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Insecticidal activity of star anise (*Illicum verum* Hook. F.) fruits extracts against lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae)

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ABSTRACT

The insecticidal activity of star anise (*Illicum verum* Hook. F.) fruits extracts was determined against the lesser mealworm (*Alphitobius diaperinus* Panzer). In laboratory tests, 25.0, 12.5, 6.25 and 3.12 mg/ml^{-1} extract concentrations as acetone solutions were added to diet of larvae and adults. The antifeedant effects of extract were investigated using choice and no-choice tests. The most sensitive stage was 7 days old larvae followed by 14 days old. Thirty days old larvae were highly tolerant to test extract. All applied doses caused the 100% mortality of younger larvae, however, only higher concentrations (25.0 and 12.5 mg/ml^{-1}) were toxic to older larvae, whereas 25.0 mg/ml^{-1} dose caused no mortality in adults. At lower doses, some older larvae developed into pupae and adults, but their body weight was lower than control. The extract was a very good feeding deterrent against larvae, but only in choice test. In the no choice test, the antifeedant effect was very weak.

Key words: Allelochemicals, *Alphitobius diaperinus*, antifeedants, essential oils, *Illicum verum*, lesser mealworm, star anise fruits.

INTRODUCTION

The lesser mealworm (*Alphitobius diaperinus* Panzer) also called the litter beetle infests the Poultry Farma. Its rapid reproduction and spread create a serious veterinary and economic problem for many poultry breeders. It also affects the storehouses, houses and offices neighbouring the hen houses. *A. diaperinus* is a vector of many bird pathogens including *Salmonella typhimurium, S. enterica, Campylobacter jejuni* (9,23). It is also intermediate host of the cysticercoides of helminths, *Choanotaenia* and *Raillietina*, which infect poultry. Moreover, the larvae and adults bore tunnels in the insulating material of buildings causing damages and loss of heat (3). Its control is very difficult now, because (i). Development of resistance to insecticides, decrease in susceptibility of *A. diaperinus* to conventional insecticides is noted in many countries (13,14,6,29) and (ii). Avoids direct contact with insecticide by hiding in deeper litter layers and tunnels bored in walls of buildings. Hence, it is necessary to find new methods for its control.

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Allelochemicals, the secondary metabolites produced by plants, play an essential role in plant defence systems against pathogens and pests. They are of great interest to researchers for more than 30 years as a potential source of ecologically safe botanical insecticides with different acting mechanisms. Some of them, such as deterrents and repellents, affect the insects' behaviour, others disturb their development, have a negative effect on their growth by disturbing the digestion and assimilation of food. Many of them are characterized by acute toxicity (2,8,11,12). They may provide the basis for alternative strategies of pest control. However till now, little information is available about the effects of plant allelochemicals on the behaviour of *A. diaperinus*. Our earlier studies indicated that natural terpenes and their synthetic derivatives affect the pest's feeding. Some of them, such as γ- i δ- hydroxylactones obtained from pulegone and isopulegole showed strong antifeedant properties (27). Likewise halolactones obtained from piperytone strongly reduce the feeding of *A. diaperinus* (Szczepanik, unpublished data). However, other terpenes, [myrcene, (+)-3-carene, (+)-limonene and (±)-camphene] are weak food deterrents against it (26).

The extract from star anise [*Illicum verum* Hook. (Illiaceae)] shows insecticidal effects on many insect species, including Coleoptera, Diptera and Lepidoptera (4,10,12, 24). Numerous investigations have shown that the susceptibility of particular species is differentiated e.g. investigations on pests of stored products show that *Callosobruchus chinensis* is more susceptible than *Sitophilus oryzae* (12). The mortality of insects treated depended on the dose and the exposure time. No investigations have been done on the effect of star anise on the lesser mealworm. This study aimed (i). to investigate in laby conditions the effect of extract of star anise on various development stages of *A. diaperinus* and (ii). to provide the possibility of using this extract to control *A. diaperinus* populations resistant to conventional insecticides.

