https://doi.org/10.31925/farmacia.2020.4.16 **ORIGINAL ARTICLE**

INVESTIGATION OF PESTICIDE RESIDUES AND HEAVY METALS CONTENT IN VARIOUS PEPPERMINT SAMPLES

MIHAI-ALEXANDRU FLOREA [1,2]*, VERONICA DRUMEA [1], ANDREEA-ROXANA NIȚĂ [1], LAURA OLARIU [1,3], TEODORA COSTEA [2], CERASELA ELENA GÎRD [2]

[1]*Biotehnos S.A., Ilfov, Otopeni, Romania*
[2]*"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[3]*The Academy of Romanian Scientists, Bucharest, Romania*

**corresponding author: alexandru.florea@drd.umfcd.ro*

Manuscript received: December 2019

Abstract

Taking into consideration, that peppermint tea is frequently used in phytotherapy due to its wide range of therapeutic effects, ten samples of peppermint leaves were analysed in order to assess the level of pesticide residues and heavy metals. Pesticide residues were under the detection limit for six samples. However, in four peppermint samples we have determined λ-cyhalothrin (42 - 45 µg/kg), chlorpyrifos-ethyl (20 - 43 µg/kg), and cyfluthrin (78 ± 21 µg/kg), in concentrations below the maximum residues limits. In order to evaluate the negative impact of pesticides on patients health, a sample was spiked with the most common pesticide residues used in agricultural practice, by accumulation in the herbal product (diphenylamine 195.8 ± 16 µg/kg, chlorpropham 108.6 ± 12.4 µg/kg, hexachlorobenzene 76.7 ± 6 µg/kg, tolclofos-methyl 111.4 ± 39 µg/kg, cyfluthrin 472.7 ± 39.7 µg/kg). Moreover, we have observed that pesticide residues had a high transfer rate in infusion (diphenylamine 71.5%, chlorpropham 158.4%, chlordane-gamma 63%, tolclofos-methyl 61.5%). From the point of view of the heavy metals content, the sample shown concentrations over allowed limits (cadmium 98 µg/kg, copper 7140 µg/kg, lead 1500 µg/kg, mercury 170 µg/kg), some being transferable in infusion (nickel 123.3 %, copper 28%).

Rezumat

În acest studiu au fost analizate zece probe de frunze de mentă în scopul evaluării nivelului de reziduuri de pesticide și metale grele. Reziduurile de pesticide au fost sub limita de detecție pentru șase probe, iar în patru probe de mentă au fost determinate λ-cihalotrin (42 - 45 µg/kg), clorpirifos-etil (20 - 43 µg/kg), și ciflutrin (78 ± 21 µg/kg), în concentrații sub limitele maximale. Pentru a evalua impactul negativ al pesticidelor asupra sănătății pacienților, o probă a fost fortificată cu cele mai frecvent folosite pesticide în practica agricolă, constatându-se o acumulare în produsul vegetal (difenilamină 195,8 ± 16 µg/kg, clorprofam 108,6 ± 12,4 µg/kg, hexaclorbenzen 76,7 ± 6 µg/kg, tolclofos-metil 111,4 ± 39 µg/kg, ciflutrin 472,7 ± 39,7 µg/kg). Am constatat faptul că reziduurile de pesticide prezintă o rată de transfer crescută în infuzie (difenilamină 71,5%, clorprofam 158,4%, clordane-gamma 63%, tolclofos-metil 61,5%). Din punct de vedere al conținutului de metale grele, proba prezintă concentrații peste limitele permise (cadmiu 98 µg/kg, cupru 7140 µg/kg, plumb 1500 µg/kg, mercur 170 µg/kg), unele fiind transferabile în infuzie (nichel 123,3%, cupru 28%).

Keywords: peppermint leaf, pesticide residues, heavy metals

Introduction

According to Romanian national legislation (which includes the official monitoring program), many plants/ vegetables and their derived products are subjected to pesticide and heavy metals analysis, the maximum residues limits (MRL) being regulated by European Regulation (EU) 396/2005, Commission Regulation No 1881/2016, Codex Stan 193 FAO/1995, and GB 2762/2017 [7, 10, 14, 27, 32]. In some cases, from unknown reasons (failure to comply with good agriculture practice or others), the crops might be involuntary contaminated with pesticide residues and heavy metals [13, 16, 17, 20, 34, 36, 44]. Of great importance is that a large amount of plant products (which are part of food supplements) are not checked/ monitored, regarding possible residues and this can lead to serious risks for human health [3, 4, 6, 15, 25, 41, 42]. Allowing that, our research was focused on one of the most used medicinal plant, peppermint (*Mentha x piperita* L.). Peppermint leaves are widely used in a wide range of ailments such as flatulence, menstrual pain, diarrhoea, nausea, depression-related anxiety, muscle and nerve pain, common cold, indigestion and IBS (irritable bowel syndrome) [28, 30, 35].

