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Toxicological Responses of Confused Flour Beetle, *Tribolium confusum* du Val (Coleoptera: Tenebrinoidea) to Various Plant Essential Oils

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The confused flour beetle, *Tribolium confusum* du Val (Coleoptera: Tenebrionidae), is one of the most serious pests of stored cereals and processed cereal products. The essential oils obtained from rosemary (*Rosmarinus officinalis* L.), common thyme (*Thymus vulgaris* L.), common sage (*Salvia officinalis* L.), oregano (*Origanum syriacum* L), Turkish oregano (*Origanum onites* L.), sweet marjoram (*Origanum majorana* L.), Greek oregano (*Origanum vulgare* L.) and mountain oregano (*Origanum minutiflorum* L.) have been analyzed by GC and GC-MS and tested for their toxicological effects against the confused flour beetle. In the current studies, γ-terpinene and *p*-cymene from oregano, *p*-cymene from common thyme, β- thujone, 1,8-cineole and L-camphor from common sage, 1,8-cineole and camphor from rosemary, carvacrol from Turkish oregano, carvacrol and thymol from sweet marjoram, carvacrol and *p*-cymene from Greek oregano and mountain oregano have been identified as the main volatile components. The essential oil extracted from rosemary, sweet marjoram and common thyme caused significant mortality on the confused flour beetle. In addition, rosemary had the lowest lethal concentrations (LC_{50}: 1.12, 0.598) in both bioassays. The common thyme and sweet marjoram had followed it as a second lower lethal concentration. Therefore, the essential oils extracted from rosemary, sweet marjoram and common thyme can be recommended as a potential source of environment-friendly botanical insecticide in control of the confused flour beetle. However, essential oil extracted from Turkish oregano, Greek oregano and mountain oregano had the lowest mortality while the higher lethal concentration. Therefore, they can not be suggested for controlling of the confused flour beetle in the stored products.

Key Words: Essential oils, γ-Terpinene, *p*-Cymene, β-Thujone, 1,8-Cineole, *Tribolium confusum*, Stored products.

INTRODUCTION

The confused flour beetle, *Tribolium confusum* du Val (Coleoptera: Tenebrionidae), is one of the most serious pests of stored cereals and processed

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cereal products worldwide[1-3]. It is about 1/4 of an inch long, reddish-brown in colour and its antennae end club-like, the 'club' consisting of four segments[1]. Even under unsuitable conditions, they can easily survive, develop, reproduce and build up high population densities[4]. The control of these pests mostly relies on use of chemical insecticides[5-8]. The continuous use of chemical insecticides for control of pests has resulted in serious problems such as resistance to the insecticides, pest resurgence, elimination of economically beneficial insects, toxicity to humans and wildlife[9]. These problems and demand for pesticide-free foods have increased efforts to find alternative management options.

Essential oils are potential alternatives to current stored-grain fumigants due to their low toxicity to warm-blooded mammals and their high volatility[10]. The plants belonging to the Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, Aristolochiaceae and Malvaceae families are important sources of natural insecticides[11]. Their components show ovicidal, repellent, antifeedant, sterilization and toxic effects to insects[11,12]. The fumigant activity of essential oils on adults, larve and eggs of stored-product insects have been reported by Huang *et al.*[13,14] and Tunc *et al.*[15]. In addition, plants in Labiatae family possess essential oils which act as fumigants and use against stored-product insects[10,15-21]. Therefore, understandings of the plant biochemistry of natural products have shown that the secondary metabolites may be used for pest control to overcome the above problems associated with the synthetic pesticides. The purpose of this study is to investigate toxicological responses of the confused flour beetle to essential oils extracted from eight Labiatae plants.

EXPERIMENTAL

Two bioassays were conducted at laboratory of the University of Mustafa Kemal, Hatay, Turkey.

Rearing insects: For both bioassays, adults of confused flour beetle were reared at laboratory cultures on wheat grains at 26 ºC, 65 % relative humidity with a photoperiod of 12 h light-dark.

