
5 µL	0.64±0.09 Ba	0±0 Cb	0±0 Bb	$F_{2,447}$=42.58 P<0.0001
10 µL	0.11±0.02 Da	0±0 Cb	0±0 Bb	$F_{2,447}$=14.32 P<0.0001
20 µL	0.03±0.01 Da	0±0 Cb	0±0 Bb	$F_{2,447}$=6.19 P<0.0001
Control	1.58±0.14 Aa	1.61±0.12 Ab	0.83±0.07 Ab	$F_{2,447}$=18.57 P<0.0001
Positive control	0.36±0.03 Ca	0.19±0.03 Bb	0.06±0.01 Bc	$F_{2,447}$=31.22 P<0.0001
P and F	$F_{4,475}$=60.12 P<0.0001	$F_{4,475}$=156.84 P<0.0001	$F_{4,475}$=134.10 P<0.0001	

ANOVA was applied to the data, and the differences between the mean values were given at the 5% significance level according to DUNCAN test.

Eucalyptus essential oil was determined as ineffective on stem growth of *C. arvensis* at 5, 10 and 20 µL concentrations in 7 days (Table 13), while it was effective on stem growth of *M. officinalis* and *A. retroflexus* at the same concentrations and treatment length (Dose: $F_{4,2235}$=234.27, P<0.0001).

Table 13. The effe**ct** of eucalyptus essential oil concentrations and treatment durations on stem growth of *C. arvensis, M. officinalis* and *A.retroflexus*

Stem (cm)	***C. arvensis***	***M. officinalis***	***A.retroflexus***	**P and F**
5 µL	0.24±0.04 Ba	0±0 Bb	0±0 Bb	$F_{2,447}$= 25.78 P<0.0001
10 µL	0.03±0.01 Ca	0±0 Ba	0±0 Ba	$F_{2,447}$= 3.12 P<0.05
20 µL	0±0 Ca	0±0 Ba	0±0 Ba	$F_{2,447}$= - P= -
Control	1.4±0.12 Ab	1.82±0.14 Aa	0.78±0.08 Ac	$F_{2,447}$= 69.04 P<0.0001
Positive control	0.37±0.04 Ba	0.06±0.02 Bb	0.05±0.01 Ab	$F_{2,447}$=40.52 P<0.0001
P and F	$F_{4.745}$=79.33 P<0.0001	$F_{4.745}$= 158.89 P<0.0001	$F_{4.745}$= 10.01 P<0.0001	

ANOVA was applied to the data, and the differences between the mean values were given at the 5% significance level according to DUNCAN test.

Synthetic pesticides have caused many serious economical and environmental problems due to their broad spectrum toxicity. Therefore eucalyptus essential oil compounds were investigated as a natural alternative to be used against storage pests, pathogenic fungi and weeds at different concentrations and in 1-7 days, in vitro. The tested essential oil (*E. camaldulensis* Dehnh.) was very effective against all the insect pests used in this study. The indicated a potential for this essential oil to be used to control these storage pests.

Essential oils can easily be obtained from plant materials by vapor distillation method. They are preferable because they exhibit low toxicity for mammals, while are highly toxic to storage pests [27]. In earlier studies, toxic effects of some essential oils were assessed to determine possible fumigant, contact and ingestion activity against *R. dominica*, *S. oryzae* and *T. castaneum* [28]. It was reported that the essential oils obtained from *Chenopodium ambrosioides* leaves showed high insecticidal toxicity against *Prostephanus truncatus, Callosobruchus chinensis, C. maculatus, A. obtectus* and *S. granarius* [8]. In

