Composition of volatile compounds in male and female *Tenebrio molitor* and *Leptinotarsa decemlineata* before and after the application of insecticides

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Abstract Some insects can be used as food, while others can affect the destruction of crops and the reduction of food reserves. The studies described in this article showed quantitative and qualitative changes in the composition of volatile compounds contained in adult *Tenebrio molitor* and *Leptinotarsa decemlineata* insects after applying insecticides. The samples were prepared by SPME and the final determinations were carried out by GC/MS. The volatile compounds that were present only in the insects after the application of insecticides (or in a larger quantities) could be due to the insecticides, as an insect defense response to these insecticides. For example, in adult *T. molitor* insects, the percentage of alkanes ranged from 38.63 to 59.05% (male), and from 38.89% to 60.20 (female), depending on the insecticide used and the time elapsed since its application. In contrast, in *L. decemlineata*, the percentage of alkanes ranged from 43.84 to 61.85% (female), and from 42.41 to 60.11% (male). The results of the

present study can be useful in understanding the i defense strategies of insects against insecticides.

Keywords Insecticides · Harmful insects · GC/MS · SPME · Saturated hydrocarbons · Unsaturated hydrocarbons

Introduction

Insects are commonly present in any environment, even those which are very extreme in terms of conditions. This is the largest group of animals on Earth. In addition, they are characterized by a high biodiversity of species (Alyokhin et al., 2007). Harmful insects can adversely affect food resources (Bazok et al., 2012). Some of them destroy crops, while others destroy stored products. Examples of such insects are *L. decemlineata* and *T. molitor*. *L. decemlineata* is a beetle of the Chrysomelidae family, which destroys crops from the Solanaceae family, mainly potatoes, but also feeds on tomatoes. *L. decemlineata*, is a harmful insect that may affect the availability of food and cultivation to a varying degree (Jaques & Fasulo 2012). *T. molitor* is a beetle of the Tenebrionidae family that destroys flour products stored in warehouses.

In order to reduce the number of harmful insects and their reproduction, chemistry insecticidal spraying is often used (Rose & Leathers 2006; Engindeniz & Yücel Engindeniz 2006; Sauphanor et al., 2007; Laznik et al., 2010; Hamaidia et al., 2018;

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Gołębiowski et al., 2016; Paszkiewicz et al., 2016a, b; Cerkowniak et al., 2020; Khan 2020; Gołębiowski et al., 2020a, b). Some insects, e.g. *Tenebrio molitor*, are considered to be a good source of fat and protein, and according to research, they can be introduced into people's and animals' diets (Goulet et al., 1978; Yoo et al., 2013). Oleic acid C18:1, contained in these insects, especially in larvae, has properties that reduce the level of LDL and increase the level of HDL in the blood (Finke 2002). These insects are also a good source of fiber, which improves metabolism. In addition, they contain quite large amounts of calcium, which can affect bone mineralization (Klasing et al., 2000). Unfortunately intensive use of insecticides can negatively affect food, e.g. animal meat. It turns out that some of the chemicals used to protect plants may remain in the diet, e.g. deltamethrin. According to Dallegrave et al. these are usually trace amounts of no hazard (Dallegrave et al., 2018). In some animals, food-borne insecticides can cause stress and negative effects on the brain (El-Demerdash 2011). Insecticides can act on insects through the digestive tract, through holes in the body (e.g. spiracles), and through the cuticles.

Insect cuticles are a physical barrier between the external environment and the interior of the insect. They protect against mechanical damage and chemical and environmental factors. The insect epidermis consists of three layers: envelope, epicuticle, exocuticle. The epidermis is resistant to enzyme degradation and has water barrier properties (Drijfhout et al., 2009; Gołębiowski et al., 2012). A wax layer is present on the surface of the epidermis, which can act as a protective barrier (Hillerton & Vincent 1983; Genin et al., 1986; Greene & Gordon 2003). It has been shown that this structure of the epidermis provides insects with protection to some extent against entomopathogenic factors. In the epidermis of the insect, various compounds are present, e.g. lipids, waxes, esters, sterols and glycerol (Ginzel & Blomquist 2016; Gołębiowski et al., 2020a, b; Paszkiewicz et al., 2016a, b). The role of these compounds varies and often depends on the species and developmental stage of the insects. For example, fatty acids contained in the epidermis and body of insects have antifungal activity (Drijfhout et al., 2009). It depends on the length of carbon chains and the number

of unsaturated bonds. Acids with a shorter chain and unsaturated bonds exhibit stronger antifungal activity (Toolson et al., 1990; Wagner et al., 1998). Compounds from the group of alkanes can perform various functions, e.g. pheromones and attractants. Tricosan acts as an attractant in the species *Mycodiplosis ligulata* (Young & Severson 1994), and in *Luciol cruciata* (Shibue et al., 2004) as a feromon. Tetracosan, pentacosan and heptacosan as attractants have been identified in cuticle compounds in *Heterotermes tenuis* (Batista-Pereira et al., 2004) and *Mycodiplosis ligulata* (Young & Severson 1994). These compounds can also act as fermones as, for example, in *Agelastica alni* (Geiselhardt et al., 2011), *Cailoma pumida* (Bergmann, 2002) and *Lucidota atra* (Shibue et al., 2004). Among the aldehydes there are also compounds with the nature of attractants and pheromones. An example is hexanal acting as a pheromone in *Cimex lectularius* (Feldlaufer et al., 2010) and an attractant in *Psila rosae* (Degen et al., 1999). Another example of a compound present in insects is glycerol, which has an affinity for water and can protect macromolecules of proteins corresponding to, i.a. for binding ice particles. Thanks to this, insects can survive low temperatures. Some aldehydes are components of pheromones with, for example, an attraction role (attractants) (Brey et al., 1985; Drijfhout et al., 2009).

The aim of the study was the quantitative and qualitative analysis of volatile compounds emitted by insects before and after the application of insecticides. The volatile compounds synthesized by insects after the application of insecticides could be due to these insecticides, as defence reaction of the insect. The results of the present study can be useful in understanding the defense strategies of insects against insecticides.

