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Assessment of pesticide residues in honey and their prospective risk to consumers in Egypt

Tarek, A. Abdel-Rahman[1] ; Asmaa, A. Eissa[2] and Hatem, M. Mahfouz[3]

[1]*Pesticides Residues and Environmental Pollution Dept., Central Agricultural Pesticides Laboratory, Agricultural Research Center, Dokki, Giza, Egypt.*
[2]*Plant Protection Research Institute, Giemeza, Agricultural Research Center, Dokki, Giza, Egypt.*
[3]*Dept. Plant Production, Fac. of Environ. Agric. Sci., Arish, Univ., Egypt.*

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Abstract:

Honeybees, *Apis mellifera* L. (Hymenoptera: Apidae) is one of the most important tasks of pollinating agricultural crops. The aim of this study is to detect of the pesticides remain in honeybee samples gathered from Egypt and compare the obtained results to the published Maximum Residue Limits (MRL's) values. Fifty honey samples were randomly gathered from local marketplaces of five Governorates viz: (Cairo, Giza, Qalyubia, Alexandria and Gharbia) in Egypt during 2020. The current study was performed to assess the residues levels of 38 pesticides in the major groups of pesticides (organophosphorus, organochlorine and Synthetic Pyrethroids) representative, using method based on the QuEChERS**.** The recovery results found ranged from 80% to 104 %. The results indicated that, total contamination with pesticides residues was 24%, dominant pesticides that were in the samples belonged to the organochlorine and organophosphorus groups. There are no demonstrable residues of synthetic pyrethroids pesticides in all gathered samples. All samples of honey are matched to MRLs. Data showed that, exposing to these pesticides in highly connected with evaluating the potential health risks. The acceptable daily intakes of the pesticide (ADIs) were so higher than estimated daily intake (EDI), which prove that consume honey has the lowest toxicological risk. This study confirms the need for recurrently monitoring scheme for pesticide residues in honey at the national concentration to protect the health of consume.

Introduction

Honey is one of the natural sweeties, as honeybees produced it from the blossom nectar. Honeybees, *Apis mellifera* L. (Hymenoptera: Apidae) , perform the essential task of pollinating agricultural crops and native species and are important to produce of honey and beeswax. 10,000–25,000 honeybee workers carry out about 10 trips daily to see the sights of about seven km^2 in the region around their hive, to collect nectar, water, and pollen from blooms. Throughout this trip, a lot

of microorganisms, chemical materials, and particles, hanging in the air, are captured by these workers and kept in their body surface hair, or respired and detained to their trachea (Hassan *et al* ., 2015). Thus, these easy to breed, almost omnipresent organisms, with simple food requirements, are highly sensetive to many factors that are physical, biological, and chemical, for instance fleas, industrial polluting materials, or insecticides and may be employed as one of the bio indicators to control the environmental stress (Celli and Maccagnani , 2003).

Honey is one of the natural sweet materials, that is created by honeybee. Regarding to its constant use, it may be polluted by pesticide remains. Nearly, no studies have dealt with pesticide remains in honey, estimated the risks, or argued their expected reproductive poisoning (El-Nahhal, 2020). Currently, with growing awareness of consumers over pesticide materials in food and the effect of crop safety practices on the environment, pesticide residues in agricultural crops should not cause a real health threat. Since the beginning of 1950s, organochlorine pesticides were used broadly in Egypt . Nevertheless, their use was legally forbidden since 1980. These compounds are characterized by stability, deep absorbance on sediments and land, so their residues may still occur in certain foods such as honey.

Organophosphorus compounds that have small perseverance and were readily decaying and used broadly nowadays for insect control all over the country as well as synthetic pyrethroid compounds which were used in cotton crop only. (Anonymous,1995). The existence of pesticide remains in honey revealed the necessity of creating control programs to evaluate properly the human exposure to pesticides and the possibility of taking policy decisions to avoid health hazards (Wallner, 1999). Accordingly, the aim of this study is to detect of the organochlorine, organophosphorus and synthetic pyrethroid pesticide remains in honeybee samples gathered from Egypt in 2020 and compare the obtained results to the published Maximum Residue Limits (MRL's) values

