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Insecticidal activities of *Thymus vulgaris* essential oil and its components (thymol and carvacrol) against larvae of lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae)

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ABSTRACT

The insecticidal activity of *Thymus vulgaris* essential oil, thymol and carvacrol was evaluated in laboratory against different larval stages of lesser mealworm (*Alphitobius diaperinus* Panzer, Coleoptera, Tenebrionidae). The earlier and later larval stages were reared on diets containing 1 or 2% acetone solutions of tested compounds. Insecticidal activity of thyme essential oil and pure monoterpenes against *A. diaperinus* larvae depended on the dose and age of larvae. The growth of younger larvae was significantly affected, while those of older larval stage was less influenced and only by pure oil components. In young larvae the application 1% thyme oil, thymol and carvacrol, caused mortality of 50.0, 86.67 and 85 %, respectively. However, the mortality was less in old larvae (17.5, 27.5 and 27.5%, respectively). At the highest dose (2%) thyme oil, thymol and carvacrol killed 62.5, 91.67 and 97.5% of young larvae, respectively. These results showed that thymol and carvacrol were more active against *A. diaperinus* larvae than thyme oil, thus these two pure components (thymol and carvacrol) can effective control this pest.

Key words: Allelochemicals, *Alphitobius diaperinus*, carvacrol, essential oils, lesser mealworm, thymol, *Thymus vulgaris*

INTRODUCTION

Essential oils (EOs) and their major components, mainly the monoterpenoids are potential source of ecologically safe botanical insecticides. These oils are formed by aromatic plants as secondary metabolites and are widely used in medicine, food and perfume industries and for crop protection. In nature, essential oils play major role in protection of plants as antibacterials, antivirals, antifungals (10,15,26) and insecticides. These secondary compounds are larvicidal, pupicidal and adulticidal, most being repellents, oviposition deterrents, or antifeedants against both agricultural pests and medically/veterinary important insects (2,23). They also may attract some insects to favour the dispersion of pollens and seeds. Essential oils are very complex mixtures (contains

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about 20-60 components in different concentrations). They typically consists of two or three major components present in high concentrations (20-70%) than other components present in trace amounts (4). Generally, the major components determine the biological properties of essential oils from which they were isolated. Carvacrol shows a strong antimutagenic activity similar to *Origanum onites* L. essential oil, because carvacrol is the main component in this oil (13). On the other hand, the insecticidal activity of mint essential oils against *Drosophila melanogaster* larvae is not in accordance with their major constituents (pulegone, menthone and carvone). Among the compounds studied, pulegone was more toxic than *Mentha pulegium* essential oil containing 75.7% pulegone (8). Thymol was 1.6-folds more toxic against malarial vector, *Anopheles stephensi* than the essential oil of *Trachyspermum ammi* (23). Contrarily, the *Majorana hortensis* essential oil was more toxic against *Spodoptera littoralis* and *Aphis fabae* than the isolated compounds, γ-terpinene and terpinen-4-ol (1). Thus the activity of major components may be modulated by other minor molecules and the biological activity of essential oil is due to synergistic/antagonistic interactions of all molecules present.

The insecticidal activity of essential oils has been reviewed recently (25). The sensitivity of different insect species to the same substance can vary. The effects of natural plant-derived compounds on survivability, behaviour, growth and development of lesser mealworm, *Alphitobius diaperinus* Panzer, a cosmopolitan pest inhabiting chicken and broiler houses are little studied than other species of family *Tenebrionidae* (14,22,30). The essential oils isolated from sassafras (*Ocotea odorifera*) and eucalyptus (*Eucalyptus viminalis*) are insecticidal to lesser mealworm larvae, *A. diaperinus* (24). The extracts from *Ruta graveolens, Chenopodium ambrosioides* and star anise, *Illicium verum* are very toxic to *A. diaperinus* (21,33). Both natural terpenes and their synthetic derivatives are deterrents to larvae and adults of *A. diaperinus*. While hydroxy-γ-spirolactones obtained from the pulegone and bicyclic γ-hydroxy-δ-lactones obtained from isopulegol are good feeding deterrents (31,32).

The *A. diaperinus* in poultry houses is controlled using chemical insecticides (synthetic pyrethroids, organophosphates, chitin synthesis inhibitors), but complete control is very difficult, due to resistance and inaccessibility of all developmental stages that occur in soil (8,9). For its control, the use of plant extracts, including essential oils with known insecticidal effects, may be an alternative method to the heavy use of insecticides. The compounds in these extracts through a synergistic action may also enhance the activity of insecticides used (28) and could also be used as flavour supplements to increase the feed intake in poultry and hence becoming safe alternatives to antibiotic growth stimulators (6). The European Union directives have prohibited the use of these stimulators in feed stuff from January 2006. Introducing essential oils into poultry houses may help in improving the poultry sanitary conditions and eliminate the poultry pathogens and parasites. The chicken bred on litter sprayed with solutions of thymol and cinnamaldehyde had better health and lower mortality (36).

