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RESEARCH ARTICLE

The content of fatty acids in the tissues of honey bees after feeding with herbicide

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Changes in agroecological and environmental conditions in the result of the use of pesticides can affect the life cycle and functional activity of honey bees. In acute oral study we investigated the influence of herbicide on honey bees *A. mellifera* L. Active ingredients of the herbicide are: desmedipham, fenmediphim, ethofumesate, 71+91+112 g/dm^3, that are related to the chemical groups of carbamates and benzofuranyl alkanesulfonates. Under the action of herbicide in indicated doses the temporary inhibition of *A. mellifera* L honey bees' activity was observed. Biochemical pathways of insects' response to the influence of the herbicide were evaluated during the study of the fatty acid spectrum of lipids in the tissues of the thoracic and abdominal segments of worker bees. Using the method of high-sensitivity gas chromatography, we detected and quantitatively identified 17 fatty acids (FAs) in the tissues of bees in the control and experimental groups. The redistribution of the content of individual FAs (saturated and unsaturated) was revealed during the action of the herbicide. In particular, a decrease in the total content of saturated FAs may indicate a lower level of energetic and structural fatty acid reserves. Besides, the revealed decrease in the content of monoenoic FAs, particularly oleic acid, as well as short-chain saturated FAs in the conditions of herbicidal load, likely affects the protective functions of bee organism. The increase of the content of long-chain polyunsaturated FAs may have an adaptive significance in response to exogenous effects. This is particularly true for arachidonic and docosahexaenoic acids, which are involved in the regulation of a wide range of physiological processes. It is assumed that the decrease in the content of the FAs of ω-6 family, as well as the increase of ω-3 and ratio value of ω-3 / ω-6, characterizes the type of eicosanoids synthesized in organism in herbicide load conditions. This is probably due to the activity of the acyl-lipid desaturase ω-3 and ω-6, as evidenced by the increase of the ratio of 22:6ω3 /18:3ω3 and 20:4ω6/18:2ω6. The revealed modifications of the spectrum of the lipids' FAs in the tissues of honey bee can be explained by the participation of the FAs in the reorganization of reactivity system of the bee organism as the response to herbicide load, since the redistribution of fatty acids in bee organism is one of the important indicative characteristics of the insects' physiological status and viability.
Keywords: Herbicide; honey bees; fatty acids; polyenoic polyunsaturated fatty acids

Introduction

Fatty acids contained in the natural feed (pollen, bee bread) are deposited in the fat body of honey bees and are known to be amongst major sources of energy and structural material. The fat storage in the body of honey bees plays an important role, in particular in heat formation, as it is used by bees at low temperatures (Taranov, 1986; Lebedev et al., 1991). It should be noted that in the process of heat formation bees constantly use and restore their fat reserve, even if they have sufficient supplies of natural feed. In addition, the bees' life cycle is dependent directly on the degree of the fat body development (Eskov, 1981; Taranov, 1995). The formation of the fat storage of honey bees in unfavorable environmental conditions, in particular, under the man-caused impact (Strogov et al., 2009; Kovalchuk, 2013), can affect functional activity and viability of insects.

Thus, the dynamics of content of lipids and their fatty acids in the body of honey bees is an important indicator of the functional state (the course of physiological and biochemical processes). In turn, it can to some extent characterize the influence of exogenous factors, in particular, the change of environmental conditions due to the use of pesticides. In our previous studies we showed the modification of the fatty acid composition of lipids in tissues and organs of fish (Khyzhnyak et al., 2018) or mammals (Khyzhnyak et al., 2016) under influence of exogenous factors on the animal organism. The aim of this research is to study the fatty acid composition of the tissues of the thoracic and abdominal segments of the honey bee *A. mellifera* L. after feeding with the herbicide (active substances: desmedipham, phenmedipham, ethofumesate, 71+91+112 g/dm^3) for assessing the biochemical pathways of the response of the insects to the use of the drug.

Materials and methods

The herbicide in the form of an emulsifiable concentrate contains three active substances: desmedipham - [ethyl-3-phenylcarbamoyloxyphenylcarbamate; ethyl-3-phenyl-phenylcarbamoyl-carbanylate; 3-ethoxycarbonyl-aminophenyl phenylcarbamate] and phenmedipham - [methyl-3- (3-methylcarbanoyloxy) carbanyl] refer to the chemical group of carbamate, and ethofumesate - [(+/-) - 2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate] belongs to the chemical group of benzofuranyl alkanesulfonates (Global Access to IUPAC Agrochemical Information).