Materials and methods

1. Samples:

Fifty honey samples were randomly gathered from local markets of five governorates viz: (Cairo, Giza, Qalyubia, Alexandria and Gharbia) in Egypt in 2020. Samples were titled according to the name of Governorate then transmitted immediately to the laboratory

2. Pesticides detection:

The samples were analysed to identify and quantify of 38 pesticides. The organophosphorus residues include: Azinphos-ethyl, chloropyrifos, chlorpyrifos-methyl, cadusafos, diazinon, dichlorovs, disulfoton, cyanophos, ethion, ethoprophos, phorate, phenthoate, pirimiphos-ethyl, pirimiphos-methyl, profenofos, prothiofos, fenitrothion, fenamiphos, methamidophos, and triazophos. The organochlorine insecticides include: alpha-HCH, beta-HCH , gama-HCH , heptachlor , heptachlor-epoxide , aldrin , dieldrin , p,p-DDE , endrin , o.p-DDT , p,p-DDD , p,p-DDT. artificial Pyrethroids contained fenpropathrin, permethrin, lambda-Cyhalothrin, cypermethrin, fenvalerate, and deltamethrin.

3. Pesticides analysis:

The samples were mingled, (10 g) of each was put into 50 mL polyethylene tube. Extraction and cleaning up were instantly done after sampling using QuEChERS method (Anastassiades *et al.,* 2003 and Eissa *et al.,* 2014) . Acetonitrile (15 mL) was put in all tubes. The samples were well blended using a vortex mixer at the

utmost speed. Thereafter, 6 g of anhydrous magnesium sulfate and 1.5 g of sodium chloride were put, afterward extract by mingling strongly on vortex for 5 min and centrifuged for 10 min at 4,000 rpm. A portion of 4 mL was withdrawn from the supernatant to a new clean 15 mL centrifuge tube containing 100 mg PSA and 600 mg anhydrous magnesium sulfate. Once again, the samples were vortexed for 3 min, afterwards, centrifuged for 10 min at 4,000 rpm.

4. Gas chromatographic analysis:

Gas chromatography (GC) Hewlett Packard (HP) serial 6890 prepared with various detectors, i.e., electron capture (ECD) and flame photometry (FPD) were used. The pesticides analysis was carried out on two capillary columns, i.e., HP-5 (5%-Phenylmethylpolysiloxane) and DB-35 (35%-Phenyl-methylpolysiloxane).

The dimensions of each column were 30 m length x 0.25 mm internal diameter and covered with 0.25 µm film thickness of the still phase. Nitrogen was employed as a carrier gas at a flux rate of 1ml/min. The injector and interface temperatures were 250°C and 300°C, respectively. The GC temperature program was as follows; preliminary temperature was 100°C for 1min, increased at speed of 25°C/min to 170°C, isothermal for 1 min, increased at a speed of 3°C/min to 230°C, then isothermal for 1 min, finally increased at a rate of 8°C /min to 300°C, then isothermal for 5 min

5. Quality assurance procedure:

The Codex quality guarantee standards (Codex, 1993) were applied to establish the performance of the multiresidue technique. Recoveries and limits of quantification (LOQ) were applied on samples at rising levels 0.01–0.05 mg/ kg from the pesticide blend standard. The average of recoveries ranged between 80% and 104 %, and limits of quantification between 0.001 and 0.043 mg/ kg. The analyses outcome was not adjusted for recoveries. Blank samples were supplemented with the pesticide mixture and analyzed as a normal sample with each set of samples. The results were recorded on control charts. Repetitive analysis of old models was frequently performed to control reproducibility.

Results and discussion

This study throw light upon the disparity between the pesticides concentrations in honey samples from various places. The greatest inclination for accumulation of pesticides was in heather honeys. The results of pesticides analysis in 50 samples are shown in Table (1) . Residues of 4 active compounds were found in 12 samples of bee honey. The most frequent residues identified were chloropyrifos (in 16% of samples), p,p-DDE (4%) and cadusafos, heptachlor-epoxide (2%). All collected honeybee samples (i.e. 50) were free from any detectable residues of synthetic pyrethroids pesticides. Total pollution with pesticides residues was 24%. All samples of honeybee under MRLs. Organophosphorus pesticides resiues recorded highest frequencies of residues (75%), followed by organochlorine pesticides (25%). The highest contamination of pesticides was found in Cairo and Alexandria 25% followed by Giza, Qalyubia and Gharbia recording 16.66.