Materials and Methods

Materials

As material we have used ten samples of peppermint leaves (*Menthae folium)*, one from organic crop (sample M1) and nine purchased from the market as a onecomponent medicinal tea (samples M2, M3, M4, M5, M6, M7, M8, M9, M10).

Reagents and solvents

100 pesticide residues (115 with isomers) belonging to different chemical classes (organochlorines, organophosphorus, pyrethroids, carbamates and others) frequently used in the agricultural practice, and 7 heavy metals (ICP multielement XIII) have been studied, the reference standards being purchased from LGC Standards, Merck and Sigma Aldrich. For extraction we used acetonitrile (purity > 99.95%), ultrapurified water, anhydrous magnesium sulphate (purity > 99.9%), crystalline sodium chloride (purity > 99.9%), purchased from Merck; ceramic homogenizers, 50 mL centrifuge tubes, clean-up kits with primary-secondary amine (PSA), 69% nitric acid, anhydrous magnesium sulphate and active carbon, purchased from Phenomenex.

Equipment

Thermo Scientific TRACE 1310 gas chromatograph coupled with a triple quadrupole mass spectrometer GC-MS/MS (TSQ 8000 EVO), NovAA 400p-AAS-atomic absorption spectrometry, thermostatic sand bath, laboratory mill Cyclotec 1093, angular tip shaker Multi-Bio RS-24, analytical balance Mettler Toledo Excellence XS 205 DU/M, Hettich laboratory centrifuge, water purification system Millipore Integral 3, semi-automatic pipettes Thermo. Acquisition mode: selective reaction monitoring-SRM [21].

Preparation of reference standard solutions for quantitative analysis

The solutions, including the limit of quantification (LOQ) levels, were prepared by mixing exactly measured volumes of individual/mixed stock solutions in volumetric flasks and diluted with acetonitrile for pesticide, and 1% acidified water for heavy metals. In case of pesticide residues, one portion of 1 g of peppermint blank matrix was fortified with 1 mL of standard solution, at LOQ level and processed with the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method (a single-point calibration has been used). The final concentration reported at samples weight (mg/kg) was correlated to maximum residues level from EU No.396/2005. For heavy metals determination, the calibration curve it was made in the extraction solvent (1% acidified water).

Extraction and quantitative assay for multipesticide residues and heavy metals from peppermint leaves and extractive solutions

The method used for pesticide residues analysis from medicinal plants has been validated according to Santé guidelines [39]. 1 g of peppermint leaves was subjected to acetonitrile extraction and clean-up using the QuEChERS method [9]. The resulting supernatant was filtered using 0.2 µm PTFE (polytetrafluoroethylene) filters directly in auto-sampler vials and analysed. For heavy metals analysis, 1 g of peppermint leaves was treated with a solution of 69% nitric acid for 2 hours at room temperature, and then it was subjected to thermal heating (250°C) for one hour on sand bath. The obtained solutions were filtered and brought to the mark with distilled water.

1 g of peppermint (M1 and M10 spiked) was infused in 50 mL of hot water for 15 min (for heavy metals content, 25 mL of infusion was filtered, acidified and analysed). 10 mL of extract was transferred in a 50 mL tube and subjected to extraction and clean-up using a modified QuEChERS method. Using a sterile syringe, the amount of purified extract was quantitatively transferred in laboratory tubes and dried-up by evaporation under a N_2 stream. The dried residue was resuspended in 0.2 mL acetonitrile and analysed by GC-MS/MS (samples were analysed in duplicate). Quantification of sample results, was performed using the same calibration curves as for the leaves.