The insects were analyzed by gender. Two different insecticides containing different active substances were used for each insect: *T. molitor* - cyfluthrin and deltamethrin, *L. decemlineata* - thiamethoxam and acetamiprid. Samples were prepared by solid-phase microextraction (SPME) using PDMS/CAR/DVB fiber. For the final determinations, a gas chromatography technique coupled with mass spectrometry (GC/MS) was used, which is characterized by high sensitivity and selectivity.

Materials and methods

Insects

Adults, males and females, of two harmful insect species: *T. molitor* and *L. decemlineata*, were used for the study. Both species were kept for a short time in the same conditions with access to food. The insects were kept at a temperature of around 25 °C. *T. molitor* was grown on oatmeal with the addition of carrots to provide access to water. *L. decemlineata* was grown on potato leaves which provided food and water. The aim was to eliminate starvation as a cause of insect deaths. 100 males and 100 females from each species were used to carry out the research. 10 insects were sprayed with water and 45 insects were sprayed with each insecticide. The insects were divided into males and females using a stereoscopic microscope. Then the insects were divided into samples (15 insects each) that were sprayed with insecticides and left for 24, 48 and 72 h. Control samples were sprayed with water.

Insecticides

Insecticides, which are insect stress agents, were used at the commercial concentrations according to the labels. The insecticides used on *T. molitor* are Cyflok 50EW at a concentration of 0.8% and K-othrine 25WG at a concentration of 1.0% (Bayer, CropScience AG, Germany). The active substance in Cyflok 50EW is cyfluthrin $C_{22}H_{18}Cl_2FNO_3$. The active substance contained in K-othrine 25WG is deltamethrin $C_{22}H_{19}Br_2NO_3$. Both compounds belong to the group of synthetic pyrethroids. They are soluble in organic solvents (Thany et al., 2015). The insecticides that were applied to *L. decemlineata* are Actara 25WG at a concentration of 0.04% (Syngenta, Switzerland) and Mospilan 20SP at a concentration of 0.04% (Nippon Soda Co. Ltd., Japan). The active substance in Actara 25WG is thiamethoxam $C_8H_{10}ClN_5O_3S$. The active substance contained in Mospilan 20 SP is acetamiprid $C_{10}H_{11}ClN_4$. Both compounds belong to the group of neonicotinoids. These substances are harmful to insects as well as to aquatic and soil organisms (Alyokhin et al., 2007).

Solid-Phase Microextraction (SPME)

Solid-phase microextraction (SPME) was used to extract volatile compounds contained in *T. molitor* and *L. decemlineata* adults. For this aim, PDMS/CAR/DVB fiber (polydimethylsiloxane/carboxen/divinylbenzene) was used to obtain the volatile compounds from the free space of the samples. The sprayed and weighed whole insects were placed in a test tube which was closed with a stopper. The sample was placed on a heating block. The fiber was inserted into the test tube. The analysis conditions were: extraction time - 40 min, extraction temperature - 105 °C (Pawliszyn 2000; Banel et al., 2011; Cerkowniak et al., 2018).

GC/MS

To achieve the final determinations, a gas chromatography technique coupled with mass spectrometry (GC/MS) was used. This technique is characterized by high selectivity and sensitivity. A Shimadzu QP 2010 s gas chromatograph with a ZB5 capillary column (Zebtron 30 m x 0.25 mm x 0.25 µm) was used for the analysis. Analysis conditions: carrier gas - helium, analysis time - 55 min, desorption time - 5 min, analysis temperature - 250 °C, column temperature - 40 °C, analysis temperature - 40 °C to 250 °C with an increase of 6 °C/min, ion source temperature - 200 °C, interface temperature - 250 °C (Cerkowniak et al., 2018).

All samples were prepared in triplicate, which allowed the calculation of the arithmetic mean. The aim of the analysis was to determine the composition of compounds contained in the insects before and after applying insecticides. All described compounds were identified based on characteristic ions and retention times. The NIST_54k computer library was used to compare the compounds.

Results

Tenebrio molitor

The research was conducted to analyze the composition of volatile compounds contained in *T. molitor* insects after applying insecticides. The insects were divided into males and females. Insecticides

containing cyfluthrin and deltamethrin were used. A total of 27 compounds, compiled in Table 1, were identified. The compounds belonged to different groups: esters, alcohols, aldehydes, alkanes, alkenes, aromatic aldehydes, fatty acids, ketones, phenols, ketones and phenols. In qualitative terms, alkanes, alkenes and aldehydes were the most numerous. In quantitative terms, alkanes and alkenes were identified the most. The content of other compounds ranged from $0.002 \pm 0.001\%$ (heptanal) in females 72 h after the application of the cyfluthrin-containing insecticide to $9.00 \pm 0.67\%$ (3-methyl-phenol) in males 72 h after the application of the deltamethrin-containing insecticide. The most 3-methyl-phenol was identified in females and males in the control samples, $24.21 \pm 2.12\%$ and $23.15 \pm 1.69\%$, respectively. (E)-7-dodecen-1-ol acetate and heptadecanol were absent in the control *T. molitor*. (E)-7-dodecen-1-ol acetate was present only in female *T. molitor* 72 h after the application of Cyflok 50EW (Table 1). Heptadecanol was present in male *T. molitor* 48 and 72 h after the application of Cyflok 50EW and in female 72 h after the application of this pesticide.

