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Insecticidal effects of essential oils from Labiatae and Lauraceae families against cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored pea seeds

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ABSTRACT

The cowpea weevil, Callosobruchus maculatus (F.) (Coleoptera: Bruchidae), is one of the most serious pests of stored pulse seeds. The essential oils obtained from rosemary (Rosmarinus officinalis L.), Turkish oregano (Origanum onites L.), sweet marjoram (Origanum majorana L.), Greek oregano (Origanum vulgare L.), mountain oregano (Origanum minutiflorum L.) and laurel (Laurus nobilis L.) have been analyzed by GC and GC-MS and tested for their insecticides effects against the cowpea weevil. In the current study, 1,8 cineole (21.45%), camphor (19.70%) from rosemary, 1,8 cineole (37.84%), α-terpinyl acetate (15.33%) from laurel, carvacrol (57.01%) from Turkish oregano, carvacrol (34.14%), thymol (20.36%), terpinene-4-ol (12.31%) from sweet marjoram, carvacrol (59.87%), p-cymene (17.55%) from Greek oregano, carvacrol (52.04%), p-cymene (22.87%) from mountain oregano have been identified as the main volatile components. In both bioassays, essential oil extracted from rosemary and laurel resulted in significant mortality on the cowpea weevil with 1.6 (µg mL^{-1}) concentrations comparing with the sweet marjoram, the Turkish oregano, the Greek oregano and mountain oregano. The essential oil extracted from the laurel and rosemary had the lower lethal concentrations compared with the Turkish oregano, sweet marjoram, Greek oregano and mountain oregano in both bioassays. The major components of 1,8 cineole, camphor, α-terpinyl acetate, carvacrol, thymol, terpinene-4-ol, p-cymene act as insecticides to the cowpea weevil. The essential oils extracted from rosemary and laurel can be recommended as a potential source of environment-friendly botanical insecticide in control of the cowpea weevil.

Keywords: Essential oils, Labiatae, Lauraceae, *Callosobruchus maculatus,* stored pulse.

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INTRODUCTION

Pea, *Pisum sativum* L., is a food legume, which was first cultivated in southwest Asia [72]. Pea is exclusively grown in the temperate regions of the world and its production rank in the world pulses is third with a value of 11.3 million ton following bean and chickpea [31]. It is used in human diet as fresh pods, dry seeds and, canned and frozen food. The pea grain contains about 23% protein [38], which makes it a valuable protein crop. In addition, it is an important source of carbohydrate [25] vitamins and minerals [39]. The cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), is one of the most serious pests of stored pulse seeds [4,28,31] causing significant damages [33,42,48,65]. Chemical control using fumigants and synthetic insecticides has dominated the control of the stored pests [16,21,22,36]. However, fumigants and synthetic insecticides cause serious problems such as resistance to the insecticides, pest resurgence, elimination of economically beneficial insects, toxicity to humans and wildlife [23,31,46]. These kinds of problems and demand for pesticide-free foods have increased efforts to find alternative management for the stored pests. Some plants contain compounds that act as insecticides for many pests [1,54]. As such, essential oils are potential alternatives to control stored pests due to their low toxicity to warm-blooded mammals and their high volatility [57, 64]. The components of essential

oils show ovicidal, repellent, contact insecticides, antifeedant, sterilization and toxic effects to insects [1,2,55,59,63-65,70]. The purpose of this study was to investigate insecticidal effects of essential oils from Labiatae and Lauraceae families against the cowpea weevil*, C. maculatus* (F.) (Coleoptera: Bruchidae) in stored pea seeds.

MATERIALS AND METHODS

Two laboratory bioassays were conducted at the University of Mustafa Kemal, Hatay, Turkey. For both bioassays, adults of the cowpea weevil were reared at laboratory cultures on pea grains at 26 ºC, 65% relative humidity with a photoperiod of 12 h light–dark.

Essential oil: Five labiatae family plants, rosemary *(Rosmarinus officinalis* L.), Turkish oregano (*Origanum onites* L.), sweet marjoram (*Origanum majorana* L.), Greek oregano (*Origanum vulgare* L.), mountain oregano (*Origanum minutiflorum* L.), were grown in Telkalis Research Farm of Mustafa Kemal University, Hatay-Turkey. A lauraceae plant, laurel (*Laurus nobilis* L.), were bought from DEO (Kırıkhan/Hatay). Essential oils of five labiatae plants and lauraceae plant were extracted by water distillation for 3 h from air-dried leaves of each individual species, using a Clevenger-type apparatus. 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.