Table(1): Minimum, maximum, mean, frequency, contamination and violation of pesticides residues monitored in 50 samples of honeybee.

Governora-te	Total no. of sample	Pesticides Found	Freq-uency No.	Range: Minium-maximum (mean) (mg/kg)	Contamin - ated Samples No. %.	MRLs** (mg/kg)	Isolated Samples No. %
Cairo	10	p,p-DDE	1	0.004-0.004(0.004)	3 30	0.01	0 0
		Chloropyrifos	2	0.004-0.01(0.007)		0.05	0 0
Giza	10	Cadusafos	1	0.001-0.001(0.001)	2 20	0.02	0 0
		Chloropyrifos	1	0.002-0.002(0.002)		0.05	0 0
Qalyubia	10	p,p-DDE	1	0.003-0.003(0.003)	2 20	0.01	0 0
		Heptachlor-epoxide	1	0.003-0.003(0.003)		0.01	0 0
Alexandria	10	Chloropyrifos	3	0.005-0.02(0.002)	3 30	0.05	0 0
Gharbia	10	Chloropyrifos	2	0.005-0.05(0.005)	2 20	0.05	0 0
Total	50				12 24		0 0

These findings agree with those obtained by (Chauzat and Faucon, 2007) who monitored the quality of bee colonies *(A. mellifera).* Over 2 years, Beeswax samples were gathered once a year from 125 honey bee colonies totally. Multi remains analyses were carried out for these samples to identifying of 16 insecticides and acaricides and also two fungicides residues. Fourteen of studied compounds were found in samples. The most often detected remains were taufluvalinate, coumaphos and endosulfan (61.9, 52.2 and 23.4% of samples respectively). Coumaphos was found in the highest value (792.6 μgkg−1). Remains of cypermethrin, lindane and deltamethrin were found in 21.9, 4.3 and 2.4% of samples respectively According to the Statistical analysis, there is no difference among years of sampling, except for the rate of recurrence of pyrethroid remains . Both in-hive acaricide handlings and, to a lesser extent, ecological pollution are the reason behind Beeswax contamination. Also, one study estimated organochlorine pesticides remains in 178 samples of Polish honey using gas chromarography and found that the pesticide remains differentiated from trace concentrations to 60 μg/kg. Aldrin (l.o.d.d and 14,27 μg/kg), Endrin (trace and 65,3 μg/kg), dieldrin (l.o.d and 5,93 μg/kg), o,p-DDT (Trace and 18,66 μg/kg), p,p-DDT (trace and 227,85 μg/kg), HCH (trace and 284,96 μg/kg) , o,p methoxychlor (trace and 7,12 μg/kg) and p,p methoxychlor (trace and 38,67 μg/kg) residues were found in 38, 13, 32, 34, 108, 113, 17, 51 of honey samples, respectively (Wilczynska and Przybylowski, 2007) . Choudhary and Sharma (2008) found that the HCH and its isomers were the most frequently found among various pesticides examined in honey followed by DDT and its isomers in various parts of Himachal Pradesh. Only cypermethrin was detected in honey samples in the studied synthetic pyrethroids. The remains that were not found are organophosphates viz. acephate, chlorpyriphos, ethion and monocrotophos; nevertheless Furthermore, honey from natural vegetation included lesser remains. Yavuz *et al*. (2010) gathered 109 different honey samples from shops and open markets in Konya, Turkey and determinated of 24 organochlorine pesticide remains by gas chromatography-electron capture detection and observed that , Aldrin, *cis*-chlordane, *trans*-chlordane, *oxy*-chlordane, 2,4′ -DDE, and 4,4′ -DDE were found in all honey samples. *oxy*-chlordane (0.0540 μg g − 1) was found in 55 samples. The