Saturated hydrocarbons

The content of alkanes ranged from 38.63% in males 72 h after using the insecticide containing deltamethrin (Fig. 1) to 60.20% in females 48 h after using the insecticide containing deltamethrin (Fig. 1). Nine compounds with carbon chains from C11 to C29 were identified from the group of alkanes (Table 1). Most were tricosane and pentacosane. The percentage of tricosane changed over time after the application of the insecticides, but in the control samples was $21.06 \pm 1.21\%$ (male) and $21.71 \pm 1.23\%$ (female). After using the insecticide containing cyfluthrin, the male tricosane content was: $28.19 \pm 2.14\%$ (24 h), $30.24 \pm 2.24\%$ (48 h) and $24.92 \pm 3.24\%$ (72 h) (Table 1). In females, the percentage tricosane content 24 h after applying the insecticide was $27.90 \pm 2.24\%$, after 48 h $23.49 \pm 2.04\%$, and after 72 h $31.12 \pm 2.57\%$ (Table 1). After using the insecticide containing the active substance deltamethrin, the male tricosane content was: $29.13 \pm 3.54\%$ (24 h), $24.55 \pm 2.22\%$ (48 h) and $24.47 \pm 1.17\%$ (72 h). The percentage of this compound in females after the application of the insecticide was: $25.77 \pm 2.21\%$ (24 h), $29.75 \pm 2.69\%$ (48 h) and $24.58 \pm 2.87\%$ (72 h) (Table 1). A fairly high

percentage in females and males was determined for pentacosane. After using the insecticide containing cyfluthrin in males, the content of pentacosane ranged from $13.48 \pm 1.01\%$ (72 h) to $19.95 \pm 1.54\%$ (Table 1). In females, the percentage of pentacosane after the application of the insecticide was: $18.84 \pm 1.14\%$ (24 h), $12.72 \pm 1.16\%$ (48 h) and $20.43 \pm 1.89\%$ (72 h). After using the insecticide containing deltamethrin in males, the percentage of pentacosane decreased over time and was: $20.22 \pm 1.58\%$ (24 h), $11.64 \pm 1.00\%$ (48 h) and $11.09 \pm 1.12\%$ (72 h) (Table 1). In females, after using the same insecticide, the percentage of pentacosane was: $12.14 \pm 0.84\%$ (24 h), $20.62 \pm 2.56\%$ (48 h) and $11.19 \pm 1.06\%$ (72 h) (Table 1). In males and females, the percentage of pentacosane increased 24 h after the application of both insecticides compared to the control samples $17.63 \pm 1.24\%$ - males and $18.11 \pm 1.11\%$ - females.

Unsaturated hydrocarbons

In the group of unsaturated hydrocarbons with a double bond, compounds with carbon chains from C23 to C27 were determined. The highest content in this group of compounds was determined for pentacosene. After using the insecticide containing cyfluthrin, the content of pentacosene in males was: $28.02 \pm 2.28\%$ (24 h), $26.52 \pm 2.45\%$ (48 h) and $39.51 \pm 3.89\%$ (72 h) (Table 1). In females after using the same insecticide, the percentages of pentacosene were: $28.12 \pm 2.59\%$ (24 h), $31.64 \pm 2.36\%$ (48 h) and $25.45 \pm 3.01\%$ (72 h) (Table 1). In males, after the application of the insecticide in which the active substance was deltamethrin, the percentage of pentacosene increased during the time elapsed after spraying. The percentage of pentacosene in males was: $26.93 \pm 2.54\%$ (24 h), $44.96 \pm 4.56\%$ (48 h) and $45.40 \pm 3.46\%$ (72 h) (Table 1). In females, after using the same insecticide, the percentage of pentacosene after 24 h was $43.30 \pm 3.54\%$, after 48 h $25.92 \pm 2.14\%$ and after 72 h $45.47 \pm 3.52\%$ (Table 1). In males ($25.56 \pm 3.56\%$) and females ($23.11 \pm 2.21\%$), the percentage of pentacosene increased 24 h after the application of both insecticides compared to the control samples. Other compounds from this group were present in small quantities. Their percentage content in males ranged from $0.12 \pm 0.01\%$ (heptacosene, deltamethrin, 72 h) to $2.09 \pm 0.14\%$ (tricosene, cyfluthrin, 72 h) (Table 1). In females, it ranged from $0.04 \pm 0.01\%$ (heptacosene,