Analysis of essential oil: The GC analyses were carried out using Hewlett-Packard 6890 GC with FID. A HP-5 MS capillary column (30 m x 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 220 °C and 250 °C, respectively. Injection was carried out automatic mode. Samples (0.5µl 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.

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 70eV 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 [7, 8].

Laboratory Bioassay: Two different bioassays were set up under standard laboratory conditions**.** Five essential oil concentrations (0.1, 0.2, 0.4, 0.8, 1.6 µg /100 ml jar) from five labiatae plants and a lauraceae plant were used in both bioassays conducted completely randomized design with four replications. A 100-ml glass jar with screwed plastic cap was used in both bioassays. Each of the jar contained 10 g of pea grains and 15 adults of the cowpea weevil. A small piece of the filter paper was attached to under surface of the each cap to serve as a diffuser, on which varying doses of the essential oils applied. However, the control diffuser was left as untreated. The insects had no contact with the diffuser and stayed at the bottom of the chamber throughout the experiment. Both bioassays were evaluated at 24-h later and number of dead cowpea weevils were counted for each of the glass jar. Data were analyzed by analysis of variance (ANOVA) with using the SAS software and the Probit analysis ($P < 0.05$). Means were separated by using the Least Significant Difference (LSD) Multiple Comparison Tests ($P < 0.05$) [9].

RESULTS

The essential oil extracted from rosemary resulted in a hunhred percent mortality on the cowpea weevil with the highest concentration in both bioassays (F: 23.47, P: 0.0001; F: 28.16, P: 0.0001, respectively) (Tables 1, 2). In the first bioassay, the essential oil extracted from laurel had a 90 percent mortality on cowpea weevil with 1.6 (µg mL^{-1}) concentration (F: 9.62, P: 0.0004) (Table 1). In the following bioassay, the laurel had a 93.33 percent mortality at the same concentration (F: 20.15, P: 0.0001) (Table 2). In the first bioassay, the sweet marjoram, the Turkish oregano, and the Greek oregano had lower rates of mortality on the cowpea weevils comparing with rosemary and laurel (Table 1). However, in the following bioassay, the essential oil extracted from the sweet marjoram, the Turkish oregano, and the Greek oregano resulted in significantly higher mortality on cowpea weevils with the highest concentration (F: 10.03, P: 0.0003; F: 8.14, P: 0.0009; F: 4.58, P: 0.0105, respectively) (Table 2).

Table 1: Effect of various essential oils on *Callosobruchus maculatus* under standard laboratory conditions.

Percent of *C. maculatus* mortality / Dose (µg mL^{-1})[x]

Lauraceae/Labiatae	0.1	0.2	0.4	0.8	1.6	n[y]	Intercept (±SE)	Slope (±SE)	X^2	[z]LC_{50}	P<0.05
Laurus nobilis L.	50.00c	58.33c	61.66c	76.66b	90.00a	300	-0.21 ± 0.23	2.39 ± 0.51	21.77	1.23	0.0001
Rosmarinus officinalis L.	43.33d	65.00 c	78.33 b	83.33b	100.00a	300	-0.43 ± 0.24	4.06 ± 0.59	46.86	1.27	0.0001
Origanum onites L.	43.33 d	48.33cd	55.00bc	61.66b	83.33a	300	-0.49 ± 0.23	2.04 ± 0.49	17.20	1.75	0.0001
Origanum majorana L.	36.66d	45.00d	58.33c	71.67b	84.99a	300	-0.80 ± 0.24	2.92 ± 0.51	31.74	1.88	0.0001
Origanum vulgare L.	31.66 c	48.33b	51.66b	54.99b	73.33a	300	-0.79 ± 0.24	2.11 ± 0.49	18.00	2.39	0.0001
Origanum minutiflorum L.	33.33d	38.33cd	50.00bc	56.66ab	63.33a	300	-0.82 ± 0.24	1.81 ± 0.49	13.45	2.86	0.0002

[x]Numbers within a row not followed by the same letter are significantly different (P<0.05) by LSD and Probit Analysis. [y]Number of C. maculatus tested. LC_{50} Lethal concentrations of essential oils (in µg mL^{-1}) at the 50 % (LC_{50}) levels of probit mortality.

Table 2: Effect of various essential oils on *Callosobruchus maculatus* under standard laboratory conditions.

