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CONFORMITY OF RAPI, PEAS AND MAIZE FLOWERS, CONCERNING PESTICIDE RESIDUES FOR ORGANIC BEEKEEPING

Valentina CEBOTARI, Ion BUZU

Institute of Zoology of Academy of Sciences of Moldova (ASM),
1 Academiei Street, MD 2028, Chisinau, Republic of Moldova

Corresponding author email: valentinaceb@yahoo.com

Abstract

The purpose of the present paper was to investigate the degree of conformity of the flowers of some honey crops (rape, peas, maize) regarding pesticide residues, for the practice of organic beekeeping. Scientific research was carried out on the content of pesticide residues in flowers taken from industrial lands, suspected of contamination with pesticides. The results of the research demonstrate that, out of the 62 pesticides tested in the rape flower samples, detectable concentrations of residues were recorded in only 5 pesticides, which constitutes 8.1%. It has been found that rapeseed flowers are heavily polluted with the residues of the Glyphosate pesticide, in a concentration of 0.1772 mg/kg, which exceeds the maximum admissible limits, according to EU and MD standards, by 77.2%. In peas flowers, there were detectable concentrations of residues in only 7 pesticides, which constitutes 11.3%. The peas flowers in the researched industrial land were slightly polluted with the residues of the Glyphosate pesticide, in a concentration of 0.1088 mg/kg, which exceeds the maximum admissible limits, according to EU and MD norms, by 8.8%. In the maize flower samples, of the 62 pesticides tested, residues were detected in concentrations detectable only at 9 pesticides, which constitutes 14.5%. It was found that the maize flowers in the industrial land were heavily polluted with the residues of the neonicotinoid insecticide Thiametoxam at a concentration of 0.0178 mg/kg, which exceeds the maximum admissible limits, according to EU and MD standards, by 78.0%. Glyphosate residues in maize flowers were detected at hazardous (at the limit) concentrations of 0.0962 mg/kg, compared to the maximum admissible limits of 0.1 mg/kg. Therefore, the sites researched with rapeseed, peas and maize plantations are not conformity for organic beekeeping, because the flowers of these crops are polluted with residues of dangerous pesticides, banned by the EU, which can affect the health of bees and inoffensiveness bee products.

Key words: *bees, honey flowers, organic beekeeping, pesticides, residues.*

INTRODUCTION

Beekeeping, in the Republic of Moldova, has a branch of agriculture of particular importance for the national economy, due to the value and quality of the products offered by it, the creation of jobs among the vulnerable sections of the population in the rural areas, as well as for maintaining through pollination the homeostasis and biodiversity of nature ecosystems.

Totally in the country there are approximately 180 thousand families of bees, from which about 4.5-5.0 thousand tons of honey are obtained annually, of which about 4000 tons are exported to different countries, including the European Union.

Other bee products are obtained from bees, which are quite important, such as: wax, pollen, propolis, royal jelly, venom, which are used in various fields of the national economy (food, medicine, pharmaceutical, cosmetics, plastic arts).

One of the most important benefits brought to humans by bees is the additional product obtained from increasing the productivity of the entomophilous plants from the cultivated and spontaneous flora, as a result of their pollination, thus ensuring the perpetuation of nature's biodiversity. By the bees are pollinated about 600 thousand ha of land with agricultural crops, from which an additional 20-30% of harvest in annual value of over 3.6-4.0 billion lei are obtained. Moreover, much of the honey production and other bee products obtained in the country is harvested from the flowers of honeycomb agricultural plants, such as sunflower (*Helianthus annuus*), rapeseed (*Brassica napus* ssp. *oleifera* L.), lavender (*Angustifolia lavandula*), salvia (*Salvia officinalis*) and other related crops (peas, maize) that meet near agricultural fields with basic honey crops.

Regrettably, agricultural producers (cultivators of agricultural crops, agricultural specialists) largely ignore the fact that bees are the main pollinator of entomophilic agricultural crops and can serve as a decisive factor in increasing their harvest, producing organic and organic bee products, ensuring efficiency economic and sustainable development of the respective branches in the country.

