Scientific Papers. Series D. Animal Science. Vol. LXI, Number 2, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

CONTENT OF PESTICIDE RESIDUES IN THE FLOWERS OF THE ACACIA AND LINDEN TREES FROM THE MOLDAVIAN CODRI AREA

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Abstract

The purpose of this research was to assess the conformity of the forest melliferous flora with organic apiculture, regarding concentrations of systemic pesticides, to predict the production of bio apicultural products. Flowers of the forest trees of white acacia (Robinia pseudoacacia) and large-leaf linden (Tilia platyphillos), which are the main sources of nectar and pollen for honey bees, were studied for the presence of 17 more commonly spread pesticides: pyrethroid insecticides (Cypermethrin, Deltamethrin, Pyrethrin, Tau-fluvalinate), neonicotinoid insecticides (Imidacloprid, Clothianidin, Thiamethoxam), organophosphorus insecticides (Fenamifos), triazole fungicides (Difenoconazole, Fenhexamid, Mepanipyrim, Cyprodinil), acaricides (Fipronil) and herbicides (Amidosulfuron, Amitrol, Glyphosate, Sulfosulfurol). Of the 17 investigated pesticides, in the white acacia flowers, 14 pesticide residues were found: Cypermethrin, Deltamethrin, Pyrethrin, Clothianidin, Fenamifos, Difenoconazole, Fenhexamid, Mepanipyrim, Cyprodinil, Fipronil, Amidosulfuron, Amitrol, Glyphosate and Sulfosulfurol. Average concentrations of pesticides residues in acacia flowers ranged from 0.003 mg/kg for Mepanipyrim and Fipronil, to 8.75 mg/kg for Difenoconazole and 0.25 mg/kg to Glyphosate. In the large-leaf linden flowers, out of the 17 investigated pesticides, the residues of 13 pesticides were registered, including 3 pyrethroid insecticides, 1 neonicotinoid insecticide, 1 organophosphorus insecticide, 3 triazole fungicides, 1 acaricide and 4 herbicides. Average concentrations of pesticide residues in the linden flowers ranged from 0.001 mg/kg for Fipronil up to 16.0 mg/kg for Difenoconazole and 1.25 mg/kg for Glyphosate. The obtained data attest that in the flowers of forest trees are detected low concentrations of residues of pyrethroid insecticides (Cypermethrin, Deltamethrin, Pyrethrin) from 0,003 up to 0,100 mg/kg, of neonicotinoid insecticide (Clothianidin) from 0,400 up to 0,425 mg/kg, of organophosphorus insecticide (Fenamifos) in an amount of 0.042 mg/ kg, triazole fungicides (Fenhexamid, Mepanipyrim, Cyprodinil) from 0,017 to 0,092 mg/kg, triazole fungicide Difenoconazole from 8,75 to 16,0 mg/kg, acaricide Fipronil from 0,001 up to 0,003 mg/kg, herbicides (Amidosulfuron, Amitrol, Sulfosulfurol) from 0,005 up to 0,013 mg/kg and herbicide Glyphosate from 0,25 up to 1,25 mg/kg. The detected concentrations of pesticide residues in the tree flowers (white acacia and large-leaf linden) was from 1.3 to 33.3 times lower than the maximum admisibil limits, according to national and EU standards. Therefore, the recorded pesticide residues in the flowers of forestry trees, are not harmful for the health of bees and fauna of studied ecosystem. In conclusion we can say that the flora of studied by us forest sector, are not polluted with pesticides, so organic beekeeping can be practiced here, with the production of organic apiculture products.

Key words*: pesticides, flowers, acacia, linden, Apis mellifera, organic apiculture.*