Compounds	Retention time [min.]	Male				Female														
		Cyfluthrin (Cyflok 50EW) (45 insects)		Deltamethrin (K-othrine 25WG) (45 insects)		Cyfluthrin (Cyflok 50EW) (45 insects)		Deltamethrin (K-othrine 25WG) (45 insects)												
		24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours										
Acetates																				
(E)-7-dodecen-1-ol acetate	18.582																			
Alcohols																				
heptadecanol	27.337																			
Aldehydes																				
heptanal	3.772	0.08 ± 0.01	0.17 ± 0.02	0.08 ± 0.02	0.02 ± 0.01	0.14 ± 0.02	0.03 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.17 ± 0.01	0.002 ± 0.001	0.07 ± 0.01	0.03 ± 0.01	0.14 ± 0.02	0.04 ± 0.01					
octanal	5.559	0.29 ± 0.05	0.33 ± 0.03	0.26 ± 0.02	0.34 ± 0.03	0.29 ± 0.04	0.21 ± 0.01	0.32 ± 0.04	0.30 ± 0.02	0.31 ± 0.04	0.32 ± 0.03	0.26 ± 0.02	0.23 ± 0.02	0.22 ± 0.02	0.28 ± 0.03					
nonanal	7.742	0.69 ± 0.04	1.61 ± 0.12	1.13 ± 0.04	1.40 ± 0.04	1.27 ± 0.11	0.93 ± 0.07	0.99 ± 0.06	0.69 ± 0.04	1.59 ± 0.11	1.32 ± 0.04	1.16 ± 0.09	0.97 ± 0.04	1.30 ± 0.12	1.01 ± 0.05					
2-nonenal	9.031	0.20 ± 0.01	0.28 ± 0.03	0.44 ± 0.01	0.25 ± 0.03	0.31 ± 0.05	0.21 ± 0.02	0.23 ± 0.02	0.21 ± 0.03	0.28 ± 0.01	0.23 ± 0.01	0.45 ± 0.5	0.22 ± 0.01	0.30 ± 0.03	0.24 ± 0.03					
decanal	10.104	0.21 ± 0.02	0.28 ± 0.02	0.27 ± 0.01	0.28 ± 0.05	0.30 ± 0.07	0.20 ± 0.03	0.20 ± 0.02	0.17 ± 0.03	0.28 ± 0.02	0.27 ± 0.02	0.29 ± 0.02	0.21 ± 0.01	0.24 ± 0.02	0.22 ± 0.02					
heptadecanal	22.968	0.09 ± 0.01	0.39 ± 0.05	2.70 ± 0.14	3.61 ± 0.21	0.24 ± 0.01	0.63 ± 0.03	0.37 ± 0.04	0.11 ± 0.01	0.41 ± 0.04	0.22 ± 0.02	0.24 ± 0.01	0.68 ± 0.03	0.25 ± 0.01	0.37 ± 0.03					
Saturated hydrocarbons																				
undecane	7.623	0.58 ± 0.02		0.45 ± 0.02					0.60 ± 0.02			0.42 ± 0.03								
9-methyl-nonadecane	27.745	0.66 ± 0.03	3.07 ± 0.05	0.79 ± 0.06	0.93 ± 0.04	4.39 ± 0.14	1.32 ± 0.04	0.73 ± 0.03	0.63 ± 0.02	2.98 ± 0.07	0.88 ± 0.04	0.71 ± 0.04	1.41 ± 0.04	4.43 ± 0.58	0.74 ± 0.04					
heneicosane	28.352	0.08 ± 0.02	0.52 ± 0.01	0.29 ± 0.02	0.13 ± 0.02	0.76 ± 0.04	0.16 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.46 ± 0.01	0.13 ± 0.04	0.30 ± 0.02	0.16 ± 0.01	0.77 ± 0.08	0.13 ± 0.01					
docosane	29.302	0.25 ± 0.04	0.47 ± 0.02	0.83 ± 0.05	0.33 ± 0.03	0.57 ± 0.03	0.39 ± 0.03	0.28 ± 0.02	0.26 ± 0.02	0.47 ± 0.03	0.31 ± 0.01	0.82 ± 0.06	0.42 ± 0.02	0.58 ± 0.06	0.29 ± 0.04					
tricosane	30.813	21.06 ± 1.21	28.19 ± 2.14	30.24 ± 2.24	24.92 ± 3.24	29.13 ± 3.54	24.55 ± 2.22	24.47 ± 1.17	21.71 ± 1.23	27.90 ± 2.24	23.49 ± 2.04	31.12 ± 2.57	25.77 ± 2.21	29.75 ± 2.69	24.58 ± 2.87					
tetracosane	32.235	2.54 ± 0.13	2.19 ± 0.05	3.90 ± 0.12	2.09 ± 0.17	3.50 ± 0.24	1.86 ± 0.11	1.65 ± 0.08	2.62 ± 0.14	2.17 ± 0.16	1.98 ± 0.14	3.93 ± 0.19	1.95 ± 0.17	3.56 ± 0.36	1.66 ± 0.21					
pentacosane	33.622	17.63 ± 1.24	19.05 ± 1.13	19.95 ± 1.54	13.48 ± 1.01	20.22 ± 1.58	11.64 ± 1.00	11.09 ± 1.12	18.11 ± 1.11	18.84 ± 1.14	12.72 ± 1.16	20.43 ± 1.89	12.14 ± 0.89	20.62 ± 2.56	11.19 ± 1.06					
heptacosane	36.215	0.48 ± 0.06	0.61 ± 0.03	0.22 ± 0.03	0.23 ± 0.01															

Table 1 (continued)

Compounds	Reten- tion time [min.]	Male			Female										
		Control Sample (10 insects)			Cyfluthrin (Cyflok 50EW) (45 insects)			Deltamethrin (K-othrine 25WG) (45 insects)							
		24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours					
nonacosane	38.640	0.89 ± 0.07	0.50 ± 0.02	0.26 ± 0.04	0.23 ± 0.01	0.25 ± 0.01	0.17 ± 0.01	0.92 ± 0.08	0.51 ± 0.01	0.22 ± 0.01	0.25 ± 0.02	0.11 ± 0.01	0.25 ± 0.04	0.17 ± 0.04	
Unsaturated hydrocarbons*															
tricosene	30.430	1.08 ± 0.05	1.40 ± 0.21	0.60 ± 0.04	2.09 ± 0.14	1.24 ± 0.13	0.98 ± 0.07	1.83 ± 0.08	1.16 ± 0.12	1.08 ± 0.06	1.98 ± 0.06	0.56 ± 0.04	1.00 ± 0.08	1.88 ± 0.05	
tetracosene	31.885	0.94 ± 0.04	0.32 ± 0.02	0.71 ± 0.07	1.22 ± 0.04	0.60 ± 0.03	2.50 ± 0.32	1.74 ± 0.07	0.79 ± 0.05	0.32 ± 0.03	0.81 ± 0.03	0.62 ± 0.02	1.35 ± 0.11	1.45 ± 0.08	
pentacosene	33.309	25.56 ± 3.56	28.02 ± 2.28	26.52 ± 2.45	39.51 ± 3.89	26.93 ± 2.54	44.96 ± 4.56	45.40 ± 3.46	23.11 ± 2.21	28.12 ± 2.59	31.64 ± 2.36	25.45 ± 3.01	43.30 ± 3.54	45.47 ± 3.52	
heptacosene	35.920	0.22 ± 0.04	0.15 ± 0.01	0.15 ± 0.01	0.26 ± 0.06	0.25 ± 0.02	0.17 ± 0.03	0.12 ± 0.01	0.20 ± 0.03	0.69 ± 0.04	0.18 ± 0.01	0.04 ± 0.01	0.18 ± 0.02	0.11 ± 0.01	
Aromatic aldehydes															
benzaldehyde	4.828	1.50 ± 0.08	1.67 ± 0.21	3.57 ± 0.34	0.86 ± 0.04	3.08 ± 0.33	0.81 ± 0.03	0.42 ± 0.02	1.62 ± 0.04	1.78 ± 0.21	0.79 ± 0.04	3.55 ± 0.56	0.89 ± 0.05	0.45 ± 0.04	
benzeneacetaldehyde	6.464	1.42 ± 0.05	1.23 ± 0.12	3.11 ± 0.08	0.84 ± 0.06	4.47 ± 0.35	1.09 ± 0.04	0.59 ± 0.04	1.47 ± 0.07	1.29 ± 0.05	0.81 ± 0.06	3.13 ± 0.08	1.17 ± 0.08	0.64 ± 0.03	
Fatty acids															
hexadecanoic acid	25.486	0.15 ± 0.02	0.41 ± 0.03	0.25 ± 0.01	0.28 ± 0.05	0.42 ± 0.03	0.42 ± 0.06	0.05 ± 0.01	0.18 ± 0.02	0.44 ± 0.03	0.26 ± 0.03	0.46 ± 0.03	0.38 ± 0.02	0.03 ± 0.01	
Ketones															
2-methyl-2-cyclohexen-1-one	6.719	0.07 ± 0.01	0.23 ± 0.02	0.27 ± 0.01	0.10 ± 0.03	0.06 ± 0.01	0.03 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.24 ± 0.02	0.10 ± 0.04	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	
Phenols															
3-methylphenol	7.099	23.15 ± 1.69	8.56 ± 0.95	2.91 ± 0.06	5.83 ± 0.36	1.24 ± 0.21	6.27 ± 0.71	9.00 ± 0.67	24.21 ± 2.12	8.60 ± 0.54	5.62 ± 0.07	2.93 ± 0.07	6.67 ± 0.45	8.85 ± 0.87	
2,4-di-tert-butylphenol	17.039	0.17 ± 0.03	0.50 ± 0.03	0.27 ± 0.01	0.54 ± 0.02	0.17 ± 0.02	0.40 ± 0.04	0.18 ± 0.03	0.18 ± 0.03	0.49 ± 0.02	0.53 ± 0.03	0.26 ± 0.02	0.47 ± 0.03	0.19 ± 0.02	