The analysis of the situation of the last years demonstrate that, the traditional technology for the growth and exploitation of the bee families does not ensure the production of organic and harmless bee products, of competitive quality, which could be marketed at advantageous prices.

According to a report by MIEPO (Moldavian Investment and Export Promotion Organization) - Organization for Investment Attraction and Export Promotion from Moldova, the purchase price of BIO (organic) honey is currently 90% higher than that of ordinary honey (Report MIEPO, 2016). This is why the beekeepers want to practice the BIO beekeeping system massively, but, not knowing the conditions and the legislation of organic farming, they apply the outdated technologies that they know, activating in the traditional system of beekeeping and, thus, the obtained production is not recognized as BIO, and its harvest decreases significantly.

Therefore, the specific technologies of the bio beekeeping system require a deep study in order to be used.

It is necessary to know the anthropic impact caused by the industrialization and intensification of agriculture, the most commonly encountered polluting factors, their spreading areas and the degree of residual pollution (their concentration in the resources of the environment), which can affect both honey bees as the main entomophilic pollinator, as well as the production obtained from them.

In the European Union, the admissible norms of pesticide residues in plants and plant production are regulated by Regulation (EC) no. 396/2005 of the European Parliament and of the Council of 23 February 2005 on the maximum contents applicable to pesticide residues in/or on foodstuffs and food of animal and plant origin for animals (Regulation (EC) No. 396/2005), as amended and subsequent additions (Regulation (EU) 2017/978; Regulation (EU) 2019/552). Based on this Regulation, national programs for monitoring pesticide residues in plant products have been developed and adopted in EU countries (National Plan, Ro, 2019).

In the Republic of Moldova, also, was elaborated the National Program for the monitoring of pesticide residues and nitrate content in food of plant origin for the years 2015-2020 (National Program, 2015-2020), approved by Government Decision no. 567 of 16.07.2014. In accordance with this Program, the National Agency for Food Safety monitors the situation regarding pesticide residues in plant and animal production in the country. According to a report of this authority, regarding the implementation of the National Program for the monitoring of pesticide residues in foodstuffs of plant origin for 2016, out of the 266 samples taken from 22 products of plant origin, 5 samples were detected (1.9%) in which the pesticide residue content exceeded the LMA, being declared non-compliant with this criterion. Non-compliant samples were contaminated with pesticide residues: Propiconazole, Thiamethoxam, Cipermetrin and others (ANSA Report, 2016).

In Romania, according to a similar report of the Laboratory for the Control of Pesticide Residues in Plants and Vegetable Products in Bucharest (2018), out of the 794 vegetable samples investigated, 7 samples (0.9%) were found to be non-compliant according to the residue content of pesticides: Chlorpyrifos, Chlorothalonil, Dimetoat and Tiametoxam (Report, madr.ro., 2018).

Although in both countries, the percentage of non-compliant samples according to the pesticide residue content in plant products is small, it indirectly explains why, in the apiculture production exported from the Republic of Moldova, they are sometimes detected, by the EU certification and control bodies (Commission Regulation 834/2009; Commission Regulation 271/2010), harmful substances and polluting residues, which leads to the compromise of the competitiveness of the branch and national image internationally (Antonescu et al, 2001; Bogdanov et al, 1999; Buruian, 2011).

Thus, to date, both agricultural producers and beekeepers have not been aware that the uncontrolled use of pesticides in the treatment of agricultural crops and honey bees has an imbalanced impact on the homeostasis of natural ecosystems, with final consequences of risk of human health security.
Therefore, researching the conformity of the honeycomb flora for the practice of organic beekeeping under the conditions of different native anthropogenic ecosystems, revealing the most widespread pesticides that produce residues in the honeycomb flora and bee products, highlighting the polluting sites and the reactive areas clean of residues of the pesticides that can ensure the organic beekeeping, they represent current problems, the solution of which would allow the elaboration of measures of adjustment of the conventional beekeeping to the organic beekeeping in the Republic of Moldova, according to the norms of the EU.
In this context, the purpose of the present paper was to investigate the degree of conformity of the flowers of some honey crops (rape, peas, maize) regarding pesticide residues, for the practice of organic beekeeping.