Therefore, these studies were performed to compare the insecticidal activity of thyme oil and its components, thymol and carvacrol against *A. diaperinus* larvae by incorporating these compounds into the food of insects.

MATERIALS AND METHODS

I. Plant material

Fresh thyme (*Thymus vulgaris* L.) was supplied by Swedeponic Polska (Kraśnicza Wola, Poland) Herbal Company. A voucher of appropriate specimen was deposited in Herbarium, Department of Chemistry Wrocław University of Environmental and Life Sciences. Only the leaves of dried plants (40^{o}C) were used for distillation. The essential oil from the dried leaves of *Thymus vulgaris* L. was obtained by hydrodistillation. The oil contained 57.44% thymol and 2.80% carvacrol.

II. Preparation of thyme essential oil

A Deryng apparatus was used to extract the volatile compounds of thyme (34). 100 g dried thyme leaves were placed in 2 L round flask with 900 mL distilled water. It was heated for 2 h after reaching the boiling point. The vapours were condensed by cold refrigerant. After 2 h distillation, 1.1 mL of essential oil (containing the volatile compounds) was collected in 2.5 mL vial and kept at -15^{o}C until the GC- MS analyses and biological tests were done.

III. Chromatographic analyses

The isolation, identification and quantification of volatile compounds was done using a gas chromatograph (GC) coupled to a mass spectrometer (MS), a Saturn 2000 MS Varian Chrompack with a DB-5 (5% phenyl methylpolysiloxane) 30 m x 0.25 mm ID x 0.25 μm film column (34). The MS was equipped with an ion-trap analyzer set at 1508 for all analyses with an electron multiplier voltage of 1350 V. Scanning (1 scan s^{-1}) was performed in the range of 39–400 m/z using electron impact ionization at 70 eV. The analyses were carried out using helium as carrier gas at flow rate of 1.0 mL min^{-1} in a split ratio of 1:20 and the following program: (a) 80^{o}C for 0 min; (b) rate of 5.0^{o}C min^{-1} from 80 to 200^{o}C; (c) rate of 25^{o}C min^{-1} from 200 to 280^{o}C and held for 5 min. The injector and detector were held at 200 and 300^{o}C, respectively. A one mL volume of sample was analyzed. Most of the compounds were identified by using 3-analytical methods: (i). Kovats indices (KI), (ii). GC-MS retention indices (authentic chemicals- standards (S) and (iii). Mass spectra (authentic chemicals and NIST05 spectral library collection (MS). The retention index standards used in this study consisted of a mixture of aliphatic hydrocarbons ranging from C-5 through C-20 dissolved in hexane.

IV. Bioassays

Insects: In this study, laboratory reared strain of *A. diaperinus* collected from Poultry Farm, Toruń (53°01′N, 18°37′E, Poland) were used. The colony was kept in glass containers on a diet consisting of one part of oat flakes, one part of wheat bran, 0.01 part of brewers´ yeast and apple halves to maintain moisture levels at ca. 55%. The colony was kept in a rearing chamber at +29°C in dark.

Ingestion toxicity and larval growth bioassay. The earlier and later larval stages were used to assess the effect of *T. vulgaris* essential oil and the isolated components on larval

mortality, growth and development. The culture method for the lesser mealworm described by Rice and Lambkin was used (27) to obtain numerous larvae of same age. The body weight of larvae was 2.25-3.09 mg [earlier larval stage (10-days old)], the second group of larvae were 3-weeks old (body mass: 13.46-14.06 mg). The test oil and its major components were added at 1 and 2% acetone solution into the diet. One g oat flakes was mixed with 1 ml test solution or acetone as control. Oat flakes (dried at room temperature) were placed together with 10-larvae in plastic containers with a capacity of 100ml. The containers were transferred into the rearing chamber and kept under the same conditions for colony maintenance. There were 4-replicates for each test, substance and concentration. Body weight gain was recorded at 3-days interval and the mortality was assessed daily. Larvae were considered dead, when they did not react to touching with a needle. When development of larvae ended, the number of living pupae and their body weight were recorded. After emergence of adults their number and body weight were also recorded.

Antifeedant no-choice bioassay. Feeding deterrent activity of test substances was assayed using larvae of same age as done in larval growth bioassay. For the feeding assays, 1 and 2% acetone solutions of test compounds were prepared. Oat flakes were used as test food. One g oat flakes was dipped in either 1 ml test solution or in acetone alone as control. After evaporation of solvent (30 min of air-drying), the oat flakes were weighed and placed in Petri dishes (9 cm dia) together with 10 larvae. Four replicates of tests for each dose and larval stage were done. The dishes were transferred into a rearing chamber and kept at 29 ± 1^{o}C in dark. After 3-days feeding, the remaining uneaten oat flakes were re-weighed and the mean weight of food eaten was calculated. Absolute deterrence coefficients A based on the amount of food consumed were calculated as under (12):

$$A= C-T/C+Tx100$$

Where, C: Weight of control, T: Weight of treated food consumed by insects.