The solution of pesticides used to fortify the sample M10 (about 100 g of peppermint was subjected to pesticides treatment), was prepared in a volumetric flask by successive dilution of the stock solutions containing diphenylamine, chlorpropham, hexachlorobenzene, tolclofos-methyl, chlorpyrifos-ethyl, chlorthal-dimethyl, chlordane, prothiofos, p, p-DDE (dichlorodiphenyl-dichloroethylene), λ-cyhalothrin, bromopropylate, cyfluthrin (isomers), etofenprox, in concentrations close to MRL.

The recovery level of the method was assessed by comparing areas of pesticide peaks from spiked herb (LOQ level) with the peaks obtained from the extractive solution fortified at the same concentration (results not shown).

Results and Discussion

Pesticide residues analysis

According to our results, samples M1, M6, M7, M8, M9 and M10 did not show quantifiable signals, all being below the detection limit of the method. For M2, M3, M4 and M5 samples, pesticide residues were below MRLs according to EU No. 396/2005 (Table I). The fortified sample (M10') showed increased values of pesticide residues (Table I), which were 2 - 4 times higher compared to the maximum allowed limits (the red colour represents pesticide residues that exceed MRL). By transposing the hypothetical situation into reality, peppermint leaves can accumulate pesticide residues as a consequence of: 1) crop management, with various pesticides (single or mixed), sometimes unauthorized; 2) misapplication of pesticides, without complying to their long-term usage or break time between sprinkling; 3) crop location near farm lands, where pesticide treatments are unavoidable and can affect nearby mint crops by leaching through the soil or air contamination; 4) other causes.

Table I

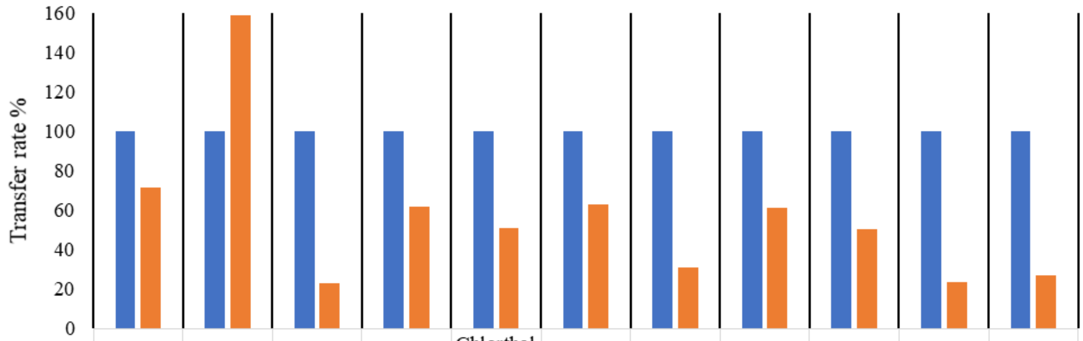
Concentration of pesticide residues in peppermint leaves from different manufacturers

Pesticides	RT (min)	LOQ-plant µg/kg	M1 µg/kg	M2 µg/kg	M3 µg/kg	M4 µg/kg	M5 µg/kg	M10' µg/kg	MRL µg/kg	ADI µg/kg bw
DFA	10.88	20	N.D.	N.D.	N.D.	N.D.	N.D.	195.8 ± 16	50	80
Chlorpropham	11.09	10	N.D.	N.D.	N.D.	N.D.	N.D.	108.6 ± 12.4	50	50
HCB	11.70	10	N.D.	N.D.	N.D.	N.D.	N.D.	76.7 ± 6	20	0.6
Tolclofos-methyl	13.68	10	N.D.	N.D.	N.D.	N.D.	N.D.	111.4 ± 39	50	50
Chlorpyrifos-ethyl	14.69	10	N.D.	43 ± 7.4	N.D.	20 ± 7.4	N.D.	24 ± 7.4	500	10
Chlorthal-dimethyl	14.85	10	N.D.	N.D.	N.D.	N.D.	N.D.	92.5 ± 9	50	10
Chlordane- gamma	16.58	10	N.D.	N.D.	N.D.	N.D.	N.D.	27.8 ± 5.6	20	0.5
Prothiofos	17.36	10	N.D.	N.D.	N.D.	N.D.	N.D.	99.6 ± 11.5	10	0.1
DDE p, p	17.64	10	N.D.	N.D.	N.D.	N.D.	N.D.	24 ± 5	500	10
Bromopropylate	21.85	10	N.D.	N.D.	N.D.	N.D.	N.D.	102.4 ±7.6	50	30
λ-cyhalothrin	23.68	5	N.D.	42 ± 6	45 ± 6	N.D.	N.D.	16.6 ± 5.6	1000	20
Cyfluthrin	24.41	40	N.D.	N.D.	N.D.	N.D.	78 ± 21	472.7 ± 39.7	100	20
Etofenprox	27.38	10	N.D.	N.D.	N.D.	N.D.	N.D.	97.5 ± 6.1	50	30