MATERIALS AND METHODS

Scientific research has been carried out on the content of pesticide residues in the flowers of honey crops in some sites, which have been suspected of contamination with pesticides. Among these crops were investigated: rapeseed (*Brassica napus* ssp. *oleifera* L.), peas (*Pisum sativum*) and maize (*Zea mays*) from industrial land.
The flower samples of the rapeseed crop were taken from the industrial land of an agricultural household in Stefan Voda, which wanted to remain confidential.
Samples of maize flowers (male and female), as well as peas were taken from the industrial land of an agricultural household in Ciadîr-Lunga, which also wanted to remain confidential.
From each site (planting land) mentioned above were taken 5 flower samples of the respective plants. For the sampling of flowers, each agricultural land was virtually divided into 5 equal parts, from each one being taken one sample. In total, 15 samples of biological material of the respective flowers were taken. Each sample of biological material collected weighed from 100 to 150 g. The samples were packaged in plastic bags and transported the same day to the accredited laboratory of the S.C. "Center of Applied Metrology and Certification", in accordance with the sanitary-veterinary norms regarding the methodology of sampling, processing, packing and transport of the samples for laboratory examinations (Sanitary-veterinary norm, 2010).
Each sample of biological material taken was tested for the most dangerous pesticide residues (62 names) most commonly encountered in our country (Table 1).

Table 1. Names of pesticides tested for contents of residues in the samples of flowers taken

No or	Name of the pesticide	No or	Name of the pesticide	No or	Name of the pesticide
1	Acetamipirid	22	Endosulfan	43	Pendimetalin
2	Azoxistribin	23	Epoxiconazol	44	Permetrin
3	Bifentrin	24	Ethion	45	Picoxistrobin
4	Bitertanol	25	Fenvalerat	46	Pirimetanil
5	Boscalid	26	Fenitrotion	47	Pirinicarb
6	Bromuco-nazol	27	Fenixicarb	48	Piridaben
7	Captan	28	Fipronil	49	Pirimitos-metil
8	Chlothianidin	29	Flutriafol	50	Procimid
9	Ciflutrin	30	Folpet	51	Protenotos
10	Cipermetrin	31	Fosalon	52	Prometrin
11	Ciproconazol	32	Haloxifop	53	Propargit
12	Ciprodinil	33	Glifosat	54	Propiconazol
13	Clorotalonil	34	Benzoton	55	Spiroxamină
14	Clorpirifos	35	Imidacloprid	56	Tau-fluvalinat
15	Deltametrin	36	Indoxacarb	57	Tebuconazol
16	Diazinon	37	Iprodion	58	Thiacloprid
17	Diclorvos	38	Kresoxim-metil	59	Tiametoxam
18	Difenoconazol	39	Lambda-Ghalotrin	60	Trifluralin
19	Dimetoat	40	Lufenuron	61	Tiadimeton
20	Dimetomorf	41	Malation	62	Vindozolin
21	Diniconazol	42	Penconazol		

The pesticide residue content was determined in the Laboratory accredited above by gas-chromatography - mass spectrometric (GC-MS) and liquid-chromatographic - mass spectrometric (LC-MS) methods, described by Lazarii I. in Collections of standard methods MS (Lazari, 2000).
The data obtained, regarding the pesticide residue content in the investigated samples, were

compared with the maximum admissible limits (MAL) norms, according to the Sanitary Regulation regarding the maximum permitted residue limits of the plant protection products from/or from food and food of plant origin and animal for animals, approved by the Government Decision of the Republic of Moldova no. 1191 of 23.12.2010 (Sanitary Regulation, 2010), adjusted to EU norms.
As a result of the comparison, conclusions regarding the conformation of the honeycomb flora of the respective sites for the practice of organic beekeeping were made.
The data obtained as a result of the researches were systematized and processed with the help of computerized software "STATISTICA - 12" and appreciated their certainty, according to the variational biometric statistics, according to the methods of Плохинский Н.А. (1989).

