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Fumigant Toxicity of Five Essential Oils Against the Eggs of *Ephestia kuehniella* Zeller and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

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The ovicidal activity of five essential oil vapours distilled from savory *Satureja thymbra* L., laurel *Laurus nobilis* L., myrtle *Myrtus communis* L., lemon *Citrus limon* L. and marjoram *Origanum majorana* L. were tested against of two stored-product moths namely the Indian meal moth *Plodia interpunctella* and the Mediterranean flour moth, *Ephestia kuehniella*. Of all the samples tested, the essential oil obtained from savory produced 100 % mortality for the eggs of both *E. kuehniella* and *P. interpunctella*. The highest mortalities caused by essential oils of myrtle, laurel, marjoram and lemon were 57.50, 45.83, 42.50 and 27.50 % for *E. kuehniella* eggs and were 41.66, 50.83, 57.50 and 26.66 % for *P. interpunctella* eggs, respectively. At the moderate dose (50 μL/L air) the LT_{99} values of the most effective essential oil savory were 158.50 and 81.88 h for the eggs of *E. kuehniella* and *P. interpunctella*, respectively.

Key Words: Mortality, Essential oils, Stored-product pests.

INTRODUCTION

The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is one of the major pests in the industrial flour mills in temperate climates[1]. Larvae reduce product quality by their presence and the production of frass and webbing and also cause direct damage by feeding[2]. The Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is a cosmopolitan pest that infests a wide range of stored-products including nuts, beans, processed foods and dried fruits[3]. The control of these pests in many storage systems depends on fumigation with methyl bromide and

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phosphine. However, methyl bromide was banned in many countries starting from 2004[4]. Many alternatives have been tested to replace methyl bromide fumigation for stored-product and quarantine uses. During recent years, some plants have been receiving global attention and certain secondary metabolites have been formulated as botanical pesticides for plant protection that do not leave residues toxic to the environment, have lower toxicity to mammals, medicinal properties for humans[5] and are non-persistent in water and soil[6].

The insecticidal activity of essential oils and plant extracts against different stored-product pests has been evaluated[7-14]. In spite of the wide recognition that many plants possess insecticidal properties, only a handful of pest control products directly obtained from plants are presently in use because the commerciali-zation of new botanicals can be hindered by a number of issues[15]. Botanicals used as insecticides presently constitute 1 % of the world insecticide market[16]. Essential oils from different plant species possess ovicidal, larvicidal and repellent properties against various insect species and are regarded as environmentally compatible pesticides[17,18]. Although much attention has been given to the fumigant activity of essential oils on adults and larvae, very little consideration has been given to the egg stage[11].

At present study we aimed to investigate the fumigant toxicity of essential oils of five aromatic plant species from Turkey against the eggs of two stored-product moths, the Mediterranean flour moth, *E. kuehniella* and Indian meal moth *P. interpunctella*.

EXPERIMENTAL

Mediterranean flour moth (MFM), *Ephestia kuehniella* Zeller, (Lepidoptera: Pyralidae) culture obtained from department of plant protection, Faculty of Agriculture of Ankara University. *E. kuehniella* larvae reared using a mixture consisting of 1 kg wheat flour, 55 g yeast and 30 g germs of wheat[22]. Indian meal moth (IMM), *Plodia interpunctella* (Hübner) used in this experiment were taken as larvae from naturally infested dried apricot collected from Kayseri province of Turkey. The larvae of *P. interpunctella* were maintained continuously on a diet containing 10 % glycerol, 50 % dried apricot and 40 % wheat flour with wheat bran mixture. Throughout the experiments insect cultures were maintained at constant temperature (27 ± 1 °C), 14L:10D photoperiod and 60 ± 5 % relative humidity.

The plants savory *Satureja thymbra* L. (Lamiaceae), laurel *Laurus nobilis* L. (Lauraceae) myrtle, *Myrtus communis* L. (Myrtaceae) and lemon *Citrus limon* L. (Rutaceae) were collected from Mersin (Southern Turkey), marjoram *Origanum majorana* L. (Lamiaceae) was collected from Canakkale (Western Turkey).