The data are presented as the mean ± standard deviation

*The position of the double bond is not specified

Fig. 1 Percentage of alkanes in *Tenebrio molitor* insects before and after applying insecticides

cyfluthrin, 72 h) to $1.98 \pm 0.06\%$ (tricosene, cyfluthrin, 48 h) (Table 1).

Leptinotarsa decemlineata

The analysis of the samples of prepared male and female *L. decemlineata* was aimed at identifying volatile compounds contained in the insects after using insecticides containing thiamethoxam and acetamiprid. A total of 27 compounds were identified in the insects. The percentage content of the identified compounds is presented in Table 2. The identified compounds belong to the groups: esters, alcohols, aldehydes, alkanes, alkenes, aromatic aldehydes, fatty acids, ketones, phenols. Alkanes and alkenes had the highest percentage. The percentage content of other compounds ranged from $0.03 \pm 0.01\%$ (heptanal, male, 48 h, thiamethoxam and acetamiprid; hexadecanoic acid, male, 72 h, thiamethoxam) to $10.23 \pm 0.98\%$ (3-methyl-phenol, male, 72 h, thiamethoxam) (Table 2). In addition, the percentage of 3-methyl-phenol in males and females in the control samples was $23.42 \pm 2.25\%$ and $24.20 \pm 2.31\%$, respectively, and after using the insecticides, the content of this compound significantly decreased (Table 2). (E)-7-dodecen-1-ol acetate and heptadecanol were absent in the control *L. decemlineata*. (E)-7-dodecen-1-ol acetate was present in male *L. decemlineata* 72 h after the application of Mospilan 20SP and in female 24 and 72 h after the application of Actara 25WG (Table 2). Heptadecanol was present in male *L. decemlineata* 72 h after the application of Mospilan 20SP and in female 24 and 72 h after the application of Actara 25WG.

Saturated hydrocarbons

Figure 2 shows the percentage of saturated hydrocarbons determined in male and female *L. decemlineata*. The percentage of alkanes increased 24 h after applying both insecticides. In females, the content of alkanes increased 24 h after applying the insecticide that contained thiamethoxam. The content decreased 24 h after applying the acetamiprid-containing insecticide, and increased after 48 h. The percentage of alkanes in the sample of control males was 44.32%, and females 45.19%. In this group of compounds, the highest percentage was determined for the compound - tricosane. A slightly lower percentage was

determined for pentacosane. The content of tricosane in males after applying the insecticide containing thiamethoxam was: $29.64 \pm 2.03\%$ (24 h), $25.83 \pm 1.67\%$ (48 h), $27.37 \pm 1.56\%$ (72 h) (Table 2). After using the same insecticide in females, the tricosane percentage after 24 h was $30.42 \pm 3.09\%$, after 48 h $27.43 \pm 2.00\%$, and after 72 h $31.70 \pm 2.87\%$ (Table 2). The percentage of pentacosane in males after the application of the insecticide with the active substance thiamethoxam was $20.60 \pm 1.28\%$ after 24 h, $12.33 \pm 0.72\%$ after 48 h and $12.44 \pm 0.98\%$ after 72 h (Table 2). When the same insecticide was used in females, the percentage of pentacosane was as follows: $19.84 \pm 1.08\%$ (24 h), $14.92 \pm 0.98\%$ (48 h) and $20.84 \pm 1.58\%$ (72 h) (Table 2). After using the second stressor, which was the acetamiprid-containing insecticide, the percentage of pentacosane in males was: $19.02 \pm 1.76\%$ (24 h), $14.26 \pm 1.01\%$ (48 h) and $19.97 \pm 1.07\%$ (72 h) (Table 2). In females, the percentage of pentacosane was: $12.16 \pm 1.01\%$ (24 h), $21.12 \pm 2.03\%$ (48 h) and $12.54 \pm 0.67\%$ (72 h) (Table 2).

In males, the percentage of pentacosane increased 24 h after the application of both insecticides compared to the control sample ($17.70 \pm 1.04\%$). In females, after using the thiamethoxam-containing insecticide, the percentage of pentacosane increased after 24 h; after using the acetamiprid-containing insecticide, it decreased, compared to the control sample ($17.99 \pm 1.04\%$). As with pentacosane, the percentage of tricosane in females and males increased 24 h after applying the insecticides, compared to the control sample ($21.48 \pm 2.03\%$ - female and $21.12 \pm 1.37\%$ - male).