Essential oil: Eight Labiatae family plants, rosemary (*Rosmarinus officinalis* L.), common thyme (*Thymus vulgaris* L.), common sage (*Salvia officinalis* L.), oregano (*Origanum syriacum* L), Turkish oregano (*Origanum onites* L.), sweet marjoram (*Origanum majorana* L.), Greek oregano (*Origanum vulgare* L.) and mountain oregano (*Origanum minutiflorum* L.), were grown in Telkalis Research Farm of Mustafa Kemal University, Hatay-Turkey in 2007. Essential oils 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 × 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 (0.5 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.

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 10-400 u. Identification of the compounds was achieved by comparing retention times and mass spectra with those of the standards in the library[22,23].

Laboratory bioassay: The first bioassay was set up on 25 August and the following on 07 September in 2008.

Five essential oil concentrations (2, 4, 8, 16, 32 µg/100 mL jar) from eight Labiatae families plants were used in both bioassays conducted completely randomized design with four replications. A 100 mL glass jar with screwed plastic caps was used in both bioassays. Each of the jar contained 30 g of wheat grains and 10 adults of the confused flour beetle. A small piece of the filter paper was attached to under surface of 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 72 h later and number of dead confused flour beetle were counted for each of the glass jar. Data were analyzed by analysis of variance (ANOVA) using the SAS software and the Probit analysis ($p < 0.05$). Means were separated by using the Student-Newman-Keuls (SNK) multiple comparison tests ($p < 0.05$)[24].

RESULTS AND DISCUSSION

The essential oil extracted from rosemary resulted in significant mortality on the confused flour beetle with all concentrations in both bioassays (F: 20.11, P: 0.0001; F: 14.49, P: 0.0001, respectively) (Tables 1 and 2). In the first bioassay, the common thyme and common sage had a 100 % mortality with 16 and 32 (µg mL^{-1}) concentrations (F: 20.11, P: 0.0001) (Table-1). However, in the following bioassay, 16 (µg mL^{-1}) concentration had a 100 % mortality on confused flour beetle (F: 14.49, P: 0.0001) (Table-2). The essential oil extracted from oregano had also significant mortality in the first bioassay but not following bioassay (Tables 1 and 2). In addition, the essential oil extracted from sweet marjoram had significant mortality with 8, 16 and 32 (µg mL^{-1}) concentrations in the first bioassay but not in

TABLE-1
EFFECT OF VARIOUS ESSENTIAL OILS ON *T. confusum* UNDER STANDARD LABORATORY CONDITIONS, CONDUCTED ON 25 AUGUST 2008

Labiatae plants	Per cent of *T. confusum* mortality / Dose (µg mL^{-1})^x					n^y	Intercept (±SE)	Slope (±SE)	X^2	$^zLC_{50}$	p < 0.05
	2	4	8	16	32						
Rosmarinus officinalis L.	70.00ab	85.00a	87.50a	100.00a	100.00a	200	-0.21 ± 0.48	3.19 ± 0.77	16.81	1.17	0.0001
Thymus vulgaris L.	7.50d	17.50d	22.50d	100.00a	100.00a	200	-5.93 ± 0.82	6.51 ± 0.86	56.71	8.14	0.0001
Salvia officinalis L.	17.50d	37.50cd	67.50ab	100.00a	100.00a	200	-3.59 ± 0.55	5.31 ± 0.72	53.87	4.74	0.0001
Origanum syriacum L.	0.00d	7.50d	10.00d	90.00a	97.50a	200	-9.57 ± 1.40	9.21 ± 1.33	47.45	10.93	0.0001
Origanum onites L.	0.00d	0.00d	10.00d	15.00d	30.00cd	200	-5.91 ± 1.09	3.43 ± 0.83	16.89	52.91	0.0001
Origanum majorana L.	32.50cd	52.50bc	82.50a	97.50a	100.00a	200	-2.33 ± 0.47	4.48 ± 0.68	43.77	3.32	0.0001
Origanum vulgare L.	0.00d	0.00d	0.00d	2.50d	0.00d	200	-7.30 ± 3.56	1.89 ± 2.84	0.44	72.75	0.5060
Origanum minutiflorum L.	0.00d	0.00d	0.00d	0.00d	5.00d	200	-134.99 ± 187.22	87.72 ± 124.38	0.00	34.571	0.9990