Extraction of essential oil: The leaves of plants were air dried at room temperature and chopped into small pieces using a mill with rotary knives. The essential oil from the plants was extracted using a Clevenger-type water steam distillation apparatus. The distilled essential oils were stored in a refrigerator at 4 °C until treatments.

Collection of eggs: To secure effective pupation the strips of corrugated card board were placed on the surface of the moth diet along the inside of the rearing jars. After adult emergence sufficient number of test adults of *E. kuehniella* and *P. interpunctella* were transferred to plexiglas cylinders with wire mesh covering one end for oviposition. The eggs were collected from Petri dishes placed under plexiglas cylinders.

Fumigant toxicity: In order to test the toxicity of essential oils on the *E. kuehniella* and *P. interpunctella* eggs freshly laid host eggs (< 24 h) were glued on the cardboards (40 eggs on each) and these cards were placed in the 300 mL glass jars. Essential oils were applied on a blotting paper strip measuring 3 cm × 3 cm which was attached to the lower side of the jar's lid. The eggs were exposed to essential oil vapours for different exposure time (24-96 h) and doses (25-200 μL/L air). After exposure the eggs were transferred to glass Petri dishes containing larval diet. The number of egg hatch was counted and the LT_{50} and LT_{99} values were estimated for each treatment. Three replicates were set up for each dose and exposure time.

Statistical analysis: The data were corrected for the mortalities in the controls and were subjected to probit analysis using SPSS[20] for Windows to estimate LT_{50} and LT_{99} values for each plant materials and doses.

RESULTS AND DISCUSSION

Effects of lemon: The data obtained from the two stored-product moth eggs exposed to lemon were summarized in Table-1. The lowest effective essential oil was obtained from lemon plant. At the highest dose (200 μL/L air) and exposure time (96 h) the per cent mortality of the eggs was 27.5 and 26.66 % for *E. kuehniella* and *P. interpunctella* showing the similar ovicidal effects (Figs. 1 and 2). To secure 99 % mortality times (LT_{99}) needed were 185.65 (134.60-365.31) and 193.26 (141.24-361.65) h. for the eggs of *E. kuehniella* and *P. interpunctella*, respectively.

Effects of laurel: Ovicidal activity of the laurel on the *P. interpunctella* eggs was higher than *E. kuehniella* eggs and percentages of unhatched eggs were 50.83 and 45.83 %, respectively (Figs. 1 and 2). To kill 99 % of the eggs LT_{99} values were estimated as 120.23 (99.88-158.96) and 108.87 (82.11-194.00) h at the highest dose and exposure time for the eggs of *E. kuehniella* and *P. interpunctella*, respectively (Table-2).