INTRODUCTION

Forest tree flowers has a particular interest as a source of harvesting for *Apis mellifera* bees. Usually, in apiculture of the Republic of Moldova, the first harvesting occurs in acacia or linden trees. The honey obtained from these harvests has a superior quality. The beekeeping branch has an essential importance for the national economy due to the value and quality of its products, the creation of jobs among vulnerable populations of rural areas, and conservation through pollination of homeostasis and of biodiversity of natural ecosystems. There are about 120 thousand families of bees in the country, from which about 2.0 to 2.2 thousand tons of honey is obtained annually, of which 600 tons are exported to different countries. Other bee products, quite important, such as wax, pollen, propolis, royal jelly, venom, are used in various fields of national economy (food, medicine, pharmaceuticals, cosmetics, plastic art, etc.). Analysis of the situation in recent years demonstrates that traditional technology of breeding and exploitation of bee families does not provide everywhere the production of organic and safety bee products of competitive quality, which could be sold at reasonable prices (Siceanu, 2012). EU certification and control bodies (EC Regulation 834/2009, EU OJ L241, 2009, EC Regulation 271/2010, EU OJ L84, 2010) often detect residues of polluting substances in apiculture production, exported from the Republic of Moldova, which leads to its embargo and compromising the competitiveness of the branch and the national image internationally (Antonescu et al., 2001; Bogdanov et al., 1999; Buruian, 2011). Both farmers and beekeepers, until now haven't realized, that the uncontrolled using of pesticides for crops treating and of honey bees, has an unbalanced impact on the homeostasis of natural ecosystems, wich will finally diminish the health of bees and human.

In this context, the research of environmental pollutants and the identification of their impact on honey bees and apiculture products, the study of the conformity of organic apiculture in the conditions of different autochthonous anthropogenic ecosystems, the organic nutrition of bees during critical periods of harvest in nature, are current problems, and solving them it would allow the development of measures to adjust conventional apiculture to organic beekeeping, acoording to EU standards. The importance of organic beekeeping at current stage was demonstrated by some authors from EU (Antonescu et al., 2001; Siceanu, 2012).

Bee products, as well as agricultural, bio-certified products from officially accredited units are sold on the markets at more advantageous prices. These have a higher demand on the market because its ensures the product's safety, its are of superior quality and have a more distinctive origin. In the EU, organic apiculture is governed by a number of European Commission (EC) regulations, the most important of them are: EC Regulation no. 834/28.06.2007 on organic production and labeling of organic products, EC Regulation no. 834/2009, EC Regulation no. 271/2010 and EC Regulation no. 392/2013, which provides the accreditation and certification by the European international bodies of apiaries and bee products of the conventional category into the bio (organic) category, according to unique criteria and standards.

The adjustment (transformation) of beekeeping of the Republic of Moldova to the organic category, requires a thorough compliance study of its main components such as: bee families, the environment, bee breeding and care infrastructure, methods of feeding and nutrition of bee families during of poor harvest in nature, the logistic of beekeeping collection (tools, equipment, machinery), primary processing and storage of apiculture products, the quality and safety of apiculture products.

The ecological status of melliferous base from ecosystems of productive activity of honey bee is a particular problem. Under the melliferous base are meant melliferous flowers that serve as harvesting source of nectar and pollen.

In this context, the investigation of pollution degree of of melliferous flowers, from the production area of bees, with different pesticide residues and elaboration on this base of proposals for enhancing the ecological and food safety are extremely important and current problems, both on nationally and internationally levels.

The residues, which accumulate in the inflorescence of melliferous plants, soil and water, are, in fact, the main pollutants of the environment where the honeybee carries out their vital activity. Among these are heavy metals, pesticides, antibiotics, radionuclides, nitrites and nitrates.

From the environment, pollutants reach in the bees 'food, having an impact both on bees' health and vitality, and on the quality and safety of bee products, affecting the balance of environmental factors, needed for organic apiculture.

Recently, finds particular concern in Europe and throughout the world the use of systemic pesticides in agriculture for seed treatment and spraying of crops to combat insect pests, fungi and weeds.