^{x}Numbers within a row not followed by the same letter are significantly different ($p < 0.05$) by SNK and Probit Analysis. ^{y}Number of *T. confusum* 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 *T. confusum* UNDER STANDARD LABORATORY CONDITIONS, CONDUCTED ON 7 SEPTEMBER 2008

Labiatae plants	Per cent of *T. confusum* mortality / Dose (µg mL^{-1})^x					n^y	Intercept (±SE)	Slope (±SE)	X^2	$^zLC_{50}$	p < 0.05
	2	4	8	16	32						
Rosmarinus officinalis L.	72.50ab	95.00a	92.50a	100.00a	95.00a	200	0.53 ± 0.51	2.40 ± 0.75	10.23	0.598	0.0014
Thymus vulgaris L.	17.50de	22.50de	57.50a-d	100.00a	95.00a	200	-3.66 ± 0.53	4.77 ± 0.63	57.07	5.860	0.0001
Salvia officinalis L.	0.00e	17.50de	40.00b-e	100.00a	97.50a	200	-6.76 ± 0.97	7.61 ± 1.05	51.87	7.720	0.0001
Origanum syriacum L.	2.50e	0.00e	5.00e	70.00a-c	82.50ab	200	-7.73 ± 1.10	6.55 ± 0.93	49.46	15.12	0.0001
Origanum onites L.	5.00e	0.00e	12.50de	27.50de	50.00b-e	200	-4.96 ± 0.78	3.28 ± 0.62	27.48	32.35	0.0001
Origanum majorana L.	25.00de	25.00de	57.50a-d	75.00ab	75.00ab	200	-1.95 ± 0.38	2.24 ± 0.39	31.93	7.420	0.0001
Origanum vulgare L.	5.00e	10.00e	7.50e	15.00de	42.50 b-e	200	-4.02 ± 0.68	2.28 ± 0.56	16.41	57.63	0.0001
Origanum minutiflorum L.	2.50e	2.50e	30.00c-e	2.50e	0.00e	200	-2.30 ± 0.61	-0.24 ± 0.63	0.144	2.586E-10	0.7045

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

the following bioassay. However, the essential oil extracted from Turkish oregano, Greek oregano and mountain oregano had the lowest mortality on the confused flour beetle in both bioassays (Tables 1 and 2).

The essential oil extracted from rosemary had the lowest lethal concentrations (LC_{50}: 1.12, 0.598) in both bioassays (Tables 1 and 2). In the first bioassay, sweet marjoram had the second lowest lethal concentration (Table-1). The common sage, common thyme, oregano (*O. syriacum*), pot marjoram, oregano (*O. vulgare*) and Turkish oregano were followed. In the following bioassay, common thyme had the second lowest lethal concentration (Table-2). The sweet marjoram, common sage, oregano (*O. syriacum*), Turkish oregano, Greek oregano and mountain oregano were followed.

More than 25 components were identified in each of essential oil, but most of them constituted less than 1 % (data not given). Only 6 components detected in essential oil extracted from oregano. The components of the myrcene, α-terpinene, γ-terpinene, terpinene-4-ol, β-caryophyllene and *p*-cymene were greater than 2 % (Table-3). Nine components, 1,8-cineole, linalool, myrcene, myrcene α-terpinene, γ-terpinene, terpinene-4-ol, thymol, *p*-cymene and carvacrol, were detected from common thyme. Nine components, 1,8-cineole, α-humulene, β-pinene, α-thujone, β-thujone, L-camphor, β-caryophyllene, viridiflorol and *p*-cymene, were detected from common sage. Eight components, 1,8-cineole, linalool, endobornyl acetate, terpinene-4-ol, *p*-cymene, borneol, camphor and 1,3-dimethylbicyclo were detected from rosemary. 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 oregano, common thyme, common sage, rosemary, Turkish oregano, sweet marjoram, Greek oregano and mountain oregano were γ-terpinene, *p*-cymene, thymol, 1,8-cineole, β-thujone, L-camphor, camphor, carvacrol and terpinene-4-ol, respectively (Tables 3 and 4).