resulted levels of organochlorine pesticide remain of *oxy*-chlordane were decides to be higher than those of Turkish Alimentarius Codex maximum residual limits (MRLs), excepting *cis*-heptachlor epoxide and *α*-hexachlorocyclohexane. It is necessary for consumer health to control organochlorine pesticide residues in honey, as all of the honey specimens are contaminated found and most of these samples exceeded MRLs. Bargan_ska *et al*. (2013) studied the levels of 30 insecticides remains in honey samples gathered from apiaries in northern part of Poland using method based on the QuEChERS extraction then, by fluid chromatography cycle mass spectrometry with electron spray ionization (LC-ESI-MS/MS). The percentage of positive samples is 29% for some of the target compounds. The compounds exceeded maximum residue standards are bifenthrin, fenpyroximate, methidathion, spinosad, thiamethoxam, and triazophos (MRL) in five samples (11%), the kind of the remains that associated with agricultural practices in the region. The maximum values of these pesticides were 14.5, 16.3, 25.7, 20.6, 20.2 and 20.3 ng/g, respectively. The most dominant pesticide was Profenofos, as it ranged from <LOQ to 17.2 ng/g.

1. Dietary intake evaluation and hazard specification:

To estimate the toxicological importance of human exposure to the pesticide remains in honey, it is necessary to compare acceptable daily intakes (ADI) established by the FAO/WHO organization with the estimated daily intake (EDI). The comparison between EDI and the acceptable daily intake (ADI), proved that the daily dosage of a chemical which, during the entire lifetime, appears to be without appreciable risk on the basis of all the facts known at the time (FAO, 1965). The integration of pesticide residue analysis data and food consumption assumptions is the basis of health risk assessment, which seeks to reflect the actual residue concentrations in food taken by the common people , with a weight of 60 kg. The source of food consuming data was WHO/Global Environment Monitoring System-Food Contamination Monitoring and assessment program average consumption cluster B diets (WHO/GEMS/FOODS, 2006). EDI was calculated using obtained findings and expressed as microgram pesticides per kilogram body weight per day (µg/kg b.w/day). The EDI is the actual evaluation of exposing to pesticide that was evaluated for each one of the pesticides on honey in accordance with the international guiding principles (WHO, 1997 and FAO, 2002), applying this equation: EDI = ΣC×F/D×W

Where C : The mean of pesticide residues concentration in honey (µg·kg-1), F : mean annual intake of honey per person (2 kg per person approximately), D i: Number of days in a year (365), and W : Mean body weight (60 kg). As shown in Table (2) , the ADIs were higher than the estimated daily intakes of detected pesticides, which proves that honey intake has a very low toxicological risk. These results match that those obtained by Blasco *et al.* (2003). So, in case the danger of a pesticide remains is not over unity, the user is sufficiently protected. Accordingly, the hazard index values show that all the intakes of pesticide remain undoubtedly are safe. Subsequently, data obtained from **Eissa *et al*. (2014)** were used for evaluating the expected health risks that could result from exposure to these insecticides. The acceptable daily intakes (ADIs) were higher than Estimated daily intake (EDI) of the observed pesticides. This finding

proves that honey intake has a very low toxicological risk.

Table (2): Estimated daily intakes (EDIs) and ADIs of pesticide remains in honey.

Pesticide	ADI* (mg/kg body weight/day)	EDI (µg/kg body weight/day)	Hazard index (EDI/ADI, %	Health risk
p,p-DDE	0.05	6.33 e-6	0.006	No
Chloropyrifos	0.01	5.02 e-5	0.05	No
Cadusafos	0.01	1.29 e-6	0.001	No
Heptachlor-epoxide	0.01	1.01e-6	0.0009	No

***Established by Codex Alimentarius Commission on Pesticide Residues, JMPR (Joint FAO/WHO Meeting on Pesticide Residues), EPA (Environmental Protection Agency) and EFSA (European Food Safety Authority).**

This study confirms the necessity of repeated monitoring programs for pesticide remains in honey at the national level to care for user health. Also, this study highlights the fact that dietary pesticide amount calculated related only to honey exposures and excludes other food products such as grains, vegetables, fruits, dairy, fish, and meats. Accordingly, assessments do not relate to total dietary exposition to the pesticides, nor to drinking water, inhabited, or professional exposures.

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