another study, the toxic effects of essential oils of *Lavandula angustifolia*, *Rosmarinus officinalis*, *Thymus vulgaris* and *Laurus nobilis* were observed to be effective against stored pests. The group has reported that 1,8-cineole, thymol and borneol were toxic at high doses on *S. oryzae* after 24 hours (at 0.1 µL/720 mL) and 100% of the main components of camphor and linalool applied on *R. dominica* and *T. castaneum* caused approximately 20% of deaths [29]. The greatest fumigant toxicity against *A. obtectus* was seen with *F. vulgare* essential oil, followed by *T. spicata* and *L. stoechas* essential oils. The main components of plant essential oils of three plants showing high response were determined by GC-MS analysis. *F. vulgare* essential oil's main components were anisole (79%) and L-fenchone (13%). *T. spicata* and *L. stoechas* contain L-fenchone (55%, 57%), camphor (24%, 24%) and 1,8-cineole (13%, 13%), respective. Main components L-fenchone and camphor caused about 100% mortality at 80 µL/L dose in 48 hours. The results indicate that *F. vulgare* essential oil or its components may have a potential for controlling of *A. obtectus* [30]. *A. obtectus* on the other hand was declared to be the most tolerant species against the essential oils [31]. As reported by these researchers were similar results with the present study.

In a previous investigation, the mycelial growth of most fungi used in the study, was affected by the essential oil which indicates the potential of this oil and its inhibitory effect against some important pathogenic fungi. It was found that four species of eucalyptus essential oil had inhibitory effect on some fungi; such as *T. cucumeris* 100% at 5 mg/mL, *F. oxysporum* more than 84% at 5 mg/mL and *C. globosum* 100% at 10 mg/mL [11]. In an another report, β-citronellol, nerol, menthol, terpinen-4-ol, α-terpineol, carvone, borneol compounds and commercial benomyl were determined as antifungal compounds, and a high concentration of *E. camaldulensis* was found to cause a remarkable inhibition against pathogenic fungi *F. solani* [15]. Of all the compounds in another study, Thymol was pointed the most strong antifungal compound against the four fungi (*F. oxysporum, R. solani, A. niger* and *P. digitatum*) [24]. When phenols, alkaloids and terpenes were extracted from *E. camaldulensis* and applied to the fungi, the results showed that terpene extract was the most active against fungi and alkaloids extract had less antifungal activity where the percentage of mycelial radial growths calculated as 99.55 and 72.44% respectively [25]. Additionally, in a study where *Myrtus communus* volatile oil was used against 19 phytopathogenic fungi, the effect of antifungal activity was determined as 10-100% [26]. Essential oil of *E. camaldulensis* was shown to inhibit mycelial growth of fungi, *F. oxysporum, F. verticillioides, F. solani, F. subglutinans* and *F. proliferatum*. It was observed as effective at 7, 8 and 10 µL/mL on the 5th day [32]. The results revealed that *E. camaldulensis* leaf oils provided 100% inhibition of the mycelial growth of *Thanatephorus cucumeris* (5 mg/mL), and *Chaetomium globosum* (10 mg/mL). No inhibition effect was observed against *R. oryzae* even at the concentration of 10 mg/mL [33]. Eucalyptus of essential oil presented high antifungal activity against *S. sclerotiorum* and *Colletotrichum circinans* fungus species at 10 and 50 µL/petri, but was not found effective against *F. oxysporum, Alternaria mali* and *Botrytis cinerea* in vitro conditions [34].

In this study, eucalyptus essential oil was ineffective on root and stem growth of *C. arvensis* at 5, 10 and 20 µL concentrations in 7 days, while it was effective on root growth of *M. officinalis* and *A. retroflexus* at the same concentrations in 7 days. In other studies, the following results were obtained, the herbicidal activity of *E. globulus* essential oil was also determined and the viabilities of *A. blitoides*, *A. viridis* and *C. dactylon* were found to be significantly lower than the control group [35] *Eucalyptus tereticornis* essential oil on the other hand, was reported to inhibit the germination of *A. viridis* [36]. The herbicidal effects of the oils on the seed germination and seedling growth of *A. retroflexus, C. album, L. serriola* and *R. crispus* were also determined [37]. The essential oil of *Nepeta meyeri* inhibited the germination of the seeds of weed species including *A. retroflexus* L., *C. album* L., *C. arvense* L. and *S. arvensis* L. [38].

Our results suggest that *E. camaldulensis* essential oil might have potential to be used as a natural insecticide, fungicide, as well as herbicide.

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