MATERIALS AND METHODS

I. Preparation of *I. verum* extract

Dried star anise fruits (*Illicium verum)* were purchased from the Kawon-Hurt (Gostyń, Poland) herbal company. A Deryng apparatus was used to extraction the volatile compounds of *I. verum* fruits (28). A suspension of 10 g of ground dried fruits was placed in a 500 ml round flask together with 100 ml of distilled water. The sample flask was heated for 1 h after the boiling point was reached. The vapours were condensed by cold refrigerant. After 60 min of extraction, 1.2 ml of essential oil containing the volatile compounds was collected in a 2.5 ml vial and kept at -15^{o}C until the GC-MS analyses and biological tests were performed. The analyses were run in triplicate.

II. Chromatographic analyses

The isolation, identification and quantification of volatile compounds were performed on a gas chromatograph (GC), Saturn 2000 Varian Chrompack with a column TRACE TR-5 (5% phenyl methylpolysiloxane) 30 m x 0.25 mm ID x 1.0 µm film (5). The mass spectrometer (MS) equipped with an ion-trap analyzer was 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 a 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. One microlitre of the extract was always injected. Most of the compounds were identified by using three different analytical methods: (1) Kovats indices (KI), (2) GC-MS retention indices (authentic chemicals – standards (S)), and (3) mass spectra (authentic chemicals and NIST05 spectral library collection (MS)). The identification was considered tentative when it was based only on mass spectral data and Kovats indices. The Sigma retention index standard (Sigma, Saint Louis, Missouri, USA) used in this study consisted of a mixture of aliphatic hydrocarbons ranging from C8 through C32 dissolved in hexane.

III. Bioassays

***Alphitobius diaperinus* population.** The larvae and adults of *A. diaperinus* used in study were taken from a laboratory colony of commercial poultry farm near Toruń (53°01′N, 18°37′E, Poland). 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 an rearing chamber at +29°C in the dark.

Toxicity and larval growth bioassay. To assess the effects of *I. verum* extract on insect mortality, growth and development three larval stages and adults (3-4 days old) were used. To provide large numbers of larvae of approximately same age, a new culture method for the lesser mealworm described by Rice and Lambkin was applied (22). The body weight of I group of larvae (about one week old) was 1.19-1.23 mg (Larvae I, see Table 3), the II group (Larvae II) was 2-weeks old and their body weight was 3.98- 4.13 mg, and that of the III group (about 30 days old) was 14.57 -14.90 mg (Larvae III). The test extract was incorporated as acetone solution into the diet. The insects were exposed to 4-concentrations: 25.0, 12.5, 6.25 and 3.12 mg/ml^{-1}. One hundred mg of wheat bran was mixed with 1 ml of a test solution or acetone as control. After evaporation of the solvent (30 min of air-drying), the food was placed in Petri dishes together with 10 larvae or 10 adults. The dishes were transferred into the rearing chamber and held under the same conditions as for colony maintenance. Treated food was completed in accordance with requisition. Mortality was observed every day and body weight gain was recorded at 5-days intervals. When control mortality reached between 5 and 20% the mortality observed was corrected by Abbott's formula (1). The number of living pupae that transformed from survining larvae and adults that emerged from pupae were recorded. The body weights of pupae and adults (immediately after emergence) were also recorded. Each test was done in four replicates.

Antifeedant choice and no-choice tests. The antifeedant activity of *I. verum* extract was assayed as per procedure of Szczepanik *et al* (27). In these bioassays, oat flakes purchased from Kupiec (Paprotnia, Poland) were used as test food. For the feeding assays, two lower doses of acetone solutions of extract i.e. 6.25 and 3.12 mg/ml^{-1} were prepared. The oat flakes were saturated by dipping in the test solution or in acetone alone as control. After evaporation of the solvent (30 min of air-drying), the oat flakes were weighed and placed in Petri dishes together with 10 larvae. In the choice test, both control and treated oat flakes were placed together in a Petri dish, with the control flakes separated from the treated flakes by a thin glass capillary. In the no-choice test, only treated or control flakes were offered. Four replicates of tests for each dose were carried out on each larval stage. The dishes were transferred into a rearing chamber and kept at 29 ± 1°C in the dark. After 3 days of feeding, the remaining uneaten oat flakes were reweighed and the mean weight of food consumed was calculated. Basing on the amount of food consumed feeding deterrence coefficients (relative R and absolute A) were calculated using the formulae (18):

$$R = C - T/C + Tx100 \text{ (choice test)},$$
$$A = CC - TT/CC + TTx100 \text{ (no-choice test)},$$

Where, C, CC and T, TT are weights of the control and the treated food consumed by the insects in the choice and no-choice tests, respectively.