N.D.= not detected; M10' = fortified sample; DFA = diphenylamine; HCB = hexachlorobenzene; DDE = dichlorodiphenyldichloroethylene; ADI = accepted daily intake body weight

According to our data and the accepted daily intake values (Table I), the risks associated with administration of contaminated peppermint leaves extractive solutions are high (four samples of ten had pesticide residues) [19, 24]. If a patient with a body weight (BW) of 60 kg, consume about 6 g of peppermint leaves which provides, in case of prothiofos (the lowest ADI = 0.1 x 60 BW = 6 µg/60 kg), 0.6 µg of residues, the ADI values will be 10 times smaller. This value is not able to affect the patients' health for short-term. However, over an extended period of time, pesticide residues lead severe health consequences due to their bioaccumulation properties [5, 16].

Regarding the transfer rate of pesticide residues in extractive solutions (Figure 1), according to our results some pesticide residues which have been quantified in M10' sample (chlorpyrifos-ethyl, λ-cyhalothrin and cyfluthrin), were not detected in the extractive solution (infusion IM10'). These results were also confirmed by other authors [23, 31]. A high transfer rate was observed for several compounds - DFA (71.5%), chlorpropham (158.4%), chlordane-gamma (63%), tolclofos-methyl (61.5%), prothiofos (61.2%), which are responsible for a wide range of diseases [18, 26].



	DFA	Chlorpropham	HCB	Tolclofos-methyl	Chlorthal-dimethyl	Chlordane gamma	Chlorfenson	Prothiofos	DDE p, p	Bromopropylate	Etofenprox
M10'	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
IM10'	71.49	158.36	22.82	61.49	50.79	62.95	30.78	61.23	50.06	23.43	27.18

Figure 1.

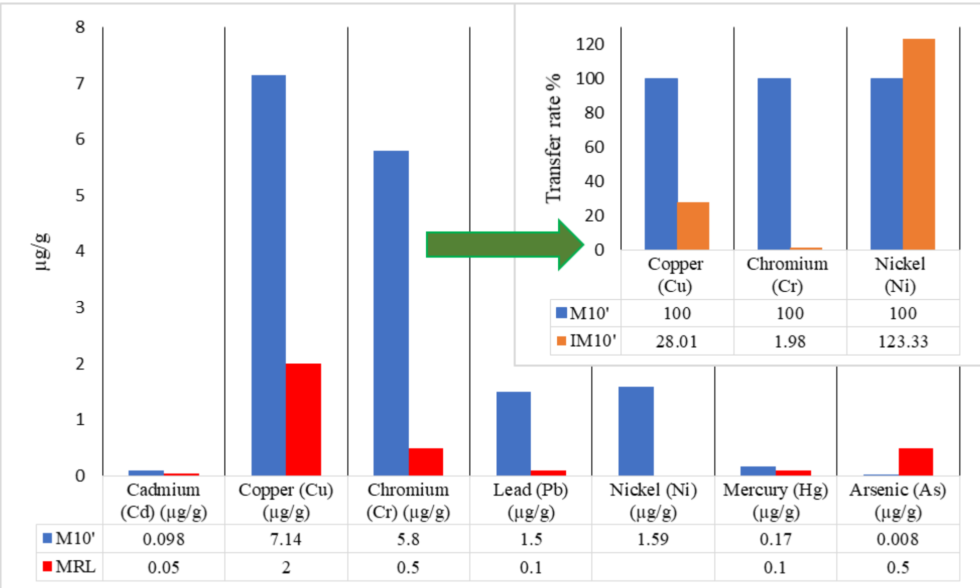
Transfer rate % of pesticide residues from M10' to IM10'

M10'= peppermint leaves (fortified sample); IM10'= infusion obtained from M10' (fortified sample); DFA = diphenylamine; HCB = hexachlorobenzene; DDE = dichlorodiphenyldichloroethylene