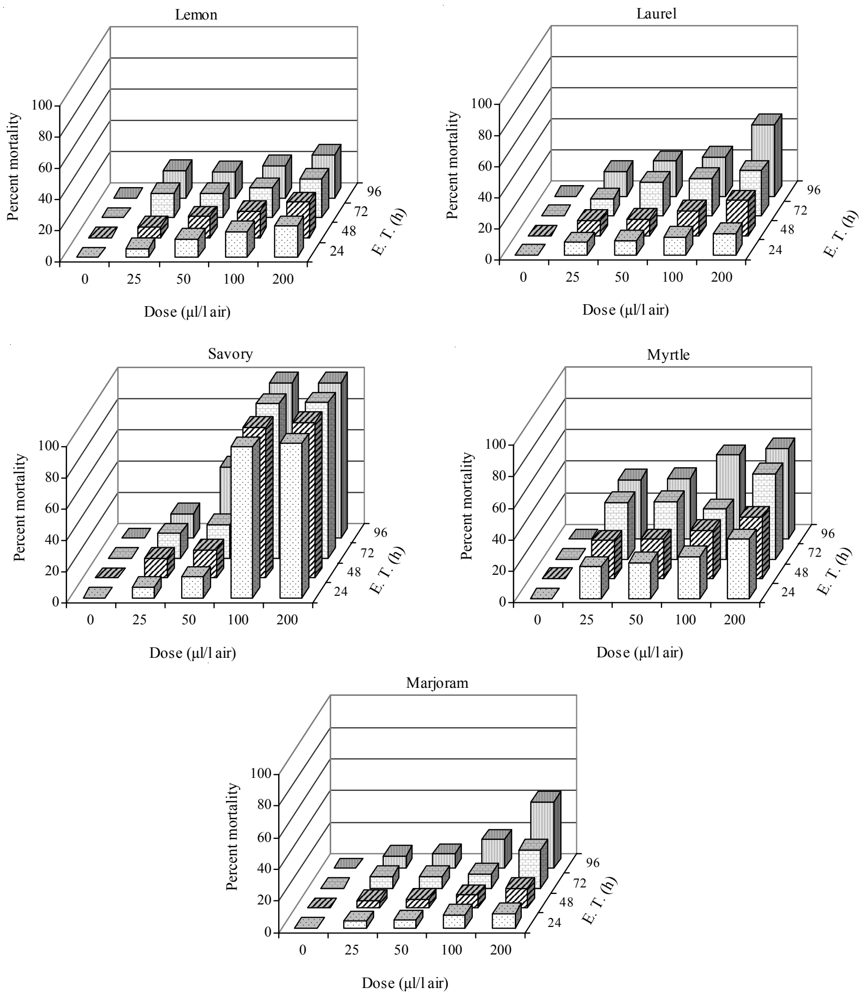

Fig. 1. Per cent mortality of the *E. kuehniella* eggs after exposure five different essential oils for different doses and exposure times. E. T. (h) : Exposure time (h)

Effects of savory: Of all the essential oils tested the highest mortality values were obtained for the savory exposed eggs. The percentage of hatched eggs of the *E. kuehniella* and *P. interpunctella* exposed to savory was negatively correlated to the doses and exposure times (Figs. 1 and 2). Mortality values of the eggs at the highest dose (200 µL/L air) and exposure time (96 h) were 100 % for both *E. kuehniella* and *P. interpunctella* eggs. The times required for 99 % mortality (LT_{99}) at 50 µL/L air doses were 158.50 (111.57-368.64) and 81.88 (63.40-136.23) h for *E. kuehniella* and *P. interpunctella* eggs, respectively (Table-3). The savory was more effective on *P. interpunctella* eggs than *E. kuehniella* eggs at 50 µL/L air dose.

TABLE-1
LT_{50} (h) AND LT_{99} (h) VALUES OF DIFFERENT CONCENTRATIONS OF ESSENTIAL OILS FROM LEMON AGAINST THE EGGS OF *E. kuehniella* AND *P. interpunctella*

Dose (µL/L air)	Lemon				
	Treated eggs (n)	LT_{50}	LT_{99}	df	χ^2
E. kuehniella					
25	120	96.63	224.29	13	56.98
50	120	96.74	280.73	13	65.04
100	120	76.53	250.40	13	65.45
200	120	52.92	185.65	13	94.35
P. interpunctella					
25	120	108.91	311.14	13	52.58
50	120	99.38	322.68	13	73.94
100	120	66.85	241.37	13	75.82
200	120	50.23	193.26	13	75.49

TABLE-2
LT_{50} (h) AND LT_{99} (h) VALUES OF DIFFERENT CONCENTRATIONS OF ESSENTIAL OILS FROM LAUREL AGAINST THE EGGS OF *E. kuehniella* AND *P. interpunctella*

Dose (µL/L air)	Laurel				
	Treated eggs (n)	LT_{50}	LT_{99}	df	χ^2
E. kuehniella					
25	120	110.99	286.830	13	34.46
50	120	77.45	196.440	13	24.48
100	120	66.98	184.770	13	33.91
200	120	46.26	120.230	10	27.46
P. interpunctella					
25	120	102.44	255.450	13	20.87
50	120	89.72	269.430	13	81.34
100	120	50.63	188.143	13	152.83
200	120	38.98	108.870	10	86.39

Effects of myrtle: Different from essential oils obtained from laurel, ovicidal activity of the myrtle oil was higher on *E. kuehniella* eggs than *P. interpunctella*. At the highest dose and exposure time percentages of the unhatched eggs were 57.5 and 41.66 % for the eggs of *E. kuehniella* and *P. interpunctella* (Figs. 1 and 2). The times required for 99 % mortality was 53.36 (35.95-213.13) and 179.33 (116.96-700.35) h at the highest dose and exposure time for the eggs of *E. kuehniella* and *P. interpunctella*, respectively (Table-4).