According to "Beyond Pesticides" – (formerly National Coalition Against the Misuse of Pesticides, is a nonprofit organization from USA) (http://www.beyondpesticides.org/programs/bee-protective-pollinators-and-pesticides/ chemicals-implicated, 2016), neonicotinoid pesticides have adverse effects on reproduction, are neurotoxic and mutagenic for insects, birds, fish, freshwater snails, earthworms, dragonflies, mosquitoes and vertebrates, mentioning that "*neonicotinoids could represent the new contemporary ecological disaster, being a threat to nature*". The World Health Organization (WHO) and the

International Agency for Research on Cancer (IARC) announced in March 2015 that the herbicide Glyphosate is carcinogenic and has negative effects on the endocrine system. Residues of these herbicides can be found in the urine and blood of animals as well as humans (http//www.maslina.slobodnadalmacija.hr/novosti/ID/19528/Glifosat, 2017). Harmful effects of systemic pesticides are confirmed by a large number of researchers (Alaux et al., 2010; http://www.greenpeace.org., 2014; Garry et al., 2016; Grill et al., 2012; Henry et al., 2012; Oliveira et al., 2014; Pettis et al., 2012; Tanner G., 2011; Schneider et al., 2012; Tome et al., 2012; Whitehorn et al., 2012).

In this context, researching pollutants and identifying their impact on honey bee and apiculture products, studying the conformity of organic beekeeping under the conditions of various native anthropogenic ecosystems, are important and current tasks.

The purpose of this research is to assess the conformity of the forest melliferous flora with organic apiculture, regarding concentrations of systemic pesticides, to predict the production of bio apicultural products.

MATERIALS AND METHODS

Residues of 17 more commonly spread systemic pesticides have been investigated, such as: pyrethroid insecticides *(Cypermethrin, Deltamethrin, Pyrethrin, Tau-fluvalinate),* neonicotinoid insecticides *(Imidacloprid, Clothianidin, Thiamethoxam),* organophosphorus insecticides *(Fenamifos),* triazole fungicides *(Difenoconazole, Fenhexamid, Mepanipyrim, Cyprodinil),* acaricides *(Fipronil)* and herbicides *(Amidosulfuron, Amitrol, Glyphosate, Sulfosulfurol)*. The investigations were carried out on the main component of the bee activity area – flowers of the entomophilous forest trees, which provide the first basic honey harvesting. Pesticide residues were determined in the flowers of white acacia (*Robinia pseudoacacia*) and large-leaf linden (*Tilia platyphillos*) trees. During the blooming period, 4 samples with mass 100-130 g each of flowers with petiole were collected from trees at different distances from beehives. Samples were collected from Ghidighici Forestry, Canton no. 9, Forest Sector no. 21, of S.E. for Silviculture Chişinău. The Ghidighici forestry is a part of Moldavian Codri area.

The sampling of the flowers has been carried out according to the sanitary-veterinary norms regarding methodology of the sampling, processing, packaging and transport of the samples for the laboratory examinations (Normă sanitar-veterinară - Sanitary-veterinary standard, 2010). The samples were packed in plastic bags and transported urgently (1-2 hours) to the laboratory for analysis.

Researches of pesticide residues were performed on the same day in the accredited Laboratory for the determination of pesticide residues of S.E. "Center of Quarantine, Identification, Arbitration on Expertise and Disinfection of Production" by Gas-Liquid Chromatography GC-MS and LC-MS Methods, described in the Collection of Standard Methods (Lazări et al., 2000).

The obtained results were compared with the maximum residue level (MRL) values according to the Sanitary Regulation on the Maximum Allowable Residue Levels of Phytosanitary Products in or on Food or Feed of Plant and Animal Origin for Animals, approved by the Decision of Government of Republic of Moldova no. 1191 of 23.12.2010, adjusted to EU requirements.

The obtained in experience data were statistically processed using computer software "STATISTICA - 6" and evaluated their certainty, according to variation biometric statistics, by methods of Plohinskiy (1989).

RESULTS AND DISCUSSIONS

Our research has shown that pyrethroid, neonicotinoid and organophosphorus insecticides, triazole fungicides, acaricides and herbicides are the most widespread pesticides in the environment (melliferous plants) where honey bees works. Among these groups and classes of pesticides, we have investigated 17 pesticides in the flowers of forest trees and we have recorded different detectable concentrations of 3 pyrethroid insecticides, 1 neonicotinoid insecticide, 1 organophosphorus insecticide, 4 triazole fungicides, 1 acaricide and 4 herbicides. In the white acacia flowers (*Robinia pseudoacacia*), from the 17 investigated pesticides, residues of 14 pesticides were found: *Cypermethrin, Deltamethrin, Pyrethrin, Clothianidin, Fenamifos, Difenoconazole, Fenhexamid, Mepanipyrim, Cyprodinil, Fipronil, Amidosulfuron, Amitrol, Glyphosate and Sulfosulfurol* (Table 1).