Statistical analysis: The percentage mortality and mean values of the deterrence coefficients were statistically analysed by means of one-way analysis of variance (ANOVA) and Tukey's test. The differences between the body weights of control and treated larvae, pupae and adults were compared using *t*-test (7).

RESULTS AND DISCUSSION

Chemical composition of essential oil

The chemical composition of obtained essential oil is presented in Table 1. Phenylopropanoid-anethole is present as volatiles as the main compound in anise seeds. In mixture of analyzed compounds, we also found terpenes: limonene, α-pinene, 1.8-cyneole, 3-carene and other aromatic compounds: p-anisaldehyde and estragol. The remaining compounds were present in trace amounts. The composition of *I. verum* essential oil is similar to the results obtained by Padmashree *et al* (20). The main difference is the lack of *trans*-ocimene and 4-terpineol in the mixture obtained.

Acute toxicity of *I. verum* extract

The mortality of lesser mealworm treated with *I. verum* extract varied according to dose, age of larvae and exposure time. Acute toxicity of extract was observed only in youngest larvae. One-week old and 2-weeks old larvae treated with a 25.0mg/ml^{-1} concentration died within 24 h (Table 2). Shortly after being transferred onto the treated food the larvae showed hyperactivity, increased mobility and convulsions ending in paralysis and death. Similar symptoms appeared in second-stage larvae of house fly, *Musca domestica* treated with an extract from star anise fruits (24). That is probably due to the pharmacological effect of anethole, the main ingredient of *I. verum*. After initial stimulation and irritation of the larva' body, it reduced the locomotory activity, drop in body temperature, hypnotic effects and paralysis. Such neurotoxic symptoms are triggered off by numerous monoterpenes occuring in the volatile oils of many plants (10). All

Table 1. Chemical composition of *I. verum* extract

No.	Compound	Retention time (min)	(%)	Identification method
1	α-Pinene	7.43	0.70	S, KI, MS
2	Sabinene	8.38	0.04	KI, MS
3	β-Myrcene	8.52	0.10	S, KI, MS
4	β-Pinene	8.66	0.09	S, KI, MS
5	α-Phelandrene	9.28	0.14	S, KI, MS
6	3-Carene	9.38	0.32	S, KI, MS
7	α-Terpinene	9.54	0.09	S, KI, MS
8	p-Cymene	9.76	0.11	S, KI, MS
9	Limonene	9.91	3.27	S, KI, MS
10	1,8 Cyneole	10.09	0.61	S, KI, MS
11	γ-Terpinene	10.67	0.14	S, KI, MS
12	Terpinolene	11.49	0.09	S, KI, MS
13	Linalool	11.62	0.67	S, KI, MS
14	4-Terpineol	14.38	0.30	S, KI, MS
15	Estragol	14.73	0.67	S, KI, MS
16	Carvone	15.92	0.02	S, KI, MS
17	*Z*-Anethole	16.31	0.13	S, KI, MS
18	p-anisaldehyde	16.55	1.45	S, KI, MS
19	**Anethole**	17.38	**90.55**	S, KI, MS
20	Metyl anisate	19.69	0.07	KI, MS
21	Arisketone	19.89	0.12	KI, MS
22	*trans*-Caryophyllene	21.42	0.03	S, KI, MS
23	4'-Methoxy propiophenone	21.75	0.02	KI, MS
24	Methyl eugenol	22.60	0.12	KI, MS
25	*trans*-nerolidole	24.17	0.08	S, KI, MS
26	Spatulenol	25.30	0.03	KI, MS
27	α-Cadinol	26.23	0.05	KI, MS

S: Authentic standard; KI : Kovats indices; MS: Mass spectrum

applied doses of *I. verum* triggered off symptoms of paralysis in one week old *A. diaperinus* larvae. However, in larvae treated with the lowest dose symptoms of recovery were observed on the following day. That shows that higher concentration of *I. verum* extract causes the “knock-down” in larvae, while with lower doses the paralysis is reversible. That, however, has no effect on the survival rate of youngest larvae. All doses applied caused 100% mortality. However, with lower doses the survival time of larvae was much longer (Table 2). The "knock-down" effect was also observed in fruit flies, *Drosophila melanogaster* M. treated with hexane extracts of *Pimpinella anisum* L. tops. Extracts representing 1.0 g of anise tops resulted in 98% mortality of fruit flies after 30 min of exposure and these flies did not revive, whereas 0.5 g anise tops caused 70% mortality after 30 min, yet after 24h all flies revived (15).