Statistical analysis: The total mortality and deterrence coefficients were statistically analysed by means of one-way analysis of variance (ANOVA) and Tukey test. *t*-Test was used for comparison the mean body weight of treated and control larvae, the number of pupae and adults and their mean body weight (11).

RESULTS AND DISCUSSION

Chemical composition of thyme essential oil

The chemical composition of essential oil is given in Table 1. Chromatographical analysis proved that the thymol chemotype of thyme was used. Besides the predominant thymol (57.44%), carvacrol (2.8%) as a monoterpenoic phenol was also present in the essential oil. The important compounds analyzed in the distillate were also monoterpenoids: γ-terpinene, *p*-cymene, and sesquiterpene caryophyllene. The content of other terpenes and terpenoids in oil was < 2%. Chemical composition and yield of essential oil was almost identical to data of Baranauskienė *et al*., (5), where the thyme cultivated in Lithuania region was used.

Table 1. Chemical composition of thyme essential oil analyzed by GC/MS and authentic chemical comparison

No.	Compound	Retention time (min)	(%)	Identification methods*
1	3-Hexen-1-ol	5.13	0.10	MS, KI, S
2	α -Thujene	6.75	1.48	MS, KI, S
3	α -Pinene	7.07	0.68	MS, KI, S
4	Camphene	7.6	0.25	MS, KI, S
5	1-Octen-3-ol	7.95	0.50	MS, KI, S
6	Sabinene	8.03	0.14	MS, KI, S
7	β - Myrcene	8.16	1.82	MS, KI, S
8	β -Pinene	8.33	1.82	MS, KI, S
9	3-Octanol	8.37	0.29	MS, KI, S
10	α - Phellandrene	8.94	0.33	MS, KI, S
11	α -Terpinene	9.19	2.37	MS, KI, S
12	*p*-Cymene	9.46	4.77	MS, KI, S
13	Limonene	9.57	0.29	MS, KI, S
14	Eucalyptol	9.72	0.35	MS, KI, S
15	*γ* -Terpinene	10.33	12.05	MS, KI, S
16	Sabinene hydrate *trans* isomer	10.78	1.33	MS, KI
17	Terpinolene	11.14	0.10	MS, KI, S
18	Linalool	11.30	1.24	MS, KI, S
19	Sabinene hydrate *cis* isomer	11.72	0.23	MS, KI
20	Camphor	13.4	0.11	MS, KI, S
21	Borneol	14.04	0.59	MS, KI, S
22	α -Terpineol	14.51	0.09	MS, KI, S
23	*trans*-Dihydrocarvone	14.65	0.05	MS, KI, S
24	Thymol methyl eter	15.15	0.35	MS, KI
25	Carvacrol methyl eter	15.44	1.10	MS, KI
26	Thymoquinon	16.09	0.08	MS, KI
27	Thymol	16.97	57.44	MS, KI, S
28	Carvacol	17.24	2.80	MS, KI, S
29	Thymol acetate	18.41	0.39	MS, KI
30	Caryophyllene	20.89	3.64	MS, KI, S
31	Germacrene D	22.46	1.00	MS, KI, S
32	Germacrene D-4-ol	24.96	0.28	MS, KI
33	τ- Cadinol	26.25	1.94	MS, KI

*MS: Mass spectrum, KI: Kovac index, S: Authentic chemicals (standard)

Growth of *A. diaperinus* larvae

Insecticidal activity of thyme essential oil and pure monoterpenes against *A. diaperinus* larvae depended on the dose and age of larvae. Significant differences in the growth of younger larvae were observed. The pure components decreased their body weight than oil. Carvacrol solutions showed high activity. During experiments, a dose-dependent manner of action was observed, but the differences between the doses were not significant. Larvae reared on diet containing 1 or 2% carvacrol solutions weighed 7.26 and 6.4 mg, respectively, after 24 days. The body weight of larvae treated thymol was only

slightly higher i.e. 8.06 and 9.14 mg for 1 or 2% concentrations, respectively (Fig. 1). While the control larvae weighed 22.7 mg and entering the pupae stage. A much lower reduction of the increase in body weight of larvae was observed with thyme essential oil After 24 days of treatment, the larvae had over 2-folds higher body mass than larvae treated with pure components. Depending on the dose of thyme oil (Fig. 1), larvae average body mass was 16.81 and 14.54 mg i.e. 74.05 and 64.05% of controls, respectively (Table 2).