Percent of *C. maculatus* mortality / Dose (µg mL^{-1})[x]

Lauraceae/Labiatae	0.1	0.2	0.4	0.8	1.6	n[y]	Intercept (±SE)	Slope (±SE)	X^2	[z]LC_{50}	P<0.05
Laurus nobilis L.	46.66d	53.33d	68.33c	82.40b	93.33a	300	-0.42 ± 0.23	3.15 ± 0.54	33.83	1.36	0.0001
Rosmarinus officinalis L.	56.66d	56.66 d	73.33 c	81.66b	100.00a	300	-0.10 ± 0.23	3.01 ± 0.55	29.83	1.08	0.0001
Origanum onites L.	43.33 c	48.33c	55.00bc	68.33b	91.66a	300	-0.59 ± 0.23	2.62 ± 0.51	26.48	1.68	0.0001
Origanum majorana L.	36.66c	41.66c	50.00c	68.33b	95.00a	300	-0.99 ± 0.24	3.26 ± 0.53	37.47	2.01	0.0001
Origanum vulgare L.	28.33 c	51.67bc	55.00b	60.00b	90.00a	300	-1.02 ± 0.25	3.19 ± 0.53	36.17	2.09	0.0001
Origanum minutiflorum L.	26.66c	33.33c	41.66c	61.66b	88.33a	300	-1.49 ± 0.27	3.56± 0.56	40.43	2.62	0.0001

[x]Numbers within a row not followed by the same letter are significantly different (P<0.05) by LSD and Probit Analysis. [y]Number of C. maculatus tested. LC_{50} Lethal concentrations of essential oils (in µg mL^{-1}) at the 50 % (LC_{50}) levels of probit mortality.

Table 3: Chemical components in essential oils of *Rosmarinus officinalis* and *Laurus nobilis*

Components	*R. officinalis*	*L.nobilis*
1,8 cineole	21.45	37.84
Linalool	5.88	1.26
Endobornyl acetate	2.44	---
Methyl eugenol	1.02	---
Myrcene	1.90	----
Sabinene	--	11.66
α-terpinene	1.00	....
γ-terpinene	1.07	1.32
Terpinene-4-ol	3.12	3.20
β-pinene	...	5.34
β-caryophyllene	....	---
Limonene	--	2.10
p-cymene	3.08	---
Borneol	8.58	---
Verbenene	1.79	---
Camphor	19.70	---
6,6-Trimethylbicyclo	1.91	---
1,3-Dimethylbicyclo	8.24	----
Nopol	1.27	----
α-pinene	--	7.57
Benzene, methyl(1-methylethyl)	---	1.61
Caryophyllene	---	1.06
α-Terpinyl acetate	---	15.33
α-Terpineol	---	2.42

The essential oil extracted from mountain oregano had the lowest mortality on the cowpea weevil in both bioassays (Tables 1, 2). In the first bioassay, the essential oil extracted from the laurel had the lowest lethal concentrations (LC_{50}: 1.23, 0.0001) and rosemary was followed in the second lethal concentrations (LC_{50}: 1.27, 0.0001) (Table 1). In the second bioassay, the essential oil extracted from rosemary had the lowest lethal concentrations (LC_{50}: 1.08, 0.0001) and the laurel was followed the second lethal concentrations (LC_{50}: 1.36, 0.0001) (Table 2). The Turkish oregano, sweet marjoram, Greek oregano and mountain oregano had the higher lethal concentrations comparing with rosemary and laurel in both bioassay (Tables 1, 2).

More than 25 components were identified in each of essential oil, but most of them constituted less than 1% (data not given). Eight components, 1,8 cineole, linalool, endobornyl acetate, terpinene-4-ol, p-cymene, borneol, camphor and 1,3-dimethylbicyclo, were detected from rosemary (Table 3).

Table 4: Chemical components in essential oils of *Origanum onites*, *Origanum majorana*, *Origanum vulgare* and *Origanum minutiflorum*.