The sensitivity of *A. diaperinus* larvae to the *I. verum* extract decreased with age. Only the highest concentration of *I. verum* extract caused 100% mortality in 2-weeks-old larvae. At 12.5 mg/ml^{-1} dose a considerable drop in mortality of these larvae was observed in the initial stage of experiment, the symptoms of neurotoxicity were also much weaker. The insects’ dying process was extended in time and 100% mortality was registered after

45 days. With lower doses the survival rate of 2-weeks-old larvae increased, a dose of 3.12 mg/ml^{-1} caused only 37.5% mortality. In those cases paralysis was observed only in isolated individuals.

Thirty days old larvae showed a much higher resistance to the extract applied. Only the highest dose, 25.0 mg/ml^{-1} caused 77.33% mortality. Half that dose, 12.5 mg/ml^{-1} showed low activity towards that stage. The mortality of larvae 2-weeks after the application of the extract was 14.85% (Table 2). The treated larvae evolved into pupae and continued their development. Susceptibility of other species of Tenebrionidae to monoterpenoids also varied with age. Older larvae of *Tribolium confusum* du Val can tolerate higher concentrations of linalool, limonene and 1.8-cineole than younger ones (25). Likewise, larvae *Tribolium castaneum* Herbst becomes less susceptible with age to *trans*-anethole, thymol and eugenol (17). The adults of *A. diaperinus* showed complete resistance to the applied *I. verum* extract. The mortality of imagos treated with a dose 25.0 mg/ml^{-1} was similar to control and was only 2.5%. Because of the high survival rate of the insects the experiments were terminated within two weeks. No tests were done using the lower doses of extract.

Growth inhibitory effects

The extract had an inhibitory effect on the growth of *A. diaperinus* larvae. The increase in body weight depended on their age and dose. The few surviving youngest larvae (7- day-old) treated with 12.5 and 6.25 mg/ml^{-1} after 15 days reached only 18.32 and 33.33% of the body weight of the control larvae, respectively (Fig.1). After many days all larvae died. The body weight of larvae treated with the lowest dose after 15 days was 43.49% of control but in following 2-weeks, the increase in their body weight was little and was only 25.59% of control (Table 3).

The reduction in body weight gain in 14-day-old larvae used in the experiment was smaller. Two weeks after start of treatment with doses of 12.5, 6.25 and 3.12 mg/ml^{-1} their body weights was 55.38, 53.37 and 68.67% of control, respectively (Fig.1). But 30 days after start of experiment, those differences were significant. In tests with 12.5 mg/ml^{-1} dose the larvae's body weight constituted 43.21% of the control, while in those with a dose of 3.12 mg/ml^{-1} it was 72.72% of control. At the lowest extract concentration, the surviving larvae continued their development, which was extended by 10 days than control (Table 3) and 62.5% of them developed into pupae. Morphologically they did not differ from control pupae but their body weight was lower-only 63.95% of the control (Table 4). 54.8% of those pupae developed into adults with 10.85 mg mean body weight i.e. 65.99% of control adults body weight.

The lowest reduction in body weight gain was observed in the oldest larvae. Though at 25.0 mg/ml^{-1} dose, there were fluctuations in body weight and low increase, but at 12.5 mg/ml^{-1} dose, the larvae reached 79.45% of body weight of control larvae and 85.15% of them developed into pupae with a mean body weight of 16.95 mg. The survival rate of those pupae was also higher: 73% developed into adults with a mean body weight of 15.13 mg. In the control, 91.34% larvae changed to pupae with a mean body weight of 19.97 mg (Table 4).