Table 1. The content of pesticide residues in acacia flowers from forest, mg/kg

No.	The name, class and group of pesticide	MRL*	Pesticide content (N=4), M ± m	Difference from MRL (3-4)	
				±d	times
1	2	3	4	6	7
	PYRETHROID INSECTICIDES				
1.	Cypermethrin	0.1	0.005±0.003	-0.095	-20.0
2.	Deltamethrin	0.05	0.008±0.005	-0.042	-6.3
3.	Pyrethrin	0.5	0.075±0.047	-0.425	-6.7
4.	Tau-fluvalinate (*L*)	0.01	n.d.	-	-
	NEONICOTINOID INSECTICIDES				
5.	Imidacloprid	0.05	n.d.	-	-
6.	Clothianidin	0.05	0.008±0.005	-0.042	-6.3
7.	Thiamethoxam	0.1	n.d.	-	-
	ORGANOPHOSPHORUS INSECTICIDES				
8.	Fenamifos	0.05	0.008±0.005	-0.042	-6.3
	TRIAZOLE FUNGICIDES				
9.	Difenoconazole	20.0	8.75±1.65	-11.25	-2.3
10.	Fenhexamid	0.1	0.005±0.003	-0.095	-20.0
11.	Mepanipyrim	0.02	0.003±0.002	-0.017	-6.7
12.	Cyprodinil (*L+R*)	0.05	0.014±0.004	-0.036	-3.6
	ACARICIDES				
13.	Fipronil	0.005	0.003±0.002	-0.002	-1.7
	HERBICIDES				
14.	Amidosulfuron	0.05	0.005±0.002	-0.045	-10.0
15.	Amitrol	0.02	0.005±0.002	-0.015	-4.0
16.	Glyphosate	2.0	0.250±0.240	-1.75	-8.0
17.	Sulfosulfurol (*SS*)	0.05	0.005±0.004	-0.045	-10.0

Notification: * - MRL (maximum residue level), according to the “Sanitary Regulation on the Maximum Allowable Residue Levels of Phytosanitary Products in or on Food or Feed of Plant and Animal Origin for Animals”, approved by the Decision of Government of Republic of Moldova no. 1191 of 23.12.2010, www//lex.justice.md, vizited on 06.12.2017.

From 17 studied pesticides in the acacia flowers, no residues of 3 insecticides (pyrethroid *Tau-fluvalinate* and neonicotinoids *Imidacloprid* and *Thiamethoxam*) were detected.

The lowest concentrations of residues were registrated at acaricide *Fipronil* - on average 0.003±0.002 mg/kg, triazole fungicides *Mepanipyrim* - 0.003±0.002 mg/kg and *Fenhexamid* - 0.005 ± 0.003 mg/kg, as well as the pyrethroid insecticide *Cypermethrin* - 0.005±0,003 mg/kg. The highest residual concentrations of the studied pesticides in acacia flowers were recorded at triazole fungicide *Cyprodinil* - 0.014±0.004 mg/kg, pyrethroid insecticide *Pyrethrin* - 0.075±0.047 mg/kg, herbicide *Glyphosate* - 0.25±0.24 mg/kg and triazole fungicide *Difenoconazole* - 8.75±1.65 mg/kg.

It should be noted that these relatively "high" concentrations of pesticide residues in the acacia flowers from forest, actually, are very low, compared to the maximum admissible limits according to national and EU standards, of 3.6 times for *Cyprodinil*, 6.7 times for *Pyrethrin*, 8.0 times for *Glyphosate* and 2.3 times for *Difenoconazole*.

Thus, very low concentrations of residues of pyrethroid, neonicotinoid and organophosphorus insecticides, as well as triazole fungicides, acaricides and herbicides in acacia flowers from forest, have been detected, values that are well below the maximum admissible level according to national and the EU standards.

In this context, we can conclude that white acacia flowers, from the forest studied by us, are not polluted with pesticides. Therefore, these (flowers) are safety for practicing organic beekeeping and obtaining of bio apiculture products.

