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SHORT COMMUNICATION

Identification and quantitative analysis of lipids and other organic compounds contained in eggs of Colorado potato beetle (*Leptinotarsa decemlineata*)

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Abstract
The importance of insects in the environment is huge, not always positive. Harmful insects are burdensome and cause large economic losses. One of the representatives of harmful insects is potato beetle (*Leptinotarsa decemlineata*). Researching the chemical composition of this insect can be used to more effectively protect plants against this pest. The eggs of Colorado potato beetle (*Leptinotarsa decemlineata*) were chosen for analysis because their composition is little known. Samples for analysis were prepared using the Folch method, and gas chromatography coupled with mass spectrometry (GC/MS) was selected for final determinations. As a result of the analyses, carboxylic acids, dicarboxylic acids, hydroxy acids, monoacylglycerols, alcohols, sterols and other organic compounds were identified in the tested samples. The highest content was determined for acids with a carbon chain C16:0–C18:0: palmitic (23 ± 0.88 μg/g), linoleic (32 ± 2 μg/g), oleic (83 ± 8.7 μg/g) and stearic (50 ± 2.2 μg/g). In the case of alcohols and sterols, the highest content was determined for docosanol (2.5 ± 0.04 μg/g) and cholesterol (23 ± 1.4 μg/g), respectively. The knowledge of the chemical composition of eggs can help in the work on effective insecticides, which will be effective only on a species of harmful insect.

Keywords *Leptinotarsa decemlineata* · GC/MS · Carboxylic acids · Sterols · Alcohols · Insect eggs

Introduction

Insects cause a lot of stress for plants which reduces yields and causes economic losses (Dixit et al. 2017). Such insects include Colorado potato beetle (*Leptinotarsa decemlineata*) (Ropek and Kołodziejczyk 2018; Sosnowska et al. 2009; Laznik et al. 2010). This insect occurs in the USA with the exception of Nevada, Hawaii, California and Alaska, in Mexico, Europe and part of Asia (Capinera 2001; Jacques Jr and Fasulo 2012). She got to Europe with food transport (Weber et al. 2006). The Colorado potato beetle mainly attacks plants from the *Solanaceae*. The Colorado potato beetle most likes potatoes, although it is also found on tomatoes (Kaniuczak 2013; Ahammao-Sahib et al. 1994). On potatoes, the whole development cycle passes, while on tomatoes it only feeds, treating them as extra food (Krawczyk et al. 2015). In spring, female beetles lay eggs. The eggs of Colorado potato beetle are located on the underside of the leaf blade, which is to protect them from solar radiation. The eggs of Colorado potato beetle (*Leptinotarsa decemlineata*) are light orange, oval shaped, 1.7–1.8 mm long and 0.8 mm wide. Females make an average of 20–60 eggs on the underside of the leaves, covering them with a yellowish adhesive (Capinera 2001). After hatching, the larvae feed on the leaves of the potatoes. Chemical and biological agents—insecticides—are most commonly used to fight this pest. They are used, for example, in the form of spraying and also soaking of potatoes seedlings in insecticide solutions against insect pests (Weber et al. 2006; Krawczyk et al. 2015; Rimoldi et al. 2011; Rojht et al. 2012). Different organic compounds may be present in the eggs, e.g., carboxylic acids, alcohols, alkanes. Their functions can be different. Carboxylic acids have antifungal activity. Alcohols, alkanes and carboxylic acids may also have an effect that attracts natural parasitoids, which are the natural enemy

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of a given species of insect (Frenoy et al. 1992; Qinge et al. 2016).

Analysis of the chemical composition of different species of insects can help in finding chemical compounds with a similar effect as repellents, antifeedants and attractants that can be effective in the fight against harmful insects (Adb El-Aziz 2011; Ningombam et al. 2017).

The purpose of the study was to know about the chemical composition of naturally occurring compounds in potato beetle eggs. It gives the possibility of controlling this pest, e.g., by introducing compounds that are antagonists for compounds contained in eggs that protect those eggs against the external environment.

Materials and methods

Insects

A Colorado potato beetle was harvested from a potato field, located in the village of Sąborze in the Pomeranian Voivodeship in Poland. Acquisition of material for testing from a potato field took place before the application of insecticides. This was to offset the adverse impact of plant protection products on the results obtained later. Insects were placed in containers with access to food (leaves of potatoes collected from the same field as insects). Adult insects were divided into males and females. There were 25 females in the cattery. The females laid eggs on the leaves of potatoes.

Extraction

The potato beetle (*Leptinotarsa decemlineata*) eggs were weighed and counted. For the preparation of one sample, 20–30 eggs were collected and then completely minced in a mortar. 19-methylarachidic acid as an internal standard was added. The samples were prepared using the Folch method. The solvent was chloroform–methanol (2:1; v:v) (Folch et al. 1957). All the extracts were then evaporated under a stream of nitrogen. Table 1 lists the weight of eggs and the masses of the extracts.