Unsaturated hydrocarbons

Of the determined unsaturated hydrocarbons, pentacosene had the highest percentage. After using the insecticide containing thiamethoxam, the percentage of pentacosene in males was: $25.24 \pm 2.35\%$ after 24 h, $42.61 \pm 3.65\%$ after 48 h, $38.89 \pm 3.87\%$ after 72 h. In females, the percentage of this compound was as follows: $26.04 \pm 1.51\%$ (24 h), $37.33 \pm 3.21\%$ (48 h) and $22.43 \pm 1.43\%$ (72 h) (Table 2). After using the second insecticide that contained acetamiprid, the percentage of pentacosene in males was: $27.40 \pm 2.83\%$ (24 h), $35.59 \pm 2.87\%$ (48 h) and $25.88 \pm 2.56\%$ (72 h), and in females: $40.47 \pm 3.34\%$ (24 h), $23.83 \pm 1.31\%$ (48 h) and $41.40 \pm 3.45\%$ (72 h) (Table 2). For males after

Compounds	Retention time [min.]	Male				Female							
		Thiamethoxam (Actara 25WG) (45 insects)		Acetamiprid (Mospilan 20SP) (45 insects)		Thiamethoxam (Actara 25WG) (45 insects)		Acetamiprid (Mospilan 20SP) (45 insects)					
		Control Sample (10 insects)	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours		
Acetates													
E)-7-dodecen-1-ol acetate	18.582												
Alcohols													
heptadecanol	27.337												
Aldehydes													
heptanal	3.775	0.09 ± 0.01	0.14 ± 0.02	0.03 ± 0.01	0.07 ± 0.01	0.17 ± 0.03	0.03 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.06 ± 0.01	0.15 ± 0.02	0.04 ± 0.00
octanal	5.555	0.30 ± 0.04	0.30 ± 0.04	0.24 ± 0.02	0.35 ± 0.03	0.34 ± 0.05	0.37 ± 0.04	0.27 ± 0.07	0.31 ± 0.03	0.29 ± 0.03	0.34 ± 0.05	0.30 ± 0.02	0.24 ± 0.04
nonanal	7.740	0.69 ± 0.05	1.33 ± 0.11	0.97 ± 0.05	1.15 ± 0.10	1.65 ± 0.16	1.51 ± 0.13	1.15 ± 0.09	0.69 ± 0.03	1.18 ± 0.11	1.10 ± 0.12	1.37 ± 0.14	1.01 ± 0.14
2-nonenal	9.032	0.20 ± 0.03	0.31 ± 0.04	0.23 ± 0.03	0.27 ± 0.04	0.29 ± 0.02	0.22 ± 0.02	0.45 ± 0.03	0.21 ± 0.02	0.48 ± 0.05	0.26 ± 0.03	0.26 ± 0.04	0.23 ± 0.03
decanal	10.100	0.22 ± 0.02	0.31 ± 0.02	0.23 ± 0.03	0.23 ± 0.03	0.30 ± 0.03	0.30 ± 0.05	0.29 ± 0.03	0.22 ± 0.01	0.30 ± 0.03	0.22 ± 0.04	0.31 ± 0.03	0.24 ± 0.01
heptadecanal	22.965	0.11 ± 0.01	0.25 ± 0.02	0.69 ± 0.05	0.43 ± 0.04	0.42 ± 0.02	0.25 ± 0.03	0.26 ± 0.04	0.22 ± 0.03	0.28 ± 0.06	0.42 ± 0.03	0.26 ± 0.04	0.67 ± 0.06
Saturated hydrocarbons - Alkanes													
undecane	7.623	0.58 ± 0.06											
heneicosane	27.747	0.08 ± 0.02	0.78 ± 0.03	0.17 ± 0.02	0.15 ± 0.03	0.52 ± 0.04	0.15 ± 0.01	0.30 ± 0.03	0.08 ± 0.02	0.31 ± 0.02	0.14 ± 0.03	0.80 ± 0.05	0.17 ± 0.03
9-methyl-nonadecane	28.345	0.66 ± 0.04	4.47 ± 0.13	1.43 ± 0.21	0.83 ± 0.06	3.05 ± 0.05	0.99 ± 0.05	0.77 ± 0.02	0.68 ± 0.04	0.79 ± 0.04	0.81 ± 0.02	4.59 ± 0.34	1.46 ± 0.07
docosane	29.302	0.26 ± 0.02	0.59 ± 0.05	0.43 ± 0.03	0.32 ± 0.02	0.47 ± 0.05	0.36 ± 0.03	0.85 ± 0.05	0.30 ± 0.02	0.87 ± 0.06	0.31 ± 0.03	0.61 ± 0.03	0.44 ± 0.02
tricosane	30.838	21.12 ± 1.37	29.64 ± 2.03	25.83 ± 1.67	27.37 ± 1.56	28.13 ± 2.35	26.31 ± 2.45	30.20 ± 2.59	21.48 ± 2.03	31.70 ± 2.87	26.70 ± 1.27	30.56 ± 2.65	26.62 ± 2.07
tetracosane	32.236	2.56 ± 0.12	3.54 ± 0.11	1.97 ± 0.07	1.85 ± 0.12	2.17 ± 0.14	2.22 ± 0.22	3.90 ± 0.25	2.64 ± 0.08	4.07 ± 0.37	1.74 ± 0.14	3.66 ± 0.43	2.02 ± 0.08