Such results were obtained by other authors (Ilavarasan and Vadivelu, 2017). The researchers have shown that *Acacia nilotica* and *Acacia leucophloea* flowers in the Thanjavur region, Tamilnadu, India, do not contain detectable concentrations of organochlorine and organophosphorus pesticide residues, which indicates their absence in the flowers of forest trees.

In the large-leaf linden flowers (*Tilia platyphillos*), out of the 17 investigated pesticides, the residues of 13 pesticides were registered, including 3 pyrethroid insecticides, 1 neonicotinoid in secticide, 1 organophosphorus insecticide, 3 triazole fungicides, 1 acaricide and 4 herbicides. The lowest residue concentration was registered at the acaricide *Fipronil* - 0.001 mg/kg and at the pyrethroid insecticide *Cypermethrin* - 0.003 mg/kg (Table 2).

Table 2. The content of pesticide residues in large-leaf linden flowers from the forest, mg/kg

Nr.	The name, class and group of pesticide	MRL*	Pesticide content, (N=4), M ± m	Difference from MRL (3-4)	
				±d	times
1	2	3	5	6	7
	PYRETHROIDE INSECTICIDES				
1.	Cypermethrin	0.1	0.003±0.002	-0.097	-33.3
2.	Deltametrine	0.05	0.020±0.004	-0.030	-2.5
3.	Pyrethrine	0.5	0.100±0.041	-0.400	-5.0
4.	Tau-fluvalinate (*L*)	0.01	n.d.	-	-
	NEONICOTINOID INSECTICIDES				
5.	Imidacloprid	0.05	n.d.	-	-
6.	Clothianidin	0.05	0.008±0.005	-0.042	-6.2
7.	Thiamethoxam	0.1	n.d.	-	-
	ORGANOPHOSPHORUS INSECTICIDES				
8.	Fenamifos	0.05	0.008±0.005	-0.042	-6.2
	TRIAZOLE FUNGICIDES				
9.	Difenoconazole	20.0	16.00±1.22	-4.00	-1.3 ori
10.	Fenhexamid	0.1	0.008±0.005	-0.092	-12.5 ori
11.	Mepanipyrim	0.02	n.d.	-	-
12.	Cyprodinil (*L+R*)	0.05	0.019±0.006	-0.031	-2.6
	ACARICIDES				
13.	Fipronil	0.005	0.001±0.0007	-0.004	-5.0
	HERBICIDES				
14.	Amidosulfuron	0.05	0.012±0.005	-0.038	-4.2
15.	Amitrol	0.02	0.009±0.003	-0.011	-2.2
16.	Glyphosate	2.0	1.250±0.478	-0.75	-1.6
17.	Sulfosulfurol (*SS*)	0.05	0.013±0.006	-0.037	-3.8

Notification: * - MRL (maximum residue level), according to the “Sanitary Regulation on the Maximum Allowable Residue Levels of Phytosanitary Products in or on Food or Feed of Plant and Animal Origin for Animals”, approved by the Decision of Government of Republic of Moldova no. 1191 of 23.12.2010, www//lex.justice.md, vizited on 06.12.2017.

Some of the investigated insecticides in large-leaf linden flowers, in general, have not been detected. Thus, no detectable concentrations of residues of pyrethroid insecticide *Tau-fluvalinate*, neonicotinoid insecticides (*Imidacloprid, Thiamethoxam*) and triazole fungicide *Mepanipyrim* were registered.

The highest concentrations of residues in the linden flowers had been registered at triazole fungicide *Difenoconazole* - 16.00±1.22 mg/kg and controversial herbicide *Glyphosate* 1.250±0.478 mg/kg. It was observed that the linden flowers have a slight tendency to accumulate higher concentrations, compared with acacia flowers, of residues of some pyrethroid insecticides (*Cyprodinil, Deltamethrin, Pyrethrine*), triazole fungicides (*Difenoconazole, Fenhexamid*) and herbicides (*Amidosulfuron, Amitrol, Glyphosate, Sulfosulfurol*). At the same time, the detected concentrations of pesticide residues in forestry linden flowers are well below the maximum admissible level, according to the current standards, for the triazolic fugicide *Difenoconazole* on 1.3 times, herbicide *Glyphosate* on 1.6 times, up to 12.5 times for fungicide *Fenhexamid* and 33.3 times for pyrethroide insecticide *Cypermethrin*.