Table 2. Inhibitory effects of thyme oil and its components (thymol and carvacrol) on growth of *A. diaperinus* larvae

Compound	Conc (%)	Younger larvae- 24 DAT		Older larvae- 15 DAT	
		Body weight (% of control)	Reduction in body weight (%)	Body weight (% of control)	Reduction in body weight (%)
Thyme EO	1%	74.05 a	-25.95 a	96.07 a	-3.93 a
	2%	64.05 a	-35.95 a	110.96 a	+10.96 a
Thymol	1%	35.50 b	-64.50 b	85.77 ab	-14.23 cb
	2%	40.26 b	-59.74 b	69.65 b	-30.35 c
Carvacrol	1%	31.98 b	-68.02 b	96.26 a	-3.74 b
	2%	28.19 b	-71.81 b	91.16 a	-8.84 b

DAT: Days after treatment, EO: Essential oil, Means followed by the same letters within each column are not significantly different (one-way ANOVA and Tukey test $p<0.05$)

The pure components slightly disturbed the growth of later larval stage. The control larvae ended the development, 2-weeks after their average body weight was 21.16 mg. The thyme oil actually stimulated the growth of these larvae (Fig. 2A). Twelve days after the start of experiment, the body weight of some larvae reared with 1% dose was much higher than control and these larvae also developed into pupae with higher body weight than control. With pure compounds small fluctuations occurred in body weight of larvae. Larvae treated with thymol were relatively smaller. Two weeks after the start of treatment with 1% thymol, carvacrol and thyme EO, the body mass of larvae was 85.77, 96.26 and 96.07 % of control, respectively. At higher dose of test substances, these values were lower for pure compounds e.g. 69.65 % with thymol and 91.16% with carvacrol. In 2% thyme oil, the larvae body mass was increased (10.96%) than control (Table 2).

Ingestion toxicity of thyme oil and its components

Mortality of xenobiotic-treated larvae was depended mainly on the age of larvae and to a lesser extent on the dose (Fig. 3). Pure compounds were more toxic than oil for both larval stages. Mortality in younger larvae treated with 1 and 2% dose of thyme oil was 50.0 and 62.5%, respectively. Older larvae susceptibility was very low and their mortality was not different from control. Toxicity of thymol and carvacrol against younger larvae was very high. Total mortality with 1 and 2% thymol was 86.67 and 91.67%, respectively. Activity of carvacrol was similar to thymol and 2% doses caused high mortality of 85.0 and 97.5 % (Fig. 3A). The mortality in older larvae treated with pure monoterpenes was slightly higher than control, but these differences were not significant except with 2% thymol (Fig.3B).