The inhibitory effects of extract on the growth of *A. diaperinus* larvae is due to its toxicity rather than to its antifeedant activity. Though the high values of deterrence

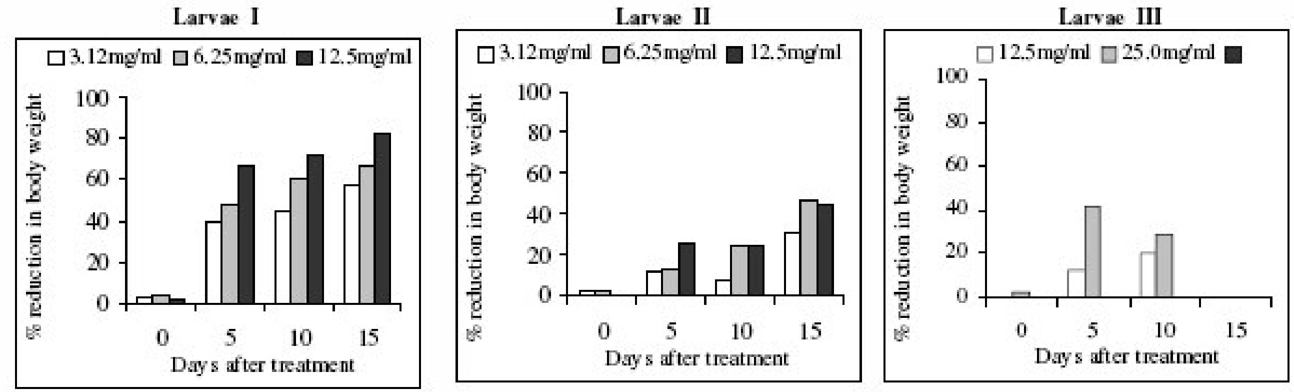

Figure 1. Inhibitory effects of *I. verum* extracts on body weight of *A. diaperinus* larvae.

Table 2. Toxicity of *I verum* extract against *Alphitobius diaperinus* larvae and adults

Instar	Dose mg/ml[-1]	Mortality (%) ±SE[a]								
		Days after treatment								
		5	10	15	20	25	30	35	40	45
Larvae I	3.12	0 d	18.06±2.73 c	46.67±7.82 b	72.71±14.5 a	80.76±9.36 a	80.76±9.36 a	88.54±7.86 a	100.0 a	
	6.25	69.44±5.47 b	74.44±4.84 a	92.5±2.5 a	100.0 a					
	12.5	85.0±8.66 b	89.44±4.55 a	89.44±4.55 a	100.0 a					
	25.0	100.0 a								
Larvae II	3.12	16.66±2.84 c	16.66±2.84 c	20.0±0.0 c	20.0±0.0 c	23.33±5.77 c	30.0±5.0 b	37.5.0±5.0 b	37.5±5.0 c	
	6.25	12.5±4.79 cd	20.0±4.08 bc	45.0±14.43 bc	55.5±20.61 bc	62.5±16.52 b	65.0±15.55 b	69.0±8.9 b	72.5±10.31 b	
	12.5	11.0±2.89 cd	37.5±7.08 b	40.0±6.29 b	55.05±6.29 b	67.50±8.16 ab	67.5±8.16 ab	85.0±6.45 a	92.11±2.63 a	100.0 a
	25.0	100.0 a								
Larvae III	12.5	7.4±4,79 cd	12.4±6.28 c	14.85±6.42 c						
	25.0	72.4±8.52 b	77.33±2.43 a	79.9±7.05 a						
Adults	25.0	2.5±0.0 d	2.5±0.0 d	2.5±0.0 d						

[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 (P< 0.05, Tukey's test))

Table 3. The effect of *I. verum* extract on the *Alphitobius diaperinus* body weight