To set up an experiment (acute experiment), three groups of adult worker honey bees *A. mellifera* L. were formed (50 individuals in each group). Individuals in the control group consumed sugar syrup, in experimental groups - herbicide containing sugar syrup in the quantity of 733 μg per individual (experimental group 1) and 1465 μg per individual (experimental group 2). After feeding the drug (4-6 hours), the worker bees were kept for 72 h fed with sugar syrup under the following conditions: temperature 25 ± 2 ºC and relative humidity 50-70% (OECD, Tests, 1998).

At the end of the experiment samples of bee tissues (total chest and abdominal segments) were taken, homogenization and lipid extraction were performed with a chloroform-methanol mixture. Hydrolysis of lipids and methylation of fatty acids (FAs) were performed in accordance with (DSTU ISO 5509-2002). Methyl esters of FAs were analyzed on a gas chromatograph "Trace GC Ultra" (USA), detector type: flame ionization. The separation was carried out on a high-polar capillary chromatography column "SPTM-2560" (Supelco, USA). For the identification of acids, a standard mixture of fatty acid methyl esters "37 Component FAME Mix" ("Supelco") was used. For the quantitative assessment of individual FAs we used the method of internal normalization and represented the relative content of FAs in percentage ratio to their total quantity (Rivis et al., 2010). Using the method of high-sensitivity gas chromatography, we detected and quantitatively identified 17 FAs in the tissues of bees in the control and experimental groups.

The residual amount of herbicide in bee organism was evaluated on the basis of one of the active ingredients - ethofumesate according to QuEChERS-method, 2008. The content of ethofumesate in insects in experimental group 2 remained twice as high as that as in experimental group 1. According to the results of toxicological studies of the herbicidal drug, the value of LD50 was estimated, which at the 48th hour of exposure was 1187.4 μg of the drug per bee (the herbicidal drug can be classified as low toxic, 3rd grade of hazard).

Methods of statistical analysis based on Student's criterion were used to process the primary results of the research. For calculations we used the computer programs Origin 6.0 and Microsoft Office Excel 2007 (USA).

Results and discussion

Fatty acid composition of the total lipids of the honey bees *A. mellifera* L. is characterized by high content of unsaturated fatty acids (UFAs) both monoenoic (palmitoleic (C16:1), oleic (C18:1ω9)) and polyenoic (linoleic (C18:2ω6), linolenic (C18:3ω3), arachidonic (C20:4ω6), docosahexaenoic (C22:6ω3)) acids (Table 1). High content of UFAs, which is 85.24% of the total amount of FAs, was confirmed in other scientific reports (Polischuk et al., 2002). This explains the possibility of permeability increasing of the lipid structural components in the body of bees for water and water-soluble substances (Mizyurev, 2004). Feeding bees with the herbicide drug reduces the total content of saturated fatty acids (SFAs) by 76 % and 87 % , respectively, for the experimental groups 1 and 2 in comparison with the control (Table 2). The obtained results, especially those with respect to palmitic (C16:0) and stearic (C18:0) acids, may indicate lower level of energetic and structural reserves of bees' organism. It is known that FAs have protective, antitoxic, antibacterial and antifungal action. This is particularly the case of saturated (caprylic (C8:0) and capric (C10:0)) and unsaturated (oleic (C18:1ω9) and linoleic (C18:2ω6)) acids. The shorter the carbon chain of SFAs is, the more antibacterial and antifungal protection of bees is provided (Manning, 2001). Under the conditions of the experiment, the average reduction by 42 % of the content of short-chain SFAs was determined, as well as monoenoic FAs (MFAs), particularly oleic (C18:1ω9) – by 22 and 38 % (experimental group 1 and 2, respectively) as to the control group. The content of linoleic (C18:2ω6) and linolenic (C18:3ω3) acids, which are most exposed to oxidation, in the conditions of the experiment varies in a different direction (Table 2). An important point is the increase in the content of long chain polyenoic FAs (PFAs) of total lipids (Tables 1 & 2), which may have an adaptive significance, since PFAs expand the range of tolerance of the organism. PFAs are the precursors of biologically active substances that are regulators of a wide range of physiological processes, including stress. In particular, this applies to arachidonic (C20:4ω6) acid, the content of which in experiments 1 and 2 increases on an average 6.5 and 6.2 times, respectively, compared to control.

Table 1. The content of fatty acids (%) in the tissues of thoracic and abdominal segments of honey bees after feeding with herbicide (active substances: desmedipham, phenmedipham, ethofumesate, 71+91+112 g/dm^3) M ± m, n=3.