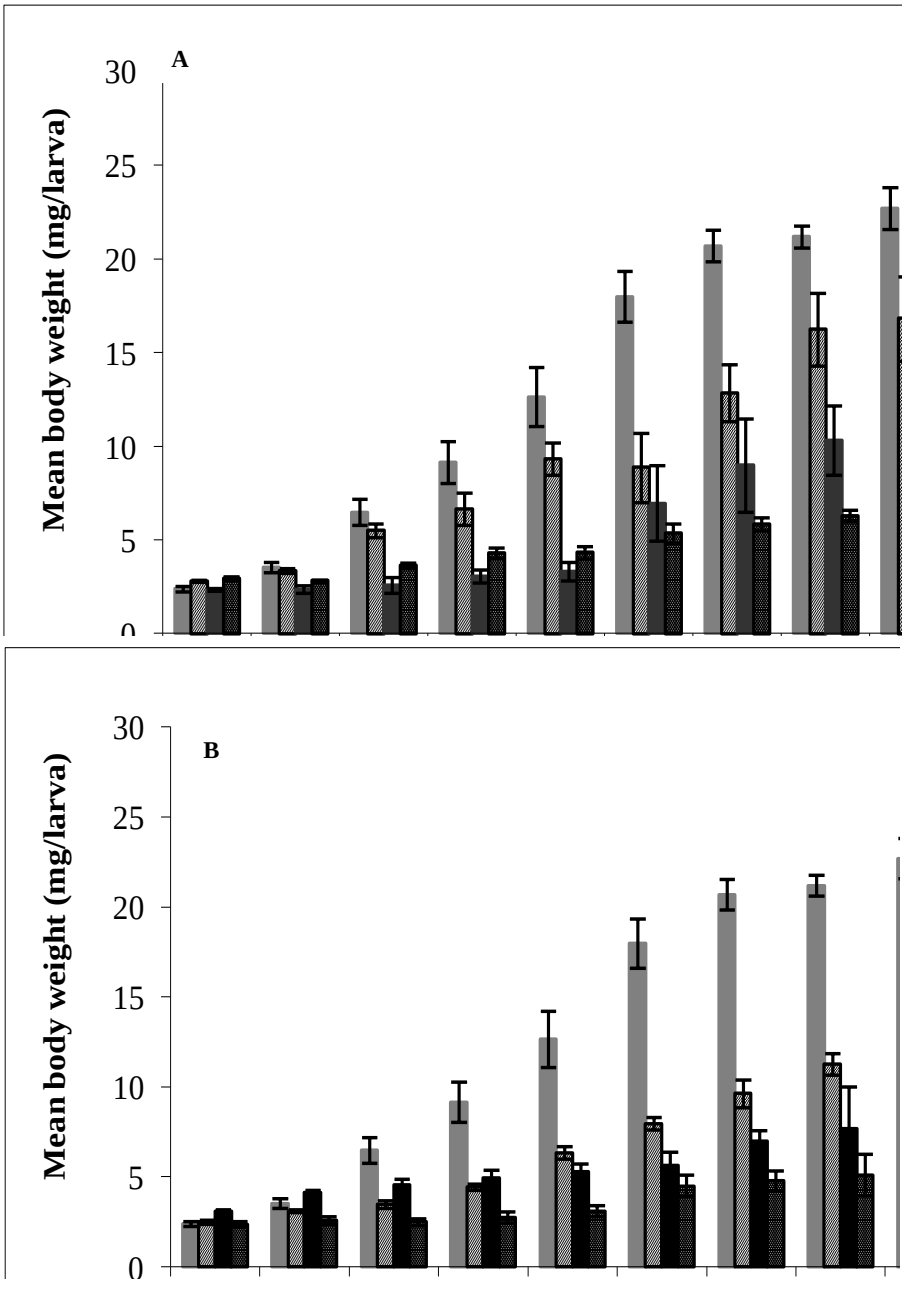

Figure 1 A and B. Effects of thyme oil, thymol and carvacrol on the growth of younger larvae of *A. diaperinus* : A: 1% Concentration, B: 2% Concentration. The standard error is indicated on the bar.