Instar	Dose mg/ml[-1]	Body weight (mg) ±SE[a,b]								
		Days after treatment								
		0	5	10	15	20	25	30	35	40
Larvae I	Control	1.23±0.03	4.04±0.21	6.88±0.21	10.53±0.28	14.49±0.26	17.49±0.24	23.21±0,51	24.70±0.28	
	3.12	1.20±0.01	2.45±0.16***	3.85±0.29***	4.58±0.10***	5.89±0.89***	5.79±0.72***	5.94±0.96***	6.23±0.60***	
	6.25	1.19±0.01	2.13±0.22***	2.71±0.24***	3.51±0.22***					
	12.5	1.21±0.02	1.34±0.08***	1.90±0.38***	1.93±0.40***					
Larvae II	Control	4.09±0.27	6.50±0.35	8.52±0.49	13.92±0.37	17.02±0.27	21.93±0.51	23.77±0.32		
	3.12	3.98±0.21	5.75±0.74NS	7.90±0,41NS	9.56±0.83**	11.60±0.55***	12.34±0.38***	14.68±1.06***	16.21±0.51	17.70±0.0
	6.25	4.01±0.18	5.68±0.49 NS	6.46±0.39**	7.43±0.57***	8.83±0.44***	9.27±0.94***	10.45±0.91***	10.88±0.56	10.99±0.26
	12.5	4.13±0.26	4.86±0.32**	6.46±0.43**	7.71±0.45***	8.19±0.57***	8.44±1.07***	9.17±0.90***	8.83±0.25	9.33±0.28
Larvae III	Control	14.90±0.34	21.67±0.41	24.43±0.51						
	12.5	14.85±0.15	18.92±0.86*	19.41±0.27*						
	25.0	14.57±0.47	12.63±0.93***	13.77±0.57***						
Adults	Control	14.70±0.07	15.17±0.06	16.03±0.14	16.61±0.09					
	25.0	14.40±0.20	15.27±0.24NS	15.99±0.27NS	16.37±0.25NS					

[a] Each value represents the mean of four replicates, each set up with 10 insects (n=40). [b] Values are expressed in mg/1larva or adult. 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

Table 4. Effects of star anise fruits extracts on number and body weight of pupae and adults developed from treated larvae[a]

Instar/dose(mg/ml^{-1})	No of pupae (%) mean ± SE	Pupa body weight (mg) mean ± SE	No of adults(%) mean ± SE	Adult body weight (mg) mean ± SE
Control	91.34±1.74	19.67±0.79	85.72±1.15	16.44±0.36
Larvae II/3.12	62.5±8.36* (-31.6)	12.58±0.61* (-36.0)	54.8±7.69* (-36.0)	10.85±0.39* (-34.0)
Larvae III/12.5	85.15±3.71 NS (-6,8)	16.95±0.87 NS (-13.8)	73.0±4.32 NS (-14.8)	15.13±0.75 NS (-8.0)

[a] Each value represents the mean of four replicates, each set up with 10 insects (n=40). For comparison of means with the control the *t*-test was used. Differences statistically significant at $^{*}P < 0.05$; NS-not significant. The data in parenthesis indicates % inhibition over control.

coefficients obtained in the choice tests can suggest high deterrent activity of *I. verum*, in the no-choice tests the insects quickly overcame their reluctance to feeding, an indication of which are the low values of the absolute deterrence coefficients (Table 5).

Table 5. Feeding deterrent activity of *I.verum* extract against *A. diaperinus*

Instar	Dose mg/ml^{-1}	Deterrence coefficients ± SE[a] R	A
Larvae I	6.25	96.77 ± 2.28 a	12.54 ± 2.40 ab
	3.12	69.53 ± 5.83 ab	14.13 ± 2.29 ab
Larvae II	6.25	66.54 ± 7.90 ab	13.62 ± 4.63 ab
	3.12	90.68 ± 2.25 a	2.62 ± 1.57 a
Larvae III	12.5	44.09 ± 19.23 b	18.75 ± 2.15 b

[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 (P<0.05, Tukey's test)

The deterrent activity of anethole against other insect species differs. It is relatively weak deterrent against *Trichoplusia ni* (2). On the other hand, it shows deterrence as well as toxicity to *Spodoptera litura* and inhibitis growth in larvae after topical administration (10). The reduced increase in body weight of *A. diaperinus* larvae may be caused by irritation and necrosis of intestinal epithelium cells, which may be caused by the continuous administration of anethole and the high concentration of its metabolite, anethole epoxide, with cytotoxic properties (19).