RESULTS AND DISCUSSIONS

According to us, honey flowers are the main link in the spread of pesticides in nature in the food chain of honey bees. Without diminishing the importance of air, water and soil, honey flowers occupy the dominant segment in the ecology of bee products, because of these nectar and pollen are collected, as the main predecessors of bee products.
In our previous research (Cebotari et al, 2018; 2019) we studied the content of pesticide residues in forest tree flowers (acacia, linden) and important agricultural crops from different sites with different anthropogenic impact (sunflower flowers from the industrial land and the domestic garden from the village, apple flowers from the industrial orchard and from the domestic garden of the villagers).
At the same time, the situation regarding pesticide residues in the flowers of several entomophilic agricultural crops remains, until now, unknown. Therefore, in this paper we undertook research to identify the degree of conformity of the honeycomb flora from some agricultural sites for organic beekeeping.

Pesticide content in rapeseed flowers (*Brassica napus* ssp. *oleifera* L.). The results of the research showed that out of the 62 pesticides tested, detectable concentrations of residues were recorded in only 5 pesticides in the samples of rapeseed flowers from the industrial land of an agricultural household in Stefan Voda, which constitutes 8.1% (Table 2).
These pesticides include 1 pyrethroid insecticide (Cipermetrin), 3 triazole fungicides (Difenoconazole, Flutriafol, Benzoton) and 1 herbicide (Glyphosate).

Table 2. Pesticide residue content in flowers of rapeseed (N = 5), mg/kg

Name of the pesticide	MAL	M ± m	d (M-MAL)	d, % to MAL
Pyrethroid insecticide				
Cipermetrin	0.2	0.0929±0.0405	-0.1071	-53.6
Triazole fungicides				
Difenoconazol	0.05	0.0398±0.0148	-0.0102	-20.4
Flutriafol	0.2	0.1004±0.0569	-0.0996	-49.8
Benzoton	0.02	0.0190±0.0076	-0.001	-5.0
Herbicides				
Glyphosate	0.1	0.1772±0.0838	+0.0772	+77.2

It was found that the residue concentrations of pyrethroid insecticide and triazole fungicides are insignificant and range from 0.0190 ± 0.0076 mg/kg in Benzoton to 0.1004 ± 0.0569 mg/kg in Flutriafol, those with 5.0-53.6% below the maximum admissible limits (MAL), according to national and European norms.
Quite a different situation (worrying) is the concentration in samples of rapeseed flowers Glyphosate herbicide residues.
Laboratory cross-sectional data show that the concentrations of this hazardous (carcinogenic) herbicide in rapeseed flower samples are quite polluting, accounting for 0.1772 ± 0.0838 mg/kg, which exceeds the maximum admissible limit by 77.2%. This demonstrate that cultivators use this herbicide, prohibited in the EU, in large quantities, uncontrolled by the state authorities empowered to control rape in the respective field.
From the data presented above, we can conclude that rapeseed growers treat plantations with fungicides triazole, insecticide and herbicide more frequently, which can in some cases damage the conformity of the honeycomb flora for its use in organic beekeeping. In the case of

the examined site, the rapeseed is heavily polluted with the Glyphosate pesticide and cannot be used for harvesting for organic beekeeping.

Pesticide content in peas flowers (*Pisum sativum*). The results of the research showed that, out of the 62 pesticides tested, in the samples of peas flowers from the industrial land of an agricultural household in Ceadîr-Lunga (which wanted to remain confidential) were detected detectable concentrations of residues at only 7 pesticides, which constitutes 11.3% (Table 3).