Fatty acids, %	Control group 1	Experimental group 1	Experimental group 2
C8:0	0.30 ± 0.01	0.17 ± 0.02*	0.17 ± 0.02*
C10:0	0.17 ± 0.01	0.10 ± 0.02*	0.10 ± 0.02*
C12:0	0.11 ± 0.01	0.21 ± 0.07*	0.27 ± 0.06*
C14:0	0.28 ±0.03	0.66 ± 0.03*	0.94 ± 0.03*.**
C16:0	6.96 ± 0.71	0.89 ±0.07*	0.64 ± 0.06*.**
C16:1	5.40 ± 0.46	6.25 ± 0.76	4.67 ± 0.52
C18:0	6.86 ± 0.38	1.29 ± 0.17*	0.94 ± 0.04*.**
C18:1ω9	30.24 ± 1.03	23.64 ± 0.89*	18.64 ± 0.66*

C18:2ω6	26.24 ± 0.71	10.93 ± 0.94*	10.30 ± 0.04*
C20:0	0.08 ± 0.02	0.24 ± 0.02*	0.28 ± 0.02*
C18:3ω3	16.00 ± 0.17	21.36 ± 0.95*	25.78 ± 0.89*
C20:1ω9	0.06 ± 0.01	0.07 ± 0.02	0.05 ± 0.01
C 20:2ω6	0.58 ± 0.04	0.10 ± 0.01*	0.05 ± 0.01*
C 20:3ω3	0.48 ± 0.03	1.47 ± 0.04*	3.41 ± 0.04*.**
C 20:4ω6	2.43 ± 0.25	15.90 ± 0.60*	15.10 ± 0.54*
C 20:5ω3	0.83 ± 0.09	3.13 ± 0.21*	2.09 ± 0.34*.**
C 22:6ω3	2.98 ± 0.20	13.59 ± 0.74*	16.20 ± 0.79*.**

Notes: data are presented as a mass fraction of fatty acid in percentage from the sum of fatty acids.
** – P < 0.05 vs control.*
*** – P < 0.05 vs experimental group 1.*

Table 2. The total content of fatty acids and their ratio in the tissues of thoracic and abdominal segments of honey bees after feeding with herbicide (active substances: desmedipham, phenmedipham, ethofumesate, 71+91+112 g/dm^3), % (M ± m, n=3).

Fatty acids, %	Control group	Experimental group 1	Experimental group 2
Σ SFAs	14.76 ± 1.49	3.56 ± 0.11*	1.86 ± 0.13*.**
Σ UFAs	85.24 ± 1.98	96.44 ± 1.19*	98.16 ± 2.25*
Σ MFAs	30.30 ± 1.51	20.71 ± 1.41*	18.69 ± 1.31*
Σ ω3	20.29 ± 0.78	38.4 ± 1.11*	47.48 ± 1.32*.**
Σ ω6	29.25 ± 0.94	18.08 ± 0.91*	17.33 ± 0.87*
SFAs/UFAs	0.17 ± 0.02	0.04 ± 0.01*	0.02 ± 0.01*
ω3/ω6	0.69 ± 0.03	2.12 ± 0.12*	2.74 ± 0.13*.**
22:6ω3/18:3ω3	0.19 ± 0.01	0.64 ± 0.02*	0.63 ± 0.02*
20:4ω6/18:2ω6	0.10 ± 0.01	1.45 ± 0.15*	1.50 ± 0.12*
16:0/18:1 ω9	0.23 ± 0.01	0.04 ± 0.01*	0.03 ± 0.01*

** – P<0.05 vs control.*
*** – P<0.05 vs experimental group 1.*

Conclusions

Analyzing the obtained results, it should be noted that at the oral admission of the herbicide (active substances: desmedipham, phenmedipham, ethofumesate, 71+91+112 g/dm^3) in doses similar to LD50, there is the temporary inhibition of the activity of honey bees. Under the experimental conditions a redistribution of saturated and unsaturated FAs in bee tissues (thoracic and abdominal segments) was observed. Reduction of the content of SFAs (palmitic (C16:0) and stearic (C18:0)) indicates the decrease in the energetic and structural components of the body of bees. In addition, by reducing the content of MFAs and short-chain SFAs, inhibition of the protective function of the organism is possible. In view of the direct involvement of PFAs in the regulation of majority of cellular processes, the increase of their total content and the redistribution of the fatty acid spectrum of ω-3 and ω-6 families can be considered as mobilization of adaptive reactions of the organism. The obtained results indicate the complexity of the processes of lipid metabolism in the bees' organism, in particular, with the involvement of FAs of ω-3 and ω-6 families, after oral admission of the herbicidal preparation (active substances: desmedipham, phenmedipham, ethofumesate, 71+91+112 g/dm^3) that may have an effect on the functional activity and viability of bees.

Prospective direction for further research in feeding the herbicide drug is to determine the fatty acids in the products of the life of a honey bee, taking into account the content of residual active substances in it.

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