Table 1 Weight and the masses of the samples

Sample no.	Weight of eggs (mg)	Masses of the extracts (mg)
1	44.3	1.1
2	50.2	1.2
3	48.3	1.0
4	47.6	1.1

Derivatization

All the extracts were evaporated under nitrogen to ca. 1 mg of dry weight. All samples were subjected to a derivatization process. A mixture of 100 µl 99% bis(trimethylsilyl) acetamide and 1% chlorotrimethylsilane (BSTFA) and 10 µl 19-methylarachidic acid (1 mg/mL) was added to 1 mg of each sample. Samples were heated for 1 h at 100 °C (Cerkowiak et al. 2013).

Gas chromatography coupled with mass spectrometry (GC/MS)

Gas chromatography coupled with mass spectrometry (GC/MS) was selected for the final determination. This technique provides high selectivity and sensitivity. Qualitative and quantitative analyses were performed on a gas chromatograph (Shimadzu QP 2010S), with capillary column ZB-5 (Zebron, 30 m × 0.25 mm × 0.25 µm). 1 µl of each extract was injected (a split ratio of 1:30). The column temperature was programmed from 80 (held for 8 min.) to 320 °C (held for 8 min) at a rate of 4 °C/min. The injector and transfer line temperatures were 320 °C. The carrier gas was helium at a column head pressure of 60 kPa. Electron impact was applied to the ionization (70 eV) and set to scan the 40–700 amu mass range. The ion source was kept at 210 °C. The analysis of organic compounds was carried out by GC/MS in the total ion current (TIC) mode. The purpose was to determine the quantitative and qualitative content of the chemicals contained in the test samples (Cerkowiak et al. 2013). Every sample was analyzed in three repetitions. Then the mean and standard deviation were calculated.

Results

In the analyzed samples, the presence of carboxylic, dicarboxylic and hydroxy acids, alcohols, monoacylglycerols, sterols and other organic compounds was found (Table 2). Figure 1 shows the total ion current (TIC) of identified compounds. A large number of groups of compounds in the analyzed samples were carboxylic acids. Dominant carboxylic acids were: palmitic (23 ± 0.88 µg/g), linoleic (32 ± 2 µg/g), oleic (83 ± 8.7 µg/g) and stearic (50 ± 2.2 µg/g). The acids occurring in smaller quantities (from 2 to < 10 µg/g) were: eicosadienoic, arachidic, behenic and lignoceric. The samples also contained seven carboxylic acids present in concentrations < 1 µg/g. Alcohols are the second-largest group of compounds determined in the analyzed samples of eggs Colorado potato beetle. The highest content was determined for docosanol (2.5 ± 0.04 µg/g). Four hydroxy acids and sterols

Table 2 Content of organic compounds in the analyzed samples of eggs of Colorado potato beetle

Compounds	μg/g ± SD
Fatty acids	
Caprylic acid	0.37 ± 0.02
Pelargonic acid	0.23 ± 0.02
Palmitoleic acid	0.45 ± 0.01
Palmitic acid	23 ± 0.88
Heptadecenoic acid	0.10 ± 0.02
Margaric acid	1.4 ± 0.03
Linoleic acid	32 ± 2.0
Oleic acid	83 ± 8.7
Stearic acid	50 ± 2.2
Nonadecanoic acid	1.7 ± 0.03
Eicosadienoic acid	3.5 ± 0.2
Gadoleic acid	1.1 ± 0.03
Arachidic acid	6.8 ± 0.08
Erucic acid	1.4 ± 0.05
Behenic acid	3.4 ± 0.12
Lignoceric acid	2.1 ± 0.07
Dicarboxylic acids	
Succinic acid	1.7 ± 0.05
Malic acid	1.2 ± 0.03
Azelaic acid	0.6 ± 0.04
Hydroxy acids	
Glyceric acid	0.30 ± 0.02
3,4-Dihydroxybutanoic acid	3.0 ± 0.07
2,3,4-Trihydroxybutanoic acid	3.0 ± 0.06
2-Hydroxyglutaric acid	0.75 ± 0.02
Alcohols	
Octadecanol	0.24 ± 0.02
Hexacosanol	1.3 ± 0.03
Octacosanol	1.6 ± 0.03
Docosanol	2.5 ± 0.04
Tetracosanol	1.4 ± 0.01
Acylglycerols	
Monopalmitoylglycerol	2.3 ± 0.05
Monostearoylglycerol	4.5 ± 0.08
Sterols	
Cholesterol	23 ± 1.4
Campesterol	0.74 ± 0.02
Stigmasterol	0.94 ± 0.04
β-sitosterol	6.6 ± 0.10
Other compounds	
Pyroglutamic acid	8.7 ± 0.36
Glutamic acid	11 ± 0.76
Pantothenic acid	0.56 ± 0.03
1,4-Butanediol	1.2 ± 0.02
Glycerol	6.0 ± 0.07
Undecane	1.2 ± 0.06
Dihydrofuranone	0.22 ± 0.02
Urea	1.2 ± 0.04

