Multiresidue determination of pesticides in market honey from northern India using QuEChERS approach and assessment of potential risks to consumers

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Honey has multiple beneficial properties but polluted environments have led to its contamination. Contaminated honey not only serves as a sentinel of environmental pollution, but can also pose potential risks to consumers' health. In the present study, QuEChERS (quick, easy, cheap, effective, rugged and safe) method along with gas chromatography coupled to selective detectors (ECD/FTD/MS) was used for determining 24 pesticide residues and/or their metabolites in 150 honey samples collected from markets in Northern India. The method was optimized and validated according to the European Commission's guidelines. Residues of pesticides were detected in 12% of samples, of which a majority contained organophosphate residues. Assessment of human health risks suggests that contaminated honey at current levels has minimal contribution to toxicological risks. However, precautionary measures should always be taken considering the customary honey feeding in infants and cumulative effect of these chemicals in the foreseeable future. This study highlights the importance of continuous monitoring of pesticide residues, and consumer awareness towards certified products to safeguard public health.

Keywords: Honey, northern India, pesticides, risk assessment, QuEChERS.

FOOD consumption is a potential route by which human beings are exposed to various contaminants. Therefore, concerns on food safety especially for foods of animal origin (including honey) are increasing worldwide. Apiculture or beekeeping refers to rearing of domesticated honey bee species and their management to produce honey and its by-products. It is an eco-friendly, economically viable, environmentally sustainable, prime agri-horticultural and forest based enterprise. Due to mega biodiverse regions in India, the country has an ancient history of beekeeping practices and now ranks 6th in the world honey production[1]. Honey is an important ingredient of pharmaceuticals, cosmetics and food products. Due to its clean and healthy image, it is consumed by people across the globe[2]. Therefore, honey should be pure, wholesome and safe to consume. However, these days, it is being produced in environmental settings contaminated by various pollutants, particularly pesticides and this has raised questions on its quality and safety[3].

To ensure food security and to gain enormous profits, pesticides are used in agriculture and allied sectors in India[4,5]. Although pesticides have been instrumental in the country's green revolution and play an important role in agricultural innovations and farming practices, their widespread application and indiscriminate use can contaminate blossoms from which honey bees collect nectar for honey production[6]. Furthermore, at environmental levels, bees on their foraging expeditions can also pickup and transfer pollutants from contaminated water and soil to their respective hives. This may result in transfer of pesticide residues to honey and finally to consumers[7–10]. Therefore, contaminated honey can pose serious risks to the health of consumers by causing various effects like partial or complete suppression of immune response, cancers, endocrine disruptions, neurological disorders, problems with reproduction and birth defects etc.[11–14]. Hence, determining residues of pesticides in honey has become an imperative concern for maintaining its beneficial characteristics and safeguarding consumers health.

For determining xenobiotics in food commodities various techniques have been used. However gas chromatography combined with detectors like electron capture detector (ECD), flame thermionic detector (FTD) and mass spectrometry detector (MSD), appears to be the most promising methodology for detection and quantification of pesticides[15]. Most of the sample preparation methods were traditionally based on liquid–liquid extraction (LLE) procedures[16–19]. But these LLE procedures have been found to be using up too much time, necessitating sizeable volumes of solvents and are expensive. Hence to overcome these issues, QuEChERS (quick, easy, cheap, effective, rugged, and safe) approach[20] for analysis

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of fruits and vegetables was found to be promising for multi-residue detection of pesticides in honey[21–24].

Keeping in view the aforementioned facts, QuEChERS-AOAC[25] method for simultaneous extraction and clean-up of pesticide residues from honey as well as gas chromatographic techniques for their subsequent determination was optimized and validated in the present study. The validated methods were then employed for detection and quantification of residue of pesticides in commercial honey samples collected from various retail markets. Additionally, human health risk assessments were also performed for all the detected pesticides. To the best of our knowledge, the present study is the first comprehensive multiclass, multi-residue analyses of pesticides in honey samples from Indian retail markets using the QuEChERS method.