Table 3. Pesticide residue content in peas flowers (N = 5), mg/kg

Name of the pesticide	MAL	M ± m	d (M-MAL)	d, % to MAL
Pyrethroid insecticide				
Cipermetrin	0.2	0.1201±0.0474	-0.0799	-39.9
Dimetilciclo-propan	0.05	0.0417±0.0122	-0.0083	-16.6
Fenoxibenzil	0.05	0.0251±0.0109	-0.0249	-49.8
Triazole fungicides				
Benzanton	0.1	0.0526±0.0312	-0.0474	-47.4
Difenoconazol	0.05	0.0494±0.0183	-0.0006	-1.2
Herbicides				
Glyphosate	0.1	0.1088±0.0435	+0.0088	+8.8
Imazamax	0.02	0.0113±0.0046	-0.0087	-43.5

Among the pesticides detected were 3 pyrethroid insecticides (Cipermetrin, Dimethylcyclopropane, Phenoxybenzyl), 2 triazole fungicides (Benzanton, Difenoconazole) and 2 herbicides (Glyphosate, Imazamax). It should be noted that the concentrations of pyrethroid insecticide residues in peas flowers are quite small, falling in the range from 0.0251 ± 0.0109 mg/kg - to Phenoxybenzyl, up to 0.1201 ± 0.0474 mg/kg - Cypermethrin.

These concentrations are well below the maximum admissible limits - with 16.6-49.8% and do not present any danger for both honey bees, bee products and human health.

The residual amounts of triazole fungicides are also insignificant, falling within the average values of 0.0494 ± 0.0183 mg/kg - for Difenoconazole and 0.0526 ± 0.0312 mg/kg - for Benzanton, being below the level the maximum admissible limits, according to EU norms with 1.2-47.4%. In fact, the residue content of the fungicide Difenoconazole in peas flower samples is at MAL risk level.

Of the residues of the detected herbicides, the concentration of the Imazamax herbicide is quite insignificant, with the value of 0.0113 ± 0.0046 mg/kg being below the maximum admissible limits with 43.5%.

At the same time, the same concern, as in rapeseed flowers, is the residue of the herbicide Glyphosate, which is in the samples of peas flowers 0.1088 ± 0.0435 mg/kg, which exceeds level the maximum admissible limits by 8.8%.

This level of Glyphosate residue concentration shows a slight pollution of peas flowers, according to the classification of pollution levels (Cebotari V. et al, 2016). Therefore, this site, which is part of the flowering peas land, is not conformity for practicing organic beekeeping.

Pesticide content in maize flowers (*Zea mays*). The results of the laboratory analyzes of maize flower samples showed that, out of the 62 pesticides tested, residues in detectable concentrations were recorded in only 9 pesticides, which constitutes 14.5%. Of these pesticides, 2 are pyrethroid insecticides (Cipermetrin, Dimethylcyclopropane), 3 neonicotinoid insecticides (Clothianidin, Imidacloprid, Tiametoxam), 2 triazole fungicides (Benzanton, Difenoconazole) and 2 are herbicides (Glyphosate, Imazamax) (Table 4).

We would like to mention that, in the overwhelming majority of pesticides, the detectable concentrations were quite low and did not exceed the maximum admissible limits, according to EU norms.

Thus, the concentrations of the pyrethroid insecticide residues were 0.1020 ± 0.0437 mg/kg - on Cipermetrin and 0.0446 ± 0.0109 mg/kg - on Dimethylcyclopropane, being below the maximum admissible limits with 49.0 and 10.8% respectively.

Similarly, the residue concentrations of the two neonicotinoid insecticides, from the three investigated, had non-polluting values, respectively 0.0080 ± 0.0025 mg/kg on Clothianidin and 0.0076 ± 0.0023 mg/kg on Imidacloprid, being below the maximum admissible limits with 20.0 and 24.0%, respectively.