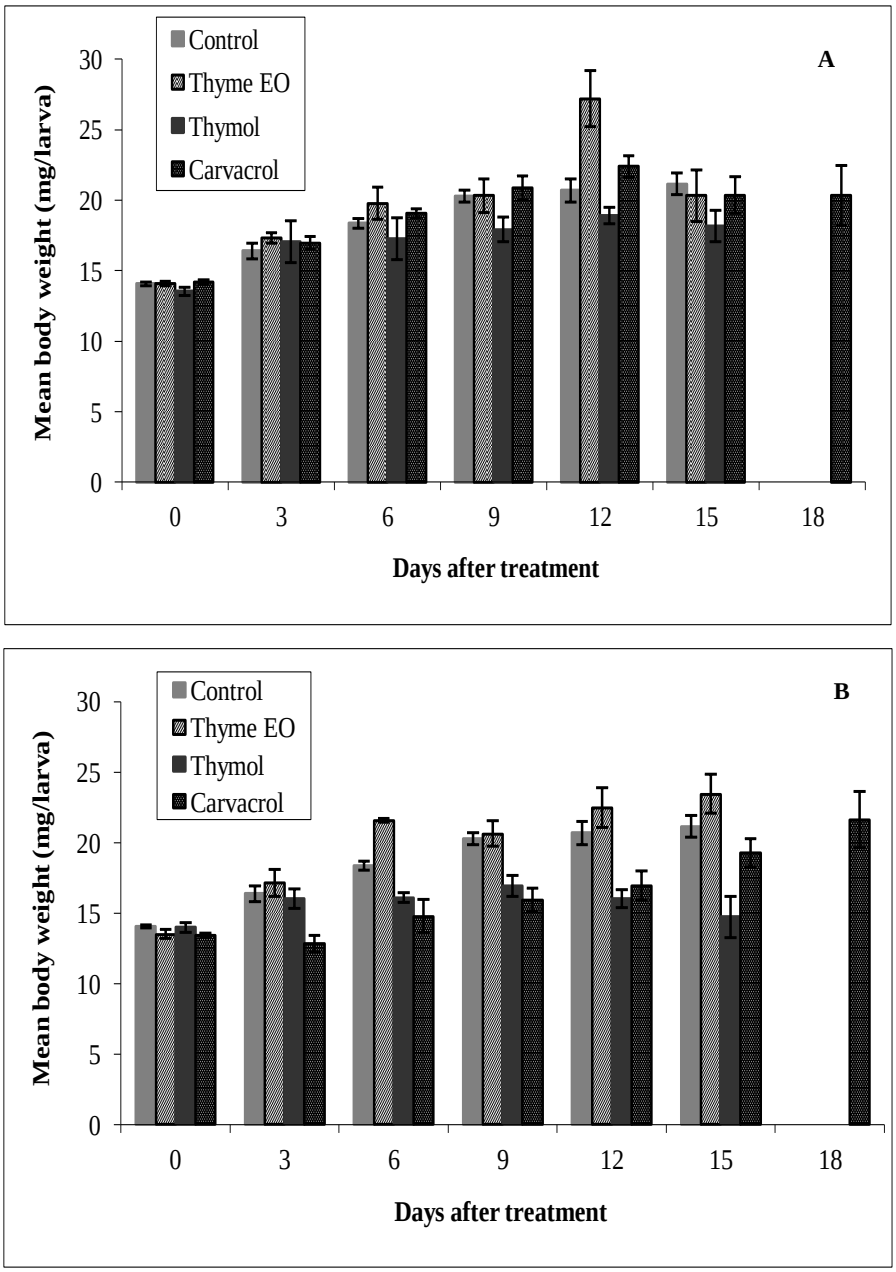

Figure 2 A and B. Effects of thyme oil, thymol and carvacrol on the growth of older larvae of *A. diaperinus*: A: 1% Concentration, B: 2% Concentration. The standard error is indicated on the bar.

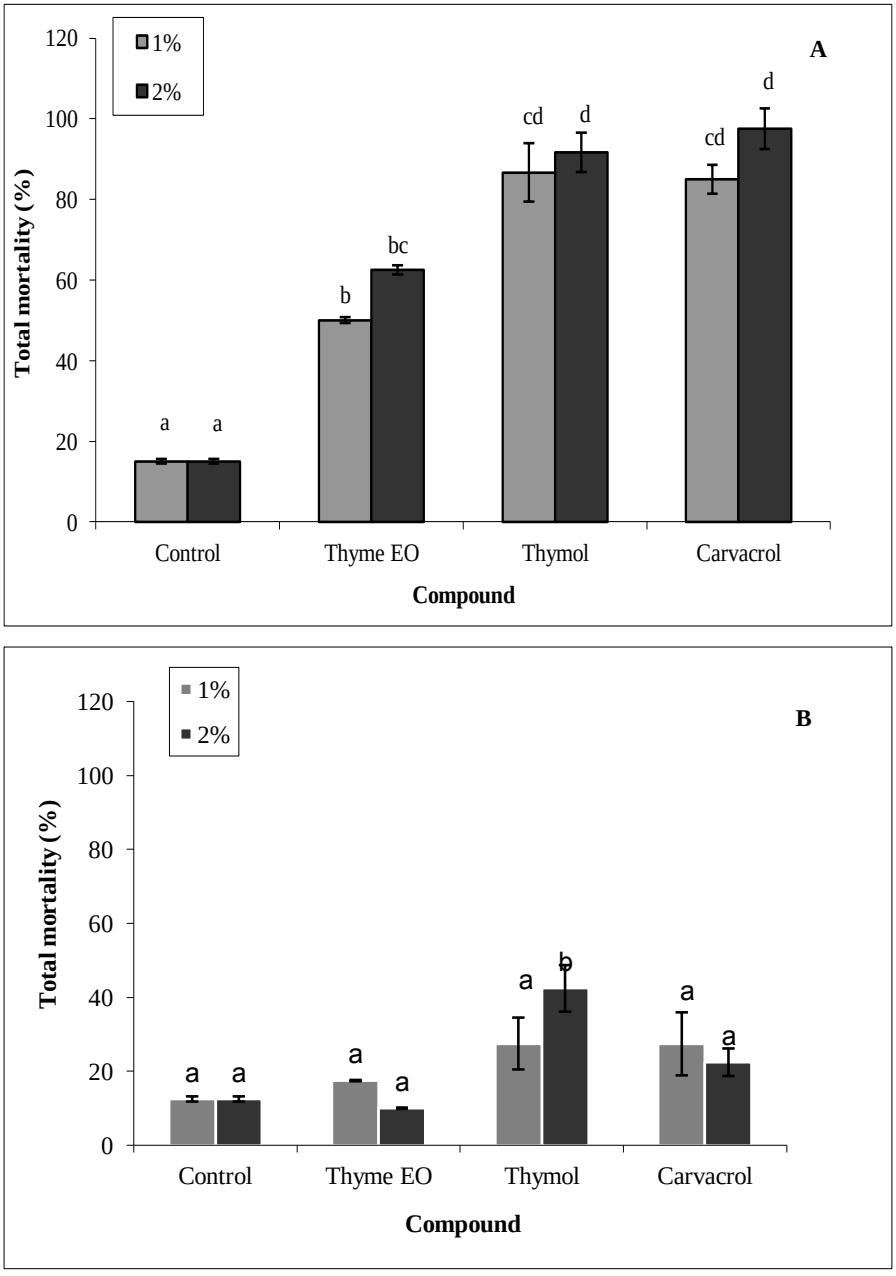

Figure 3 A and B. Mortality of *A. diaperinus* larvae treated with thyme oil, thymol and carvacrol: A: Young larvae, B: Old larvae. Means followed by the same letters are not significantly different (one-way ANOVA and Tukey test $p<0.05$). The standard error is indicated on the bar.