Also such results have been obtained in the researches of other authors (Łozowicka et al., 2014). In the North-East of Poland, the presence of 163 pesticides residues were evaluated (6 acaricides, 62 fungicides, 18 herbicides and 77 insecticides) in various herbs, including 3 samples of linden flowers. As a result pp′-DDD was found in the linden sample 0.02 mg/kg below MRL (MRL = 0.05 mg/kg). It is necessary to point out that pp′-DDD was found in one sample, and that this pesticide belongs to the chlorinated pesticide group and is a product of the breakdown of DDT, a pesticide banned for agricultural use worldwide. These results are important because lime flowers are part of medicinal plants collected from the forest. Good quality control and determination of the presence of toxic pesticides in herbs is essential to avoid their overconsumption and cumulative toxicities in long-term use.

If the presence of insecticide and fungicide residues in the flowers forest trees can be explained by their use in spray treatments against harmful insects and fungi, then, it is curious to note the fact of herbicides presence in the forest trees flowers. Where did these come from? We suppose that these pesticides could reach the flower samples by spraying them through aerosols in the nearby agricultural fields, through the vapors from the rainwater, from the groundwater and surface waters through infiltration or other less known by us pathways.

Our hypotheses find their confirmation in communications of researchers from Romania (Mihail et al., 2010). Studying the content of organochlorine insecticide residues in the forest soil (272 samples), they had found an average concentration of HCH equal to 0.03 mg/kg, ranging from 0.001 to 0.434 mg/kg, and an average of 0.032 mg/kg DDT, ranging from 0.001 to 0.264 mg/kg, compared to 0.01 mg/kg CMA according to EU standards. Thus, average concentrations of some pesticides in the soil, detected by these researchers have exceeded about 3.0 times CMA, according to EU standards.

In our research, if we compare indirectly the concentrations of pesticide residues researched in the flowers of the forest trees (acacia and linden) with the maximum residue levels (MRL), we find that these (concentrations) do not exceed the established national and EU standards.

Therefore, the recorded pesticide residues in the flowers of forestry trees, are not harmful for the health of bees and fauna of studied ecosystems.

In conclusion we can say that the flora of studied by us forest sector, are not polluted with pesticides, so organic beekeeping can be practiced here, with the production of organic apiculture products.

CONCLUSIONS

In the flowers forest trees, such as acacia white (*Robinia pseudoacacia*) and large-leaf linden (*Tilia platyphillos*) low concentrations of pesticide residues were detected, as follows: pyrethroid insecticides (*Cypermethrin, Deltamethrin, Pyrethrine*) from 0.003 to 0.100 mg/kg, neonicotinoid insecticide (*Clothianidin*) from 0.400 to 0.425 mg/kg, organophosphorus insecticide (*Fenamifos*) in the amount of 0.042 mg/kg, triazole fungicides (*Fenhexamid, Mepanipyrim, Cyprodinil*) from 0.017 to 0.092 mg/kg, triazole fungicide *Difenoconazole* from

8.75 to 16.0 mg/kg, Fipronil acaricide from 0.001 to 0.003 mg/kg, herbicides (*Amidosulfuron, Amitrol, Sulfosulfurol*) from 0.005 to 0.013 mg/kg and herbicide *Glyphosate* from 0.25 to 1.25 mg/kg. Pesticide residues concentrations in the above-mentioned forest trees flowers (acacia and linden) are 1.3 to 33.3 times lower than the maximum admissible levels under national and EU standards.

Therefore, the recorded pesticide residues in the flowers of forestry trees, are not harmful for the health of bees and fauna of studied ecosystems. In conclusion we can say that the flora of studied by us forest sector, are not polluted with pesticides, so organic beekeeping can be practiced here, with the production of organic apiculture products.

AKNOWLEDGEMENT

Scientific researches have been carried out within the fundamental institutional project 15.817.02.12F "Diversity, structure and functioning of complex natural and anthropogenic fauna in the context of strengthening of the national security strategy of the Republic of Moldova" from the state budget.

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