were also detected. Among hydroxy acids, the compounds present in the highest concentrations were 3,4-dihydroxybutanoic acid (3.0 ± 0.07 μg/g) and 2,3,4-trihydroxybutanoic acid (3.0 ± 0.06 μg/g). Among sterols, dominant compound was cholesterol (23 ± 1.4 μg/g). Three dicarboxylic acids were found in the potato beetle. These compounds were present in smaller quantities from 0.57 ± 0.04 to 1.7 ± 0.05 μg/g. Only two acylglycerols were present in analyzed samples: monopalmitoylglycerol (2.3 ± 0.05 μg/g) and monostearoylglycerol (4.5 ± 0.08 μg/g). Among other organic compounds were identified amino acid, amino acid derivative, amide between pantoic acid and β-alanine, diol, alkane, glycerol, dihydrofuranone and urea.

Discussion

The aim of the research was the quantitative and qualitative identification of chemical compounds contained in eggs of Colorado potato beetle. This is due to the use of a large amount of insecticides to control harmful insects. Insecticides are often harmful to other nontarget organisms. They are also toxic to the environment. Therefore, it is important to conduct research that may contribute to the knowledge of the chemical composition of harmful insects, which may help in the creation of effective preparations for protection against pests. These preparations should act selectively and be harmless to the environment.

A large number of groups were C8–C24 carboxylic acids. The highest content of carboxylic acids in the analyzed samples of eggs of Colorado potato beetle was identified for C16 and C18 acids. The presence of these acids was identified in other species of insects, for example in *Melanoplus sanguinipes*, *Melanoplus packardii* (Soliday et al. 1974), *Zophobas atratus* (Cerkowniak et al. 2015), *Acyrthosiphon pisum* (Brey et al. 1985), *Sarcophaga carnaria* (Gołębiowski et al. 2014), *Locusta migratoria* (Oraha and Lockey 1990), *Fannia canicularis* (Kerwin 1984), *Tenebrio molitor* (Paul et al. 2017), *Dermestes maculatus*, *Dermestes ater* (Cerkowniak et al. 2017) and *Calliphora vicina* (Gołębiowski 2012). In the analyzed samples, the following saturated and unsaturated acids were also identified: C8, C9, C17, C19, C20, C22 and C24. Their content was lower than C16:0 and C18:0. Unsaturated carboxylic acids with longer carbon chains have antimicrobial activity against entomopathogenic fungi (Gołębiowski et al. 2014). Linoleic and oleic acids inhibit the growth of Gram-positive bacteria (Dilika et al. 2000). Linoleic acid is essential in human diet because it reduces inflammation and reduces the risk of myocardial infarction (Alim et al. 2008).

Only three dicarboxylic acids were present in the analyzed samples. The presence of dicarboxylic acids was