The presence of above-mentioned pesticide residues in peppermint sample and their high transfer rate in infusion, is responsible for a series of health issues both in short-term and long-term administration. Long exposure to chlorpyrifos is associated with a high incidence of lung cancer. This pesticide is a very efficient insecticide, (usually used in the agriculture field, against insects by affecting their nervous system), still it can accumulate in medicinal plants and pollute the environment [9, 43]. The main pesticides, which have been identified in our samples belong to the organochlorines class. These pesticide residues are usually banned, due to their high toxicity and bio-accumulation potential (DDT – dichlorodiphenyltri-chloroethane and their metabolites, HCB – hexa-chlorobenzene, chlordane, aldrin, dieldrin) [1]. Compared with other pesticide classes, organo-chlorines are well-known, since they persist in the environment and accumulate in the food chain, being less acutely toxic for humans [40]. Recent studies have shown the long-term exposure risk to some organochlorines (DDE - dichlorodiphenyldichloro-ethylene, HCB - hexachlorobenzene, chlordane, HCH - hexachloro-cyclohexane isomers), due to their storage in humans fat tissues [5]. In most cases, the presence of organo-chlorines in humans is associated with breast cancer [8, 12, 33], endocrine imbalance [41], high risk of type 2 diabetes [4]. Another class of pesticides identified in our sample was that of pyrethroids, the least toxic to humans [26, 37]. These compounds accumulate in live organisms, with negative effects upon animals and human's reproductive health [22, 25]. In the last years, several compounds, which belong to this class (deltamethrin, cyfluthrin, cypermethrin, fenvalerate, bifenthrin, fenpropathrin, permethrin, λ-cyhalothrin) have been widely used like insecticides (in agriculture systems or in different veterinary products, such shampoos and mosquito-repellent perfumes) [38]. In the agriculture field, DFA is used as a fungicide and antiparasitic [2]. Moreover, it is a plant growth regulator and it is used for prevention of post-harvest breakage of apples and pears crops. It is slightly toxic to humans, only an impurity of this compound, diphenylnitrosamine, has been classified as a "probable human carcinogen" [10].

Heavy metals analysis

In the last years, the exposure of both humans and the planet's ecosystems has increased dramatically, as a result of used heavy metals in certain sectors of industry. By feeding plants with mineral substances from soil, air, water, they also accumulate toxic elements, such as heavy metals. For each element analysed it was made a calibration in 4 points, the concentration varying depending on the technique used and the maximum allowed limits (Figure 2).



	Copper (Cu)	Chromium (Cr)	Nickel (Ni)
M10'	100	100	100
IM10'	28.01	1.98	123.33

	Cadmium (Cd) (µg/g)	Copper (Cu) (µg/g)	Chromium (Cr) (µg/g)	Lead (Pb) (µg/g)	Nickel (Ni) (µg/g)	Mercury (Hg) (µg/g)	Arsenic (As) (µg/g)
M10'	0.098	7.14	5.8	1.5	1.59	0.17	0.008
MRL	0.05	2	0.5	0.1		0.1	0.5

Figure 2.

Concentration of some heavy metals in peppermint leaves and transfer rate in infusion IM10'

N.F.= not found; M10' = fortified sample; IM10'= infusion obtained from M10' (fortified sample)

Some of them have an important role in the human body, like Cr, Ni, and Cu being involved in certain metabolism processes, but in large quantities can lead to severe complications. We quantified in our sample, high amount of heavy metals, most of them being over MRL (Cd, Cu, Cr, Pb, Hg). Pb, Hg and Cd are most commonly associated with heavy metals intoxication, therefore the monitoring of them in plant products can reduce the occurrence of future diseases. In infusion it was observed high transfer rate in case of Ni 123.3%, Cu 28% and Cr 2%, the rest of elements could not be detected.

Conclusions

Customers can buy medicinal plants contaminated with pesticide residues and heavy metals. In our study, four of ten peppermint samples showed pesticide residues, still all of them, were below the accepted limits. According to our study, for some pesticide residues and heavy metals we observed a high transfer rate through tea preparation process (infusion of leaves for 15 min with hot water). The percentage of transfer rate depends in most cases on their partition coefficient in the extractive solutions. Our results are quite concerning, and highlight the need for rigorous national control of food supplements and natural products.

Future research will focus on the rate of pesticide residues and heavy metals accumulation in different organs of laboratory animals.