TABLE-3
LT_{50} (h) AND LT_{99} (h) VALUES OF DIFFERENT CONCENTRATIONS OF ESSENTIAL OILS FROM SAVORY AGAINST THE EGGS OF *E. kuehniella* AND *P. interpunctella*

Dose (µL/L air)	Savory				
	Treated eggs (n)	LT_{50}	LT_{99}	df	χ^2
		E. kuehniella			
25	120	97.75	251.24	13	64.36
50	120	58.58	158.50	10	66.49
100	120	-	-	-	-
200	120	-	-	-	-
		P. interpunctella			
25	120	71.92	190.49	13	60.59
50	120	34.64	81.88	8	56.36
100	120	-	-	-	-
200	120	-	-	-	-

- : It was impossible to estimate LT_{50} or LT_{99} values due to 100 % mortality.

TABLE-4
LT_{50} (h) AND LT_{99} (h) VALUES OF DIFFERENT CONCENTRATIONS OF ESSENTIAL OILS FROM MYRTLE AGAINST THE EGGS OF *E. kuehniella* AND *P. interpunctella*

Dose (µL/L air)	Myrtle				
	Treated eggs (n)	LT_{50}	LT_{99}	df	χ^2
		E. kuehniella			
25	120	43.39	141.15	11	86.060
50	120	40.53	132.39	11	70.180
100	120	32.11	126.90	11	148.630
200	120	22.23	53.36	5	63.920
		P. interpunctella			
25	120	116.30	314.11	13	29.470
50	120	102.08	285.97	13	61.370
100	120	99.13	299.01	13	76.343
200	120	50.42	179.33	11	112.130

Effects of marjoram: Per cent mortality values of the *E. kuehniella* and *P. interpunctella* eggs increased depending on the doses and exposure time (Figs. 1 and 2). Mortality values of the eggs at the highest dose and exposure time were 42.5 and 57.5 % for *E. kuehniella* and *P. interpunctella* eggs, respectively. To obtain 99 % mortality the times (LT_{99}) required were 127.82 (109.54-159.95) and 90.19 (69.77-147.96) h for the eggs of the *E. kuehniella* and *P. interpunctella*, respectively (Table-5).

TABLE-5
LT_{50} (h) AND LT_{99} (h) VALUES OF DIFFERENT CONCENTRATIONS OF ESSENTIAL OILS FROM MARJORAM AGAINST THE EGGS OF *E. kuehniella* AND *P. interpunctella*

Dose (µL/L air)	Marjoram				
	Treated eggs (n)	LT_{50}	LT_{99}	df	χ^2
		E. kuehniella			
25	120	156.70	361.40	13	17.97
50	120	154.28	366.09	13	28.16
100	120	106.62	264.03	13	52.13
200	120	57.98	127.82	12	37.64
		P. interpunctella			
25	120	137.26	352.62	13	38.68
50	120	92.27	258.74	13	64.09
100	120	69.86	231.30	13	55.98
200	120	35.66	90.19	9	69.05

From the essential oils tested in this experiment, savory showed the highest ovicidal activity against the eggs of the *E. kuehniella* and *P. interpunctella*. The essential oil obtained from lemon showed similar ovicidal activity against both *E. kuehniella* and *P. interpunctella* and the activity of this oil was the lowest of all the tested essential oils. Sarac and Tunc[10] reported that responses of egg and active stages of stored-product insects to the essential oils are different. In the present study the myrtle has more ovicidal activity against *E. kuehniella* eggs than *P. interpunctella* at the same exposure time and doses.

The authors used < 24 h age eggs in this experiment and ovicidal activity increased depending on the increasing exposure time (*e.g.* the percentage of unhatched eggs at 200 µL/L air was 83.33 % at 24 h exposure, this value increased to 100 % at 96 h exposure for savory). The changes in the permeability of the chorion and/or vitelline membrane may occur during embryogenesis and may facilitate the diffusion of vapours into older eggs so that vital physiological and biochemical processes are affected[21].