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Compounds	Retention time [min.]	Male	Female												
		Control Sample (10 insects)	Thiamethoxam (Actara 25WG) (45 insects)			Acetamidiprid (Mospilan 20SP)									
			24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours				
pentacosane	33.634	17.70 ± 1.04	20.60 ± 1.28	12.33 ± 0.72	12.44 ± 0.98	19.02 ± 1.76	14.26 ± 1.01	19.97 ± 1.07	17.99 ± 1.04	19.84 ± 1.08	14.92 ± 0.98	20.84 ± 1.58	12.16 ± 1.01	21.12 ± 2.03	12.54 ± 0.67
heptacosane	36.214	0.49 ± 0.05	0.24 ± 0.04	0.15 ± 0.01	0.15 ± 0.03	0.60 ± 0.03	0.25 ± 0.04	0.23 ± 0.02	0.51 ± 0.03	0.22 ± 0.01	0.25 ± 0.02	0.22 ± 0.02	0.14 ± 0.01	0.25 ± 0.02	0.17 ± 0.03
nonacosane	38.639	0.87 ± 0.05	0.26 ± 0.01	0.11 ± 0.01	0.18 ± 0.03	0.48 ± 0.02	0.25 ± 0.05	0.26 ± 0.01	0.94 ± 0.05	0.26 ± 0.03	0.25 ± 0.04	0.27 ± 0.04	0.18 ± 0.02	0.26 ± 0.03	0.12 ± 0.01
Unsaturated hydrocarbons - Alkenes*															
tricosene	30.431	1.11 ± 0.07	1.28 ± 0.08	1.01 ± 0.06	2.02 ± 0.08	1.00 ± 0.03	2.21 ± 0.04	0.63 ± 0.03	1.11 ± 0.05	0.62 ± 0.04	2.34 ± 0.14	0.71 ± 0.04	2.09 ± 0.11	1.26 ± 0.09	1.04 ± 0.08
tetracosene	31.887	0.69 ± 0.03	0.43 ± 0.03	1.48 ± 0.09	1.66 ± 0.07	0.39 ± 0.03	1.19 ± 0.05	0.84 ± 0.05	0.65 ± 0.02	0.71 ± 0.03	0.98 ± 0.04	0.87 ± 0.04	1.52 ± 0.12	0.12 ± 0.02	1.40 ± 0.34
pentacosene	33.325	25.17 ± 3.21	25.24 ± 2.35	42.61 ± 3.65	38.89 ± 3.87	27.40 ± 2.83	35.59 ± 2.87	25.88 ± 2.56	23.36 ± 1.98	26.04 ± 1.51	37.33 ± 3.21	22.43 ± 1.43	40.47 ± 3.34	23.83 ± 1.31	41.40 ± 3.45
heptacosene	35.922	0.19 ± 0.04	0.25 ± 0.03	0.18 ± 0.05	0.13 ± 0.02	0.28 ± 0.04	0.33 ± 0.03	0.16 ± 0.02	0.16 ± 0.03	0.18 ± 0.03	0.27 ± 0.09	0.13 ± 0.02	0.08 ± 0.02	0.13 ± 0.02	0.14 ± 0.04
Aromatic aldehydes															
benzaldehyde	4.825	1.57 ± 0.14	3.23 ± 0.06	0.92 ± 0.05	0.48 ± 0.02	1.84 ± 0.06	0.94 ± 0.11	3.68 ± 0.14	1.69 ± 0.14	3.65 ± 0.23	1.02 ± 0.06	3.90 ± 0.23	0.54 ± 0.05	3.14 ± 0.14	0.95 ± 0.03
benzeneacet-aldehyde	6.463	1.44 ± 0.08	4.80 ± 0.12	1.20 ± 0.08	0.72 ± 0.03	1.30 ± 0.09	0.94 ± 0.08	3.11 ± 0.09	1.47 ± 0.12	3.14 ± 0.12	0.98 ± 0.04	3.24 ± 0.32	0.71 ± 0.06	4.99 ± 0.26	1.23 ± 0.05
Fatty acids															
hexadecanoic acid	25.488	0.22 ± 0.02	0.49 ± 0.04	0.53 ± 0.03	0.04 ± 0.01	0.64 ± 0.03	0.33 ± 0.04	0.33 ± 0.04	0.24 ± 0.03	0.30 ± 0.03	0.30 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	0.52 ± 0.06	0.57 ± 0.02
Ketones															
2-methyl-2-cyclohexen-1-one	6.718	0.77 ± 0.04	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.26 ± 0.04	0.11 ± 0.02	0.11 ± 0.02	0.07 ± 0.02	0.12 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
Phenols															
3-methyl-phenol	7.099	23.42 ± 2.25	1.34 ± 0.06	6.76 ± 0.23	10.23 ± 0.98	8.77 ± 0.23	6.29 ± 0.21	3.10 ± 0.08	24.20 ± 2.31	3.16 ± 0.32	6.46 ± 0.56	3.28 ± 0.45	9.89 ± 0.56	1.30 ± 0.12	6.78 ± 0.43

Table 2 (continued)

Compounds	Retention time [min.]	Male			Female								
		Control Sample (10 insects)	Thiamethoxam (Actara 25WG) (45 insects)		Acetamiprid (Mospilan 20SP) (45 insects)		Thiamethoxam (Actara 25WG) (45 insects)		Acetamiprid (Mospilan 20SP) (45 insects)				
			24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours		
2,4-di-tert-butylphenol	17.043	0.17 ± 0.03	0.18 ± 0.02	0.46 ± 0.06	0.50 ± 0.03	0.61 ± 0.05	0.29 ± 0.04	0.22 ± 0.01	0.27 ± 0.06	0.64 ± 0.06	0.30 ± 0.03	0.22 ± 0.03	0.48 ± 0.03

The data are presented as the mean ± standard deviation

*The position of the double bond is not specified

the application of both insecticides and females after the application of the thiametoxam-containing insecticide, the percentage after 24 h increased slightly compared to the control samples ($25.17 \pm 3.21\%$ - male, $23.36 \pm 1.98\%$ - female). Then after 48 h, the percentage content of this compound increased by a few to over a dozen percent (Table 2).