Components	*O. onites*	*O. majorana*	*O. vulgare*	*O. minutiflorum*
Linalool	8.39	--	--	--
β- phellandrene	--	1.17	--	--
Myrcene	2.48	--	1.53	--
Sabinene	--	3.55	--	--
α-terpinene	2.87	4.30	1.71	1.02
γ-terpinene	8.77	6.75	6.50	--
Terpinene-4-ol	2.09	12.31	1.96	1.53
Carvacrol methyl ether	--	--	--	1.03
Thymol	--	20.36	1.00	1.00
Caryophyllene oxide	--	--	--	1.90
Terpinolene	--	1.68	--	--
β-caryophyllene	1.27	--	2.15	1.18
p-cymene	7.86	1.47	17.55	22.87
Trans-sabinene hyrate	--	5.69	--	6.33
Carvacrol	57.01	34.14	59.87	52.04
Borneol	--	--	1.63	4.25
α-terpineol	--	2.54	--	--

Eight components, 1,8 cineole, sabinene, terpinene-4-ol, β-pinene, limonene, α-pinene, α-terpinyl acetate, α-terpineol, were detected from the laurel. Seven components, linalool, myrcene, α-terpinene, γ-terpinene, terpinene-4-ol, p-cymene and carvacrol, were detected from Turkish oregano (Table 4). Eight components, sabinene, α-terpinene, γ-terpinene, terpinene-4-ol, thymol, trans-sabinene hyrate, carvacrol and α-terpineol, were detected from sweet marjoram. Four components, γ-terpinene, β-caryophyllene, p-cymene and carvacrol, were detected from Greek oregano. Four components, p-cymene, trans-sabinene hyrate, carvacrol and borneol, were detected from mountain oregano. The active ingredient for insecticidal activity in rosemary, laurel, Turkish oregano, sweet marjoram, Greek oregano and mountain oregano were 1,8 cineole, borneol, camphor, 1,3-dimethylbicyclo, sabinene, α-pinene, α-terpinyl acetate, linalool, γ-terpinene, p-cymene, carvacrol, terpinene-4-ol, and thymol, respectively (Tables 3,4).

DISCUSSION

Over 2000 species of plants are known to have some insecticidal activities [43]. The major components of essential oils show insecticides, ovicidal, repellent, antifeedant, fumigants, sterilization and toxic effects in insects [2,3,35,44,47,49,55, 60,63,64,66,69,70]. Essential oils from Patchouli, *Pogostenmon heyneanus* (Solanaceae) and *Ocimum basilicum* (Lamiaceae) showed insecticidal activity against *S. oryzae* (Coleoptera: Curculionidae), *Stegobium paniceum* (Coleoptera: Anobiidae), *T. castaneum* (Coleoptera: Tenebrionidae) and *Bruchus chinensis* (Coleoptera: Bruchidae) [26,27]. Regnault-Roger and Hamraoui [58] reported that essential oil compound of carvacrol, linalool and terpineol are more toxic on *A. obtectus* adults than p-cymene, cinnamaldehyde, anethole. Prates et al. [56] reported that essential oil from *Eucalyptus* spp., isolating 1,8-cineole and limonene have insecticidal activity for the stored pests. Ketoh et al. [41] stated that several essential oils components have shown insecticidal activity against *C. maculatus*. The leaf oil of *Cymbopogon schoenanthus,* rich in piperitone, gave 90% mortality after 24 h at a concentration of 6.7 µL/L. Negahban et al. [50] reported that essential oil of *Artemisia sieberi* (Asteraceae) was tested against *C. maculatus, S. oryzae* and *T. castaneum.* The main constituents of the oil were chrysanthenone, camphor, 1,8-cineole, camphene, borneol, cyclohexanol acetate, trans-pinocarveol,pcymene and trans-piperitol. The concentration of 1.85 µl/l and exposure time of 24 h was enough to obtain 100% mortality of the three insects. Essential oil from *Macrozamia lucida,* known major components of oxygenated monoterpenes: 1,8-cineole (43.4%), α-terpinyl acetate (14.5%), 4-terpinen-4-ol (3.4%), α-terpinol (3.4%), and monoterpene hydrocarbons: sabinene (8.2%) and β-pinene (4.0%), have insecticide and repellent activities for the stored pests [20,45,51]. Plant products from species such as *Dennitia tripetela, Cucurma longa, Piper guineensis* and *Azadirachta indica, Ocimum gratissimum, Monodora myristica, Momordica charantia* have been reported as alternatives to synthetic insecticides [24,30,34,37,52,53,67]. The essential oils from *Artemisia annua, Artemisia scoparia, Artemisia selengensis* and *Artemisia sieversiana* (Asteraceae) were tested against *Callosobruchus chinensis* (Coleoptera: Bruchidae). The four essential oils showed strong fumigant and contact activity, the major activity was obtained with *A. selengensis* oil [71]. Rozman et al. [60] stated that the insecticidal