Fumigant activity of *Origanum majorana* against *Culex pipiens* L. larvae[22] and *Laurus nobilis* on *Sitophilus oryzae* (L.), *Rhyzopertha dominica* Fabricius and *Tribolium castaneum* (Herbst) adults were reported[16]. In present study, percentage mortality of marjoram was 42.5 and 57.5 % at the highest exposure time and doses for the eggs of *E. kuehniella* and *P. interpunctella*, respectively. Laurel caused a moderate mortality for the eggs of two stored-product moths (45.83 % for *E. kuehniella* and 50.83 % for *P. interpunctella*) at the 96 h exposure time and 200 µL/L air dose.

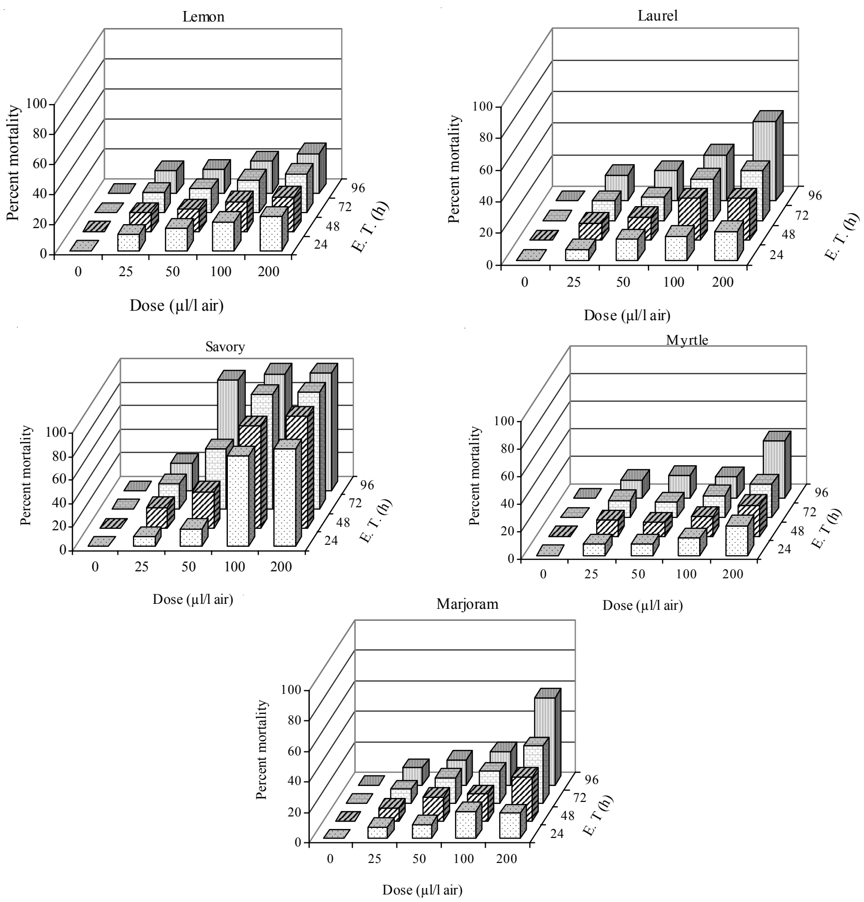

Fig. 2. Per cent mortality of the *P. interpunctella* eggs after exposure five different essential oils for different doses and exposure times E.T. (h): exposure time (h)

The ovicidal effect of these essential oils is advantageous because the destructive larval stage is never reached[23]. If the cost-effective commercial problems can be solved, the essential oils obtained from these plants can be effectively used as a part of integrated pest management strategies[16]. The essential oil content of aromatic plants is about 1-3 %. Therefore large quantities of plant material have to be processed in order to obtain essential oils in quantities sufficient for commercial scale test[11]. It would be useful to breed the plants containing desired essential oils in elevated quantity.

In the present study and those reported earlier showed variation in the activity of essential oils regarding the plant origin, stage and species of the insect[11]. All the essential oils tested in this study did not produced desired results at the treated doses and exposure times, but the essential oil of savory yielded promising results to control stored-product moths, *E. kuehniella* and *P. interpunctella* when these moths were egg stage.

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