Table 4. Pesticide residue content
in maize flowers (N = 5), mg/kg

Name of the pesticide	MAL	M ± m	d (M-MAL)	d,% to MAL
Pyrethroid insecticide				
Cipermetrin	0.2	0.1020±0.0437	-0.098	-49.0
Dimetilciclo-propan	0.05	0.0446±0.0109	-0.0054	-10.8
Neonicotinoid insecticides				
Clothianidin	0.01	0.0080±0.0025	-0.002	-20.0
Imidacloprid	0.01	0.0076±0.0023	-0.0024	-24.0
Tiametoxam	0.01	0.0178±0.0088	+0.0078	+78.0
Triazole fungicides				
Benzanton	0.1	0.0492±0.0264	-0.0508	-50.8
Difenoconazol	0.05	0.0368±0.0101	-0.0132	-26.4
Herbicides				
Glyphosate	0.1	0.0962±0.0415	-0.0038	-3.8
Imazamax	0.02	0.0148±0.0054	-0.0052	-26.0

At the same time, one of the three dangerous neonicotinoid insecticides, namely Tiametoxam, left pollutant residues in maize flowers. Thus, the concentration of Tiametoxam residues in the flowers of this plant constituted 0.0178 ± 0.0088 mg/kg, being 78.0% higher compared to national and EU norms. Therefore, the site where the corn plantation is located is heavily polluted with residues of this dangerous pesticide.

Recall that the insecticide Tiametoxam is part of the group of neonicotinoid insecticides, which are applied in maize seed treatment plants before sowing. Being a systemic pesticide, it penetrates from the seed through the strain of the grown plant, reaching up to the leaves, flowers and newly grown corn kernels, presenting a danger for both bees that collect nectar and pollen from flowers, as well as for the health of animals and humans, who consumes corn kernels, honey and pollen from corn harvesting.

The triazole fungicide residues detected in maize flower samples were recorded at insignificant concentrations of 0.0492 ± 0.0264 mg/kg - in Benzanton and 0.0368 ± 0.0101 mg/kg - in Difenoconazole, being much higher small, compared to the maximum admissible limits of the Republic of Moldova and the EU - by 50.8 and 26.4%, respectively.

Regarding herbicide residues, the situation is similar to that recorded in peas flowers, with the exception that the Glyphosate residues were detected at risky (at the limit) concentrations of 0.0962 mg/kg, compared to the maximum admissible limit of 0.1 mg/kg, according to national and EU norms. Imazamax herbicide residues, as well as peas flowers, were recorded in concentrations of 0.0148 ± 0.0054 mg/kg, being below the maximum admissible limits with 26.0%.

CONCLUSIONS

Rapeseed flowers (*Brassica napus* ssp. *oleifera* L.) from the researched industrial land are heavily polluted with the residues of the *Glyphosate* pesticide, in a concentration of 0.1772 ± 0.0838 mg/kg, which exceeds the maximum admissible limits, according to EU and MD standards, with 77.2%.

The peas flowers (*Pisum sativum*) from the researched industrial land are slightly polluted with the residues of the Glyphosate pesticide, in a concentration of 0.1088 ± 0.0435mg/kg, and exceed the maximum admissible limits, according to EU and MD norms, by 8.8%.

The maize flowers (*Zea mays*) in the researched industrial land are heavily polluted with the residues of the neonicotinoid insecticide Tiametoxam in a concentration of 0.0178 ± 0.0088 mg/kg, which exceeds the maximum admissible limits, according to EU and MD standards, by 78.0%.

Glyphosate residues in maize flowers were detected at hazardous (at the limit) concentrations of 0.0962 mg/kg, compared to the maximum admissible limit of 0.1 mg/kg.

The sites surveyed with rapeseed, peas and maize plantations are not conformity for organic beekeeping, because the flowers of these crops are polluted with residues of dangerous pesticides, banned by the EU, which can affect the health of bees and inoffensiveness of the bee products.

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