Our investigations bring information about the stomach action of *I. verum* extract on *A. diaperinus* larvae and adults. That makes it possible to assess if adding the extract to the poultry's food can help reduce the pest's population. The inclusion of ivermectin into the poultry's diet can be effective method of control of *A. diaperinus* (16). Our results show that the doses used in laboratory conditions are effective only against the youngest larval stages of lesser mealworm. Using higher doses can be difficult for economic and sanitary reasons. High anethole concentrations are toxic to vertebrates. Hepatotoxicity in female rats was reported when dietary levels of *trans*-anethole were 250 mg/kg or more body weight/day (19). An excessive content of extract in the poultry's food can endanger their health. Low doses of anethole do not constitute such danger. For example, in humans 1 mg/kg body weight is safe. The safety of low doses of anethole is supported by its occurence as a natural component of traditional food (19). The lower doses used in this study, reduced the numbers of *A. diaperinus* population by inhibiting the young development stages, on the other hand they can be more effective in the synergistic effect of anethole. Extracts from anise increase the insecticidal activity of numerous monoterpenes (10,21) as well as the toxicity of synthetic insecticides, among other things pyrethroids, which are widely used for control *A. diaperinus* (15). Considering the registered drop in the pest's sensitivity to chemical insecticides (6,29), the introduction of a synergizing factor can be important. Anethole also strongly synergizes the toxicity of thymol towards *Spodoptera litura* (10). It is therefore necessary to investigate the synergetic effects of anethole and other monoterpenes as well as chemical insecticides against *A. diaperinus*. The investigations will be continued.

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REFERENCES

1. Abbott, W.S. (1925). A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265-267.
2. Akhtar, Y. and Isman, M.B. (2004). Comparative growth inhibitory and antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species. *Journal of Applied Entomology* **128**: 32-38.
3. Axtel, R.C. and Arends J.J. (1990). Ecology and managment of arthropod pests of poultry. *Annual Review Entomology* **35**: 101-126.
4. Chaiyasit, D., Choochote, W., Rattanachanpichai E., Chaithong, U., Chaiwong, P., Jitpakdi, A., Tippawangkosol, P., Riyong, D. and Pitasawat, B. (2006). Essential oils as potential adulticides against two populations of *Aedes aegypti*, the laboratory and natural field strains, in Chiang Mai province, northern Thailand. *Parasitology Research* **99**: 715-721.
5. Figiel, A., Szumny, A., Gutiérrez-Ortíz, Á. and Carbonell-Barrachina, A. (2010). Composition of oregano essential oil (*Origanum vulgare*) as affected by drying method. *Journal of Food Engineering* **98:** 240-247.
6. Hamm, R.L., Kaufman, P.E., Reasor, C.A., Rutz, D.A. and Scott, J.G. (2006). Resistance to cyfluthrin and tetrachlorvinphos in the lesser mealworm, *Alphitobius diaperinus,* collectedfrom the eastern United States. *Pest Management Science* **62**: 673-677.
7. Hammer, Ř., Harper, D.A.T. and Ryan, P.D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* **4** (1): 9pp. http://palaeo-electronica.org/2001_1/past/issue1_01.htm
8. Han, M.K., Kim, S. and Ahn, Y.J. (2004). Insecticidal and antifeedant activities of medicanal plant extracts against *Attagenus unicilor japonicus* (Coleoptera: Dermestidae). *Journal of Stored Product Research* **42**: 15-22.
9. Hazeleger, W.C., Jacobs-Reitsma, W.F., Beumer, R.R. and Bolder, N.M. (2008). Darkling beetles (*Alphitobius diaperinus*) and their larvae as potential vectors for the transfer of *Campylobacter jejuni* and *Salmonella enterica* serovar parathypi B variant Java between successive broiler flocks. *Applied and Environmental Microbiology* **22**: 6887-6891.
10. Hummelbrunner, L.A. and Isman M.B. (2001). Acute, sublethal, antifeedant and synergistic effects of monoterpenoid essential oil compounds on the tobaco cutworm, *Spodoptera litura* (Lep., Noctuidae). *Journal of Agriculture and Food Chemistry* **49**: 715-720.
11. Isman, M.B. 2000. Plant essential oils for pest and disease management. *Crop Protection* **19**: 603-608.
12. Kim, S.I., Roh, J.Y., Kim, D.H., Lee, H.S. and Ahn, Y.J. (2003). Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis. Journal of Stored Product Research* **39**: 293-303.
13. Lambkin, T.A. (2005). Baseline responses of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) to fenitrothion and susceptibility status of populations in Queensland and New South Wales, Australia. *Journal of Economic Entomology* **98**: 38-942.
14. Lambkin, T.A. and Rice, S.J. (2007). Baseline responses of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) to Spinosad, and susceptibility of broiler populations in eastern and southern Australia. *Journal of Economic Entomology* **100**: 1423-1427.
15. Marcus, C. and Lichtenstein, E.P. (1979). Biologically active components of anise: Toxicity and interaction with insecticides in insects. *Journal of Agriculture and Food Chemistry* 27: 1217-1223.
16. Miller, R.W. and Redfern, R.E. (1988). Feed additives for control of lesser mealworm (Coleoptera: Tenebrionidae) in poultry broiler houses. *Journal of Economic Entomology* **81**: 1137-1139.
17. Mondal, M. and Khalequzzaman, M. (2010). Toxicity of naturally occurring compounds of plant essential oil against *Tribolium castaneum* (Herbst). *Journal of Biological Sciences* **10**: 10-17.