Development of *A. diaperinus* population

Application of thyme oil and its pure components in breeding of larvae *A. diaperinus* affected the number of pupae, their body weight and survival (Table 3,4). The monoterpenes were more active than essential oil and this activity was age- and dose-dependent. The number of pupae developed from younger larvae treated with 1% thymol and carvacrol was 13.33 and 15%, respectively. Higher doses of these oil components caused drastic reduction in pupae number i.e. 91.67% (2% thymol) and 97.5% (2% carvacrol). Their body weight was also lower than control. With 2% carvacrol solution the body weight of pupae was very low (3.23 mg) than 18.89 mg in control. Survival of pupae was relatively high. Differences between the number of pupae and adults were small (<10%). Body weight of adults determined immediately after the emergence, corresponded to body weight of pupae. The lowest body mass was observed in adults coming from trials treated with both doses of carvacrol i.e. 2% solution of thymol and 2% solution of thyme oil (Table 3).

Table 3. Effects of thyme oil and its components on number and body weight of pupae and adults developed from treated younger larvae of *A.diaperinus*

Compound	Conc (%)	Pupae		Adults	
		Numbers (%) ± SE	Body weight (mg) ± SE	Numbers (%) ± SE	Body weight (mg) ± SE
Control		80.00 ± 10.80	18.89 ± 0.31	80.00 ± 10.80	15.48 ± 0.85
Thyme EO	1%	50 ± 5.80*	16.38 ± 0.69 NS	43.30 ± 6.70*	14.21 ± 1.36 NS
	2%	37.50 ± 8.50**	15.67 ± 0.50*	22.5 ± 2.50**	12.33 ± 0.67*
Thymol	1%	13.33 ±2.23***	18.24 ± 1.57 NS	13.33 ± 2.23***	14.92 ± 0.94 NS
	2%	8.33 ±6.4***	14.63 ± 0.83 *	5.0 ± 2.5***	12.85 ± 1.55*
Carvacrol	1%	15.00 ± 2.90**	12.53 ± 1.18**	10.00 ± 4.10***	7.81 ± 2.76**
	2%	2.50 ± 2.50***	3.23 ± 3.23***	2.50 ± 2.50***	2.60 ± 2.60***

EO: Essential oil, For comparison of means with the control the *t*-test was used. Differences statistically significant at *p< 0.05; **p< 0.01; ***p<0.001; NS: Not significant

The effects of thyme oil and its components was not strong on the development of older larvae. Pupae body weight was not significantly different from control, except with 2% thymol solution, when pupae weighed 14.86 mg compared to 16.46 mg in controls. The pupae developed from larvae treated with both doses of thyme oil had higher body mass compared to controls. Furthermore, adults developed from them also weighed more (Table 4). The highest mortality (35.5%,) was observed in pupae developed from the larvae treated with 2% carvacrol solution.

The thymol and carvacrol were more active against *A. diaperinus* larvae than thyme oil. These monoterpenes from cymenole group, although they possess the same chemical formula and molecular weight but they differed in position of hydroxyl group on carbon ring. However, different position of hydroxyl group does not affect the activity of these compounds against *A. diaperinus* larvae. Likewise, these compounds showed similar activity against mosquito larvae, *Ochlerotatus caspius* and *Culex pipiens* (17,35)**,** but their similar effects on insects is not the rule. Thymol shows strong toxic properties when applied to *C. pipiens* larvae, while carvacrol does not cause insects mortality (7). On the

Table 4. Effects of thyme oil and its components on number and body weight of pupae and adults developed from treated older larvae of *A. diaperinus*

Compound	Conc (%)	Pupae		Adults	
		Numbers (%) ± SE	Body weight (mg) ± SE	Numbers (%) ± SE	Body weight (mg) ± SE
Control		87.5 ± 7.5	16.46 ± 0.27	80.00 ± 14.1	13.73 ± 0.35
Thyme EO	1%	82.5 ± 8.5 NS	19.39 ± 0.30**	72.5 ± 9.5 NS	16.18 ± 0.57*
	2%	90.0 ± 5.8 NS	18.34 ± 0.26*	83.3 ± 3.3 NS	16.64 ± 0.48*
Thymol	1%	72.5 ± 6.29*	16.37 ± 0.31 NS	70.0 ± 4.78*	14.25 ± 0.33 NS
	2%	57.5 ± 6.27**	14.86 ± 0.61**	27.5 ± 14.43***	11.17 ± 1.07*
Carvacrol	1%	70.0 ± 10.8*	17.94 ± 0.31*	65.0 ± 13.2*	14.88 ± 0.52 NS
	2%	77.5 ± 6.29*	16.31 ± 0.43 N S	40.0 ± 10.8**	12.98 ± 0.73 NS

EO: Essential oil, For comparison of means with the control the *t*-test was used. Differences statistically significant at *p< 0.05; **p< 0.01; ***p<0.001; NS: Not significant

other hand, insecticidal activity of carvacrol against second instar larvae of *Drosophila melanogaster* was stronger than thymol (16). Similary, carvacrol was more toxic than thymol, when tested against *Thecodiplosis japonensis* larvae (20).