Acknowledgement

Contract doctoral degree nr. 27768/ 30.09.2016, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania. This study was financed by the POS CCE 546/2013 – "Innovative solutions to determine the extent of contamination by toxic residues - heavy metals and pesticides - in products of plant origin".

Conflict of interest

The authors declare no conflict of interest.

References

1. Aktar MW, Sengupta D, Chowdhury A, Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Toxicology*, 2009; 2(1): 1-12.
2. Aoyagi M, Chiba M, Kakimoto Y, Nemoto S, Determination of diphenylamine in agricultural products by HPLC-FL. *J Food Hygienic Soc Japan*, 2016; 57(6): 201-206.
3. Anghel AI, Rădulescu V, Ilieș DC, Dinu M, Istudor V, Nencu I, Ancuceanu RV, Investigation of lipophyilic compounds from native species of *Portulaca* L. (*Portulaceae*) genus. *Farmacia*, 2019; 67(3): 517-521.
4. Azandjeme CS, Bouchard M, Fayomi B, Djrolo F, Houinato D, Delisle H, Growing burden of diabetes in Sub-saharan Africa: contribution of pesticides? *Curr Diabetes.*, 2013; 9(6): 437-449.
5. Bräuner E, Raaschou-Nielsen O, Gaudreau E, Predictors of adipose tissue concentrations of organochlorine pesticides in a general Danish population. *J Expo Sci Environ Epidemiol.*, 2012; 22: 52-59.
6. Chen Z, Lin Z, Tea and human health: biomedical functions of tea active components and current issues. *J Zhejiang Univ Sci B*, 2015; 16(2): 87-102.
7. Codex Stan 193-1995 FAO, Codex General Standard for Contaminants and Toxins in Food and Feed, 1995.
8. Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H, Hypothesis, xenoestrogens as preventable causes of breast cancer. *Environ. Health Perspect.*, 1993; 101: 372-377.
9. Drumea V, Nita AR, Olariu L, Florea MA, The behavior of some pesticide residues in stored medicinal plants samples. *Chem Res J.*, 2018; 3: 33-43.
10. EPA-738-F-97-010. Prevention, Pesticides and toxic substances, 1998; www.3.epa.gov.
11. European Parliament and the Council, Regulation (EC) No. 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. *Official Journal of the European Union*, 2005; L70/1: 55-70, with later amendments.
12. Falck F, Ricci A, Wolff MS, Godbold J, Deckers P, Pesticides and polichlorinated biphenyl residues in human breast lipids and their relation to breast cancer. *Arch Environ Health*, 1992; 47: 143-146.
13. Ferencz L, Balog A, A pesticide survey in soil, water and foodstuffs from central Romania. *Carpathian J Earth Environ Sci.*, 2010; 5(1): 111-118.
14. GB 2762-2017, National Food Safety Standard of Maximum Levels of Contaminants in Food, 2017.
15. National research and development Institute for Alimentary Bioresources, Bucharest, Guide to dietary supplements based on aromatic herbs hive, https://bioresurse.ro (available in Romanian).
16. Grewal AS, Singla A, Kamboj P, Dua JS, Pesticide residues in food grains, vegetables and fruits: A hazard to human health. *J Med Chem Toxicol.*, 2017; 2(1): 1-7.
17. Grover A, Singh RB, Urban industrial development, environmental pollution, and human Health: A case study of east Delhi. Climate change and Human Health Scenario in South and Southeast Asia, 2016; Springer International Publishing.
18. Grunfeld HT, Bonefeld-Jorgensen EC, Effect of *in vitro* estrogenic pesticides on human estrogen receptor alpha and beta mRNA levels. *Toxicol Lett.*, 2004; 151: 467-480.
19. Guidelines for predicting dietary intake of pesticide residues – "Programme of Food Safety and Food Aid, World Health Organization". 1997.
20. Hashem T, Abbas H, Farid IM, El-Husseiny O, Abbas MHH, Accumulation of some heavy metals in plants and soils adjacent to Cairo - Alexandria agricultural highway. *Egypt J Soil Sci.*, 2017; 2: 215-232.
21. Igor F, Ciceri E, Huebschamnn HJ, Aniello C, Fast GC-Ms/MS for High Throughput pesticides analysis. *Thermo Fisher Application 52027*, 2010, www.analitica-web.com.br.
22. Jaensson A, Scott AP, Moore A, Kylin H, Håkan Olsén K, Effects of a pyrethroid pesticide on endocrine responses to female odours and reproductive behaviour in male parr of brown trout (*Salmo trutta* L.). *Aquat Toxicol*, 2007; 1: 9.
23. Jaggi S, Sood C, Kumar V, Ravindranath D, Shanker A, Leaching of pesticides in tea brew. *J Agricult Food Chem.*, 2001; 49(11): 5479-5483.
24. Joint FAO/WHO Meeting on Pesticide Residues (JMPR), 2012, https://apps.who.int
25. Jurewicz J, Radwan M, Wielgomas B, Sobala W, Piskunowicz M, Radwan P, The effect of environmental exposure to pyrethroids and DNA damage in human sperm. *Syst Biol Reprod Med.*, 2015; 61: 37-43.
26. Kolaczinski JH, Curtis CF, Chronic illness as a result of low-level exposure to synthetic pyrethroid insecticides: a review of the debate. *Food Chem Toxicol.*, 2004; 42: 697-706.
27. Manabe M, Kanda S, Fukunaga K, Tsubura A, Nishiyama T, Evaluation of the estrogenic activities of some pesticides and their combinations using MtT/Se cell proliferation assay. *Int J Hyg Environ Health*, 2006; 209: 413-421.
28. McKay DL, Blumberg JB, A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita L.*). *Phytother Res.*, 2006; 20(8): 619-633.
29. Ministry of Agriculture and Rural development, www.madr.ro, (available in Romanian).
30. Mnif W, Hassine AIH, Bouaziz A, Bartegi A, Thomas O, Roig B, Effect of endocrine disruptor pesticides:

A review. *Int J Environ Res Public Health*, 2011; 8: 2265-2303.
31. Nagayama T, Behavior of residual organophosphorus pesticides in foodstuffs during leaching and cooking. *J Agricult Food Chem.*, 1996; 44: 2388-2393.
32. Official Journal of the European Union, Maximum levels for certain contaminants in foodstuffs. *Commission Regulation (EC) No 1881/2006*, 2006, https://eur-lex.europa.eu.
33. Parron T, Alarcon R, Requena MDM, Hernandez A, Increased breast cancer risk in women with environmental exposure to pesticides. *Toxicol Lett.*, 2010; 196S: P203-024.
34. Petrescu-Mag RM, Banatean-Dunea I, Vesa SC, Copacinschi S, Petrescu DC, What do Romanian farmers think about the effects of pesticides? Perceptions and willingness to pay for bio-pesticides. *Sustainability*, 2019; 11: 1-16.
35. Peyman M, Sina M, Milad M, Shahin A, Pharmacological and therapeutic effects of *Mentha Longifolia* L. and its main constituent, menthol. *Anc Sci Life*, 2013; 33(2): 131-138.
36. Popp J, Peto K, Nagy J, Pesticide productivity and food security. A review. *Agronomy for Sustainable Development*, 2013; 33: 243-255.
37. Ray DE, Fry JR, A reassessment of the neurotoxicity of pyrethroid insecticides. *Pharmacol Ther.,* 2006; 111: 174-193.
38. Riviere JE, Papich MG. *Veterinary Pharmacology and Therapeutics*, Wiley-Blackwell Edition. 2009.
39. Sante/11813/2017, Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. *European Commission*, 2018; www.accredia.it.
40. Singh Z, Kaur J, Kaur R, Hundal SS, Toxic effects of organochlorine pesticides: A review. *American J BioSci.*, 2016; 4(3-1): 11-18.
41. Stancu E, Tăerel AE, Soroceanu V, Rais C, Ghica M, Ethical aspects of food supplements in EU and Romania. *Farmacia*, 2019; 67(4): 736-742.
42. Vranceanu N, Motelica D, Dumitru M, Gament E, Accumulation of heavy metals in soils and vegetation from Copsa Mica area. *Bulletin UASVM Agriculture*, 2008; 65(2): 189.
43. Weichenthal S, Moase C, Chan P, A review of pesticide exposure and cancer incidence in the agricultural health study cohort. *Environ Health Perspect.*, 2010; 118(8): 1117-1125.
44. Wojciechowska M, Stepnowski P, Gołębiowski M, The use of insecticides to control insect pests. *ISJ*, 2016; 13: 210-220.