activities of 1,8-cineole, camphor, eugenol, linalool, carvacrol, thymol, borneol, bornyl acetate and linalyl acetate varied with *S. oryzae*, *R. dominica* and *T.castaneum*. The most sensitive species was *S. oryzae*, followed by *R. dominica*. The compounds of 1,8-cineole, borneol and thymol were highly effective against *S. oryzae* when applied for 24 h at the lowest dose (0.1 ml/720 ml volume). The compounds of camphor and linalool were highly effective and produced 100% mortality on *R. dominica* in the same conditions. Kotan et al. [44] reported that the insecticidal activity of the essential oil isolated from *Salvia hydrangea* (Lamiaceae), was evaluated against adults of *S. granarius* and *T. confusum* and its essential oil showed 68.3 and 75.0% mortality on stored pests. The essential oil from *Cymbopogon giganteus*, rich in limonene and *p*-mentha-1(7),8-dien-2-ol, showed insecticidal activity against *C. maculatus* and *C. subinnotatus* [18]. Ekeh et al. [29] reported that *Ocimum gratissimum* had the highest insecticidal properties against *C. maculatus* and successfully used for the control of *C. maculatus*.

The previous studies have been reported that essential oil could protect stored products from the cowpea weevil by using as insecticides, fumigants and repellents. With the current study, adults of the the cowpea weevil were exposed to various concentrations of essential oils extracted from rosemary *(R. officinalis* L.), Turkish oregano (*O. onites* L.), sweet marjoram (*O. majorana* L.), Greek oregano (*O. vulgare* L.) , mountain oregano (*O. minutiflorum* L.) and laurel (*L. nobilis* L.) in two laboratory bioassays. In both bioassays, essential oil extracted from rosemary and laurel resulted in significant mortality on the cowpea weevil with 1.6 (µg mL^{-1}) concentrations comparing with the sweet marjoram, the Turkish oregano, the Greek oregano and mountain oregano. The essential oil extracted from the laurel and rosemary had the lower lethal concentrations comparing with the Turkish oregano, sweet marjoram, Greek oregano and mountain oregano in both bioassays. The toxicological effects of the essential oils can be attributed to their major components [10,35,68].

In the current study, 1,8 cineole (21.45%), camphor (19.70%) from rosemary, 1,8 cineole (37.84%), α-terpinyl acetate (15.33%) from laurel, carvacrol (57.01%) from Turkish oregano, carvacrol (34.14%), thymol (20.36%), terpinene-4-ol (12.31%) from sweet marjoram, carvacrol (59.87%), p-cymene (17.55%) from Greek oregano, carvacrol (52.04%), p-cymene (22.87%) from mountain oregano have been identified as the main volatile components. The major components of 1,8 cineole, camphor, α-terpinyl acetate, carvacrol, thymol, terpinene-4-ol, p-cymene act as insecticides to the cowpea weevil. Insecticidal activities of essential oils have been described to differentiate the major active ingredients (γ-terpinene, p-cymene, β-thujone and 1, 8 cineole). The current study also confirmed the previous studies were conducted by Regnault-Roger and Hamraoui [58], Prates et al. [56], Trypathy [11], Ketoh *et al.* [6], Tapondjou *et al.* [12], Waliwitiya *et al*. [20]; Negahban *et al*. [50], Ngamo *et al*. [51], Yuan *et al*. [71], Kouminki *et al*.[45]; Stamopoulos *et al.* [13] Kotan *et al.* [14] and Nyamador *et al*. [18]

CONCLUSION

The present study was conducted by investigate insecticidal effects of essential oils from Labiatae and Lauraceae families against the cowpea weevil*, C. maculatus* (F.) (Coleoptera: Bruchidae) in stored pea seeds. The essential oil extracted from rosemary and laurel showed significant mortality on the cowpea weevil with 1.6 (µg mL^{-1}) concentrations in both bioassays. The essential oil extracted from the sweet marjoram, the Turkish oregano, and the Greek oregano resulted in significant mortality on cowpea weevils with 1.6 (µg mL^{-1}) concentrations in the second bioassay. The essential oil extracted from the laurel and rosemary had the lower lethal concentrations comparing with the Turkish oregano, sweet marjoram, Greek oregano and mountain oregano in both bioassays.

The compounds of essential oils, 1,8 cineole, camphor, α-terpinyl acetate, carvacrol, thymol, terpinene-4-ol, p-cymene, have been identified as the main volatile components and act as insecticides to the cowpea weevil. The essential oils extracted from rosemary and laurel can be recommended as a botanical insecticide in control of the cowpea weevil.

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