Thyme oil obtained by us contained 57.44% thymol and only 2.80% carvacrol. It seems that thymol is major component responsible for the activity of this oil. Comparing the activity of *T. vulgaris* oil with thymol, revealed that the oil was less active against *A. diaperinus* larvae than the isolated components. This may suggest an antagonistic interaction between them and other EO constituents. The content of biosynthetic precursors of thymol and carvacrol, e.g. *p*-cymene and γ-terpinene can have an impact on the relatively low activity of oil. Karpouhtsis *et al*., (16) tested the insecticidal and genotoxic activities of essential oils of 3-Oregano taxa, found that *Satureja thymbra* oil was richer in precursors (40.62% of total oil) than monoterpenoic phenols (thymol + carvacrol constitutes 37.84% of oil) was most effective as insecticide aginst *D. melanogaster* larvae. Thyme oil studied by us contained only 16.82% of precursors and 60.24% thymol and carvacrol. Components of essential oils may act synergistically in some cases and cause stronger activity of oil when compared to isolated compounds. For example, the *Majorana hortensis* oil exhibited stronger toxic effect against *Spodoptera littoralis* larvae and adults of *Aphis fabae* than its major components, γ-terpinene and terpinen-4-ol (1).

In our study we did not observe acute toxicity of thyme oil and its components. All larvae were alive 24 h after treatment. The mortality after 3-days was 5-10% but the total mortality in younger larvae exposed to pure components was high (above 85%). Mortality of larvae treated with terpenes may have different reasons. These substances act in many ways on various insects - as neurotoxins, growth regulators, antifeedants, repellents etc. The total high mortality observed by us for younger larvae corresponded to small gain in their body weight. It is not likely that the reduction in body weight gain is caused by feeding deterrent activity of tested compounds. Low values of deterrence coefficients, except with 2% carvacrol solution, do not indicate strong antifeedant activity (Table 5).

Table 5. Feeding deterrent activity of thyme oil and its components in no-choice test against *A. diaperinus* larvae

Compound	Conc (%)	Deterrence coefficients ± SE[α]	
		Younger larvae	Older larvae
Thyme EO	1%	-15.85± 6.30 a	26.04 ± 3.28 ab
	2%	–1.87 ± 3.02 a	5.09 ± 5.67 ab
Thymol	1%	4.69 ± 3.29 a	29.58 ± 7.68 ab
	2%	5.44 ± 2.31 a	51.28 ± 4.98 a
Carvacrol	1%	2.81 ± 3.79 a	23.99 ± 13.41 ab
	2%	41.83 ± 3.23 b	–2.18 ± 5.70 b

EO: Essential oil, [a]Each value represents the mean of four replicates, each set up with 10 insects (n = 40). Means followed by the same letters within each column are not significantly different (one-way ANOVA and Tukey test $p<0.05$).

In the no-choice tests, large fluctuations between the trials were observed (see SE in Table 5). This may indicate that the reduction in body weight gain and high mortality among the younger larvae were due to disturbances in digestion and absorption of food. Age-related differences in larvae susceptibility may have resulted from the applied doses of compounds. The presence of better developed mechanisms of detoxication in older larvae can not be excluded. In our previous study, we observed the dose- and age-dependent differences among the larvae *A. diaperinus* treated with star anise, *Illicum verum* fruits essential oil, 7days old larvae followed by 14-days old were most sensitive stages. Thirty days old larvae were most tolerant to test oil (33). The application of thymol/thyme oil to chicken houses for poultry pest management does not contaminate the poultry, laying hens and eggs (29). Moreover, application of phytogenic feed additive containing essential oils may improve the broiler chickens nutrition (3).

CONCLUSIONS

Our study showed that the application of *T. vulgaris* EO against *A. diaperinus* larvae was not effective. Significant reduction in pest population can be achieved with use of pure components. Thymol and carvacrol inhibited the growth, particularly in younger larvae and most of treated larvae did not survive. Thymol was strong deterrent against adults of lesser mealworm (unpublished data). Therefore, thymol and carvacrol may have potential as an alternative to chemical control and can be incorporated into IPM of this pest.

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