Materials and methods

Chemicals and reagents

The QuEChERS salt packets (6.0 g $MgSO_4$ and 1.5 g CH_3COONa) and 15 ml d-SPE polypropylene tubes (0.4 g primary secondary amine sorbent and 1.2 g $MgSO_4$) were procured from Agilent Technologies, USA. The ultrapure demineralized water used was obtained from Milli-Q water purification system (Merck-Millipore Corporations, USA). The standards for organochlorines, phenylpyrazole (fipronil), organophosphates and synthetic pyrethroids chosen for the study were procured from Merck KGaA, Germany. All the reference standards were initially stored at –20°C. The individual stock standard solutions (100 mg/l) were then made by dissolving each individual standard in HPLC grade *n*-hexane : acetone (1 : 1) and stored at –20°C. Intermediate standard solutions of 10 mg/l were made by dissolving individual stock standard solutions in *n*-hexane: acetone (9 : 1) and stored at 4°C. For multi-residue analyses of pesticides, multicomponent, working calibration solutions in concentrations ranging from 5 to 500 µg/l were then made by mixing and properly diluting the calculated volumes of each intermediate standard solution with *n*-hexane and acetone (9 : 1). The multicomponent calibration mixtures obtained were used for spiking honey samples as well as for preparing matrix-matched calibration (MMC) standards. For preparation of MMC standards, suitable quantity of multicomponent calibration mixtures was added to the control negative honey matrix in the final reconstitution stage. All additional analytical reagents and solvents used were procured from standard commercial traders.

Sample collection

One hundred and fifty honey samples were collected from various retail markets of Northern Indian states namely J&K, Himachal Pradesh, Punjab, Haryana and Rajasthan during January 2016–March 2017 (Table 1). The samples comprised branded honey (certified as well as uncertified) sold in retail markets and unbranded, processed honey sold by traders, self-help groups, krishi vigyan kendras (Agricultural Universities), co-operative societies and road side vendors. All honey samples weighing 100–250 g were subsequently kept in glass bottles at –20°C in the dark prior to extraction and analyses to avoid any fermentation phenomenon in the honey matrix. One blank (reference) honey sample was also collected from a beehive located in a sparsely inhabited area of Northwestern Himalayan Region of Himachal Pradesh (India) and was checked for any contamination.

Sample preparation and extraction of analytes

Five grams of honey sample was weighed and homogenized for 60 sec by dissolving in 10 ml of demineralized water. Thereafter, 10 ml of acetonitrile acidified with 1% glacial acetic acid and contents of 'QuEChERS' salt packet were added to the homogenized sample. The sample mixture was immediately stirred vigorously for 60 sec and subsequently subjected to centrifugation at 4000 *g* for 5 min in a refrigerated centrifuge at 4°C. The supernatant (6 ml) was then decanted into the d-SPE centrifuge tube. The tube was instantly hand-shaken for 60 sec and finally subjected to centrifugation at 4000 *g* for 3 min in a refrigerated centrifuge at 4°C. Four ml of the supernatant solution were then decanted to a clean borosilicate beaker, and the contents were evaporated to complete dryness using vacuum concentrator at 40°C. Finally, the residues of pesticide in the beaker were re-dissolved in 2 ml of *n*-hexane : acetone (9 : 1). The reconstituted sample (1 µl) was then injected into a gas chromatograph coupled with flame thermionic detector (GC-FTD) and 2 µl into the gas chromatograph coupled with electron capture detector (GC-ECD).

Chromatographic analyses

The residues and metabolites of pesticides were detected and quantified by comparing the retention times and area

Table 1. Honey samples collected from markets of Northern India

Honey type	With certification marks*	Without certification marks	Total
Branded honey	59	56	115
Unbranded honey	00	35	35
Total	59	91	150

*Product certified by FSSAI/AGMARK/ISO/any other food safety organization.

under the peaks of sample chromatograms with that of MMC standards analysed under the same operating parameters. Each chromatographic sequence included: a reagent blank, MMC standards, market honey and quality control samples (blank and spiked honey samples) for identification and quantification of chromatographic peaks and for checking contaminants in the test samples.