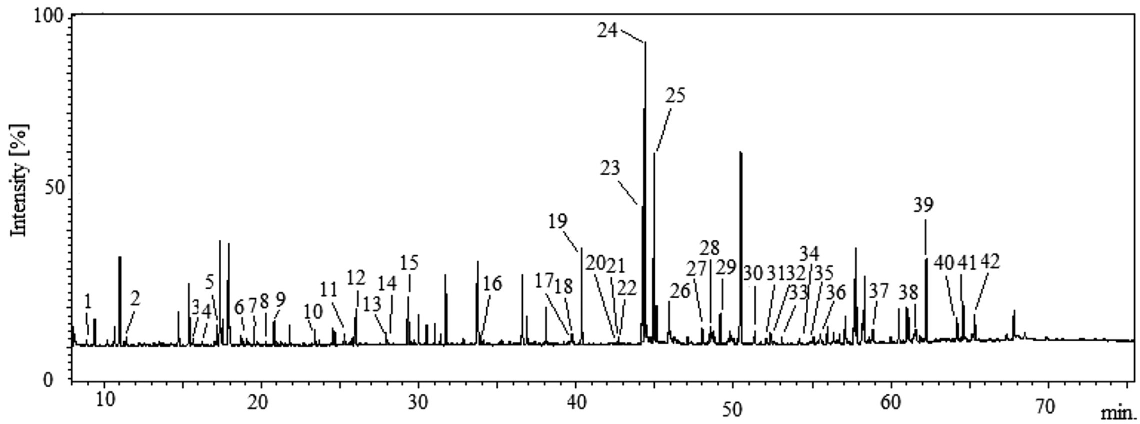

Fig. 1 TIC of identified compounds in eggs of Colorado potato beetle. 1—Undecane, 2—1,4-butanediol, 3—urea, 4—caprylic acid, 5—glycerol, 6—succinic acid, 7—glyceric acid, 8—pelargonic acid, 9—dihydrofuranone, 10—3,4-dihydroxybutanoic acid, 11—malic acid, 12—pyroglutamic acid, 13—2,3,4-trihydroxybutanoic acid, 14—2-hydroxyglutaric acid, 15—glutamic acid, 16—azelaic acid, 17—pantothenic acid, 18—palmitoleic acid, 19—palmitic acid, 20—heptadecenoic acid, 21—margaric acid, 22—octadecanol, 23—linoleic acid, 24—oleic acid, 25—stearic acid, 26—nonadecanoic acid, 27—eicosadienoic acid, 28—gadoleic acid, 29—arachidic acid, 30—docosanol, 31—monopalmitoylglycerol, 32—erucic acid, 33—behenic acid, 34—tetracosanol, 35—monostearoylglycerol, 36—lignoceric acid, 37—hexacosanol, 38—octacosanol, 39—cholesterol, 40—campesterol, 41—stigmasterol, 42—β-sitosterol

found, for example, in *Limulus polyphemus* (Karlson et al. 1969). They exhibit antibacterial activity and are often added to cosmetics (Zeichner 2013).

In the alcohol group were identified octadecanol, hexacosanol, octacosanol, docosanol and tetracosanol. The major alcohol in eggs was docosanol. The presence of alcohols is described. For example, alcohols are present in *Locusta migratoria migratoriodes* (Oraha and Lockey 1990), *Lucilia sericata* (Gołębiowski et al. 2012a), *Heliothis virescens* (Buckner et al. 1996) and *Musca domestica* (Gołębiowski et al. 2012b). Some alcohols presented antimicrobial activity (Gołębiowski et al. 2012b).

The presence of fatty acids, alcohols and alkanes was also found in *Bactrocera dorsalis eggs* (Qinge et al. 2016), *Ostrinia nubilalis* (Frenoy et al. 1992). Studies on these species have shown that these chemicals can serve as a chemical signal that attracts parasitoids, e.g., in the case of *Bactrocera dorsalis* it is *Fopius arisanus*. Using research on the chemical composition of harmful insects at various stages of development, for example, you can use these compounds for spraying plants to increase the ability to locate and identify and infect parasitoid insects with harmful ones. This could increase the effectiveness of the control of harmful insects in the future.

In the analyzed samples were identified sterols cholesterol, campesterol, stigmasterol and β-sitosterol. Cholesterol was the dominant sterol found in eggs of *Leptinotarsa decemlineata*. Sterols were determined in insects, e.g., *Melanoplus sanguinipes, Melanoplus packardii* (Soliday et al. 1974) and *Eurycotis floridana* (Roeske and Clayton 1968). Sterols are precursors of hormones and components of cell membranes (Gołębiowski et al. 2013).

Among other organic compounds, glycerol and undecane were identified. For example, glycerol was found in eggs *Bombyx mori*, larvae of *Hyalophora cecropia*, different stages of development *Eurosta solidaginis*, *Antheraea polyphemus* and *Bracon cephi.* The role of glycerol is tolerance to low temperatures (this compound prevents insects from freezing). The lower the temperature, the higher its content (Sømme 1964). Alkanes are found in many insect species, for example in *Lucilia sericata* (Gołębiowski et al. 2012c), in adults *Leptinotarsa decemlineata* (Nelson et al. 2003) and *Stenocara gracilipes* (Lockey 1988). Alkanes can serve as a chemical signal to distinguish between species (Lockey 1988).

Chemical compounds with the action of attractants can be used to lure the natural enemies of harmful insects. An example may be the use of attractants of *Cryptolaemus montrouzieri*, which is the enemy of scalar insects—ornamental plant pests and similarly, *Coccinella septempunctata*, which feeds on, e.g., aphids, maggots, which are pests of arable crops (Kundoo and Khan 2017). In contrast, antifeedant compounds can also be used as an alternative means of controlling harmful insects. Their task is to regulate the growth and reproduction of potential pests. An example may be the use of *Cabralea canjerana* extracts on *Spodoptera frugiperda* larvae (Ningombam et al. 2017).

In conclusion, the presence of carboxylic acids, dicarboxylic acids, hydroxy acids, alcohols, acylglycerols, sterols and other organic compounds was found. Their role is not known because it requires additional biological research.

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Compliance with ethical standards

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

Human and animal rights The article does not contain any human and animal rights.

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