Essential oils, consisting of complex mixtures of monoterpenoids and biogenetically related phenols, sesquiterpenes, demonstrate a wide range of bioactivities such as direct toxicity to insects, feeding deterrence, repellence and attraction. The previous studies have been reported that essential oil could protect stored products from confused flour beetle by using as fumigants and repellents. With the current study, adults of the confused flour beetle were exposed to various concentrations of essential oils extracted from oregano (*O. syriacum*), common thyme, common sage, rosemary, Turkish oregano, sweet marjoram, Greek oregano and mountain oregano in two laboratory bioassays.

TABLE-3
CHEMICAL COMPONENTS IN ESSENTIAL OILS OF *Origanum syriacum*, *Thymus vulgaris*, *Salvia officinalis* AND *Rosmarinus officinalis*

Components	*O. syriacum*	*T. vulgaris*	*S. officinalis*	*R. officinalis*
1,8-Cineole	–	2.40	22.83	21.45
Linalool	–	4.60	–	5.88
Endobornyl acetate	–	–	–	2.44
Methyl eugenol	–	–	–	1.02
α-Humulene	–	–	2.82	–
Myrcene	6.00	2.11	1.96	1.90
Sabinene	1.19	–	–	–
α-Terpinene	8.86	2.62	–	1.00
γ-Terpinene	36.17	10.07	–	1.07
Terpinene-4-ol	3.59	2.53	0.93	3.12
Thymyl methylether	–	1.31	–	–
Carvacrol methyl ether	–	1.17	–	–
Thymol	–	17.88	–	–
β-Pinene	–	–	2.63	0.92
α-Thujone	–	–	4.29	–
β-Thujone	–	–	25.05	–
L-camphor	–	–	18.38	–
β-Caryophyllene	4.52	1.71	3.83	0.94
Viridiflorol	–	–	4.51	–
α-Phellandrene	1.01	–	–	–
L?monene	1.30	–	–	–
p-Cymene	30.25	40.00	2.03	3.08
trans-Sabinene hyrate	1.52	–	–	–
Carvacrol	–	3.79	–	–
Borneol	–	–	1.30	8.58
Verbenene	–	–	–	1.79
Camphor	–	–	–	19.70
6,6-Trimethylbicyclo	–	–	–	1.91
1,3-Dimethylbicyclo	–	–	–	8.24
Nopol	–	–	–	1.27

The essential oil extracted from rosemary caused significant mortality on the confused flour beetle with all concentrations in both bioassays. The rest of the essential oils extracted from different plants species resulted in significant mortality on the confused flour beetle with 16 and 32 µL/100 mL jar. The toxicological effects of the essential oils can be attributed to their major components[15,25,26].

In present study, γ-terpinene and *p*-cymene from oregano (*O. syriacum*), *p*-cymene from common thyme, β-thujone, 1,8-cineole and L-camphor from common sage (*S. officinalis*), 1,8-cineole and camphor from rosemary, carvacrol from Turkish oregano, carvacrol and thymol from sweet marjoram, carvacrol and *p*-cymene from Greek oregano and mountain oregano have been identified as the main volatile components. Insecticidal activities of essential oils have been described to differentiate

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	–	–

the major active ingredients (γ-terpinene, *p*-cymene, β- thujone and 1,8-cineole). Present study also confirmed the previous studies conducted by several workers[27-29].

In conclusion, the essential oils extracted from rosemary, sweet marjoram and common thyme caused significant mortality on the confused flour beetle. In addition, rosemary had the lowest lethal concentrations (LC_{50}: 1.12, 0.598) in both bioassays. The common thyme and sweet marjoram followed it as the second lowest lethal concentration. Therefore, the essential oils extracted from rosemary, sweet marjoram and common thyme can be suggested for controlling the confused flour beetle in stored products. However, the essential oils extracted from Turkish oregano, Greek oregano and mountain oregano had the lowest mortality, but the higher lethal concentration on the confused flour beetle in both bioassays. Therefore, they can not be suggested for controlling of the confused flour beetle in stored products.

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