Statistical test

In order to check whether there are statistical differences in the composition of chemical compounds in the analyzed insects depending on the time elapsed since the application of insecticides, a one-way statistical ANOVA test was performed. The test showed no statistically significant differences in the chemical composition of insects 24, 48 and 72 h after the application of insecticides. The same test was used to check whether there were statistical differences in the chemical composition of the insects depending on the insecticide used. The test showed that there are significant statistical differences in the chemical composition of insects depending on the used insecticides Cyflok 50EW and K-othrine 25WG for *T. molitor* and Actara 25 WG and Mospilan 20SP for *L. decemlineata*. One-way ANOVA was also used to check whether there were statistically significant differences in the chemical composition of the two analyzed insect species after insecticide treatment, depending on sex. The test showed that there are significant statistical differences in the chemical composition of insects between males and females after treatment with insecticides. The last comparison was to check whether there were statistical differences in chemical composition after the use of insecticides between the species of the analyzed insects. For this purpose, a two-way ANOVA test was used. The chemical composition of males *T. molitor* and *L. decemlineata* and females of these two species of insects was compared with each other. In each comparison, it was found that there were statistically significant differences between the chemical composition of insects after the use of insecticides for the same sex of different species.

Discussion

Research on the composition of volatile compounds contained in insects have shown that the composition

Fig. 2 Percentage of alkanes in *Leptinotarsa decemlineata* insects before and after applying insecticides

and quality of compounds may change under the influence of insecticides. The use of a stressor, namely insecticides, mainly affected a quantitative change in the composition of volatile compounds. Differences were also visible depending on the insecticide used, the time elapsed since its application and the sex of the insects. Similar conclusions and results have been described for the larvae of *T. molitor* and *L. decemlineata* after using insecticides containing cyfluthrin and deltamethrin (Wojciechowska and Gołębowski 2020). Using the Folch method, changes in the composition of internal compounds contained in the fat body after applying insecticides have been described in the larvae, females and males of *T. molitor* (Wojciechowska et al., 2019). As it turns out, not only insecticides can be a stress factor, affecting the composition of chemical compounds contained in insects. Another example is entomopathogenic fungi, e.g. *Beauveria bassiana* fungus used on male and female *Hylobius abietis* insects (Gołębowski et al., 2020a, b).

In the conducted research, the highest percentage content was found for compounds from the alkanes group. In *T. molitor* males, the percentage of alkanes ranged from 38.63 to 59.05% depending on the insecticide used and the time elapsed since its application. In females, this range was from 38.89 to 60.20%. In contrast, in female *L. decemlineata*, the percentage of alkanes ranged from 43.84 to 61.85%, and in males, from 42.41 to 60.11%.

The content of alkanes has been described with varying lengths of carbon chains in many insect species that inhabit different ecosystems (Howard & Blomquist 2005). Carbon chain alkanes C27–C31 are indicative in *Arenivaga investigata* (Jackson 1983). In *Locusta migratoria cinerascens*, on the other hand, there are alkanes with a longer homologous series C21 – C37 (Genin et al., 1986). Species inhabiting desert areas, e.g. *Drosophila pseudoobscura* and *Drosophila mojavensis* (Blomquist et al., 1985) produce a small amount of alkanes, which constitute a barrier to water. As a result, these insects are susceptible to drying of the epidermis (Toolson et al., 1990). The presence of alkanes in insects protects adults, queens in colonies, and insect eggs against drying (Greene & Gordon 2003). In addition to the water barrier, alkanes can also have other functions depending on the species. In the species *Pogonomyrex barbatus*, individuals exposed to sunlight have

more alkanes in the epidermis than individuals living in shady places (Wagner et al., 1998). In some species of honey bee, alkanes play a signaling role (Getz & Smith 1987). In *Aphaenogaster* ants, the alkanes contained in the epidermis, mainly pentacosane, are used to signal and identify intruders (Smith et al., 2009). The chemical signal about the alarming role is undecane, whose occurrence has been described in the Dufour gland in the species *Lasius* and *Formica* (Hölldobler & Wilson 1990). In flies of the *Lycoriella mali* species, heptadecan acts as a pheromone (Blomquist et al., 1987).

The presence of unsaturated hydrocarbons with one and double bond has been described as a pheromone, e.g. 9-tricosene, e.g. in *Musca domestica*. On the other hand, 7-tricosen in *Drosophila melanogaster* is said to be a compound that inhibits same-sex individuals (Scott 1986; Tillman et al., 1999). In insects, other alkenes are also responsible for sexual signaling (Howard & Blomquist 1982). Alkenes as sex pheromones (C31:1, C33:1) at position Z9 have been identified in the *Stomoxys calcitrans* fly (Sonnet et al., 1979). The composition of the alkenes in the insect epidermis may be associated with the level of aggression in insects e.g. in *Macrotermes falciger* termites (Kaib et al., 2002).

In honey bees, compounds from the group of alcohols, aldehydes and ketones act as neurotransmitters (Sasche et al., 1999). Alcohols have also been identified in *Melanoplus pakardii* and *Acyrtosiphon pisum* (Brey et al., 1985). In addition, alcohol mixtures in *Musca domestica* showed an antimicrobial effect (Gołębowski et al., 2012a). Some fatty acids have antifungal activity in various species, e.g. *Musca domestica* (Barlow 1964), and *Blatella germanica* (Blomquist et al., 1991).

(E)-7-dodecen-1-ol acetate and heptadecanol were present in male and female *T. molitor* and *L. decemlineata* after applying insecticides and were absent from control insects, so these compounds may occur due to the application of insecticides and can be synthesized as an insect defense response. Probably these chemical compounds are not specific to these insect species and were present in many insect species. For example, (E)-7-dodecen-1-ol acetate was detected in *Actebia fennica* (Struble et al., 1989) and *Grapholita endrosias* (Ando et al., 1981). Heptadecanol was present in *Locusta migratoria migratoriodes* and *Schistocerca gregaria* (Ohara and Lockey, 1990).

Conclusions

The use of stress agents on insects, in the form of insecticides, changes the composition of compounds contained in insects, depending on the species of insect, sex, type of insecticide used to treat the insect, and time elapsed after treatment. As a result of the research, it turned out that the quantitative changes in the composition of compounds on the surface of insects range from a few to a dozen or so percent. They can be an introduction to biological tests determining whether the described changes will affect the usefulness of such areas of research as *T. molitor* in the diet of humans and animals. Will the insecticides used for *L. decemlineata* as a crop pest in the long run not affect crop efficiency and food availability?

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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