18. Nawrot, J., Błoszyk , E., Harmatha, J., Novotny, L. and Drożdż, B. (1986). Action of antifeedants of plant origin on beetles infesting stored products. *Acta Entomologica Bohemoslovakia* **83**: 327-335.

19. Newberne, P., Smith, R.L., Doull, J., Goodman, J.I., Munro, I.C., Portoghese, P.S., Wagner, B.M., Weil, C.S., Woods, L.A., Adams, T.B., Lucas, C.D. and Ford, R.A. (1999). The Fema Gras assessment of *trans* - anethole used as a flavouring substance. *Food and Chemical Toxicology* **37:** 789-811.

20. Padmashree, A., Semwal , A.D., Sharma, G.K., Agathian, G. and Bawa, A.S. (2007). Star-anise (*Illicium verum*) and black caraway (*Carum nigrum*) as natural antioxidants. *Food Chemistry* **104:** 59-66.
21. Passreiter, C.M., Wilson, J., Andersen, R. and Isman, M.B. (2004). Metabolism of thymol and *trans*-anethole in larvae of *Spodoptera litura* and *Trichoplusia ni* (Lepidoptera: Noctuidae). *Journal of Agriculture and Food Chemistry* **52**: 2549-2551.
22. Rice, S.J. and Lambkin, T.A. (2009). A new culture method for lesser mealworm, *Alphitobius diaperinus. Journal of Applied Entomology* **133**: 67-72.
23. Roche, A.J., Cason, J.A., Fairchild, B.D., Hinkie, N.C., Cox, N.A., Richardson, L.J. and Buhr, R.J. (2009). Transmission of Salmonella to broilers by contaminated larval and adult lesser mealworms, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). *Poultry Science* **88**: 44-48.
24. Sripongpun, G. (2008). Contact toxicity of the crude extract of Chinese star anise fruits to house fly larvae and their development. *Songklanakarin Journal of Science and Technology* **30**: 667-672.
25. Stamopoulos, D.C., Damos, P. and Karagianidou, G. (2007). Bioactivity of five monoterpenoid vapours to *Tribolium confusum* (du Val) (Coleoptera: Tenebrionidae). *Journal of Stored Products Research* **43:** 571-577.
26. Szczepanik, M., Szumny, A., Grudniewska, A. and Wawrzeńczyk C. (2005). Feeding deterrent activity of α-methylenelactones against the lesser mealworm (*Alphitobius diaperinus* Panzer). *Pstycydy/Pesticides* **4:** 25-32.
27. Szczepanik, M., Dams, I. and Wawrzeńczyk C. (2008). Terpenoid lactones with the *p*-menthane system as feeding deterrents to the lesser mealworm, *Alphitobius diaperinus. Entomologia Experimentalis et Applicata* **128:** 337-345.
28. Szumny, A., Figiel, A., Gutiérrez-Ortíz, Á. and Carbonell-Barrachina, A. (2010). Composition of rosemary essential oil (*Rosmarinus officinalis*) as affected by drying method. *Journal of Food Engineering* **97:** 253-260.
29. Tomberlin, J.K., Richman, D. and Myers, H.M. (2008). Susceptibility of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) from broiler facilities in Texas to four insecticides. *Journal of Economic Entomology* **101**: 480-483.