GC–ECD analysis: For detecting and quantifying the organochlorines and synthetic pyrethroids, chromatographic analysis was performed by GC 7890B equipped with a micro ECD and chromatographic column DB-5 (30 m × 0.32 mm i.d. × 0.25 µm film thickness). The temperature programming used was: 170°C (held for 13 min), a 3°C/min ramp to 270°C and held for 10 min leading to a run time of 56.33 min. The temperature of the injector and detector was set at 280°C and 300°C respectively. The flow of carrier gas (N_2) was 32.904 ml/min, maintaining 2.7 ml/min through DB-5 column at split ratio of 1 : 10. OpenLAB EZChrom chromatography software was utilized for controlling the instrument and for data processing.

GC–FTD analysis: For determining organophosphate pesticides in honey samples, chromatographic analyses were carried out by Shimadzu GC-FTD analytical instrument equipped with a capillary column RTX-5 (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The temperature programming used was: Initial temperature 180°C (held for 2 min), a 10°C/min ramp to 270°C (held for 3 min), a 5°C/min ramp to 280°C (held for 5 min), leading to a run time of 21 min. Temperature of the injection port and detector was set to 280°C. Nitrogen was used as carrier gas with flow rate 9 ml/min, 1.0 ml/min through RTX-5 column at split ratio of 1 : 5. Shimadzu GC solution software was utilized for controlling the instrument and for data processing.

GC-MS analysis: Suspected samples of honey were further investigated using GC-MS to qualitatively reconfirm the GC-ECD and GC-FTD results. GC-MS analysis was performed using GCMS-QP 2010 Plus model (Shimadzu, Japan) equipped with auto sampler AOC 20i, RTX-5MS column (30 m × 0.25 mm i.d. × 0.25 µm film thickness), using helium as carrier gas and operated through GCMS solution-software-based data acquisition. The temperature programming was as follows: Initial temperature 80°C (held for 3 min), a 20°C/min ramp to 180°C (held for 2 min), and then again a 2°C/min ramp to 190°C (held for 2 min), and then finally a 5°C/min ramp to 280°C and held for 10 min. The temperatures of the ionization source, interface and injection port were 200°C, 290°C and 285°C respectively. One µl of sample was injected in the split-less mode with 60 sec purge off. Chromatographic analyses were carried out in Selected Ion Monitoring (SIM) mode, examining particular ions of every targeted analyte. The suspected analytes were re-qualitatively confirmed based on their retention time and fragment ions (m/z) (Supplementary Table 1).

Validation parameters

The methods of extraction, clean-up, detection and quantification of 24 pesticides (or their metabolites) from the honey matrix were optimized and validated in compliance with the European Commission guidance document SANTE 11945/2015 (ref. 26) by evaluating the following performance parameters: linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy (expressed as recovery percentage), precision (% relative standard deviation), ruggedness and selectivity.

Linearity of the method was assessed by calculating five-point straight line plot with three replications on the basis of linear regression equation and coefficient of determination (R^2) values. Five-point matrix matched calibrations were performed in concentrations ranging from 5 to 100 µg/kg for organochlorines, phenylpyrazole and synthetic pyrethroid pesticides and in concentrations ranging from 10 to 200 µg/kg for each organophosphate pesticide.

The LOD and LOQ were estimated from the calibration curve using the equation[27]

$$\mathrm{LOD} = \frac{3.3 \times \sigma}{m}, \quad \mathrm{LOQ} = \frac{10 \times \sigma}{m},$$

where m is the slope of calibration curve and σ is the standard deviation of the response.

The reliability of the method was evaluated by estimating the accuracy and precision. Recovery experiments were carried out at 3–4 fortification levels by spiking blank honey (negative control sample) with working solutions of multicomponent calibration standard mixtures for assessing the accuracy. The fortification levels of 25, 50 and 100 µg/kg were used for organochlorines, phenylpyrazole and synthetic pyrethroids; and 25, 50, 100 and 200 µg/kg for organophosphates. Spiking was done such that the first fortification level corresponded close to the quantitation limit for each targeted residue, with three replications for each fortification level. The spiked honey samples were then kept at usual room temperature of 23°C for about 2 h to attain sample equilibration. The area under the peaks of the known amount of analytes in the blank honey matrix (negative control) spiked before extraction and in the sample extract spiked near the chromatographic analyses (matrix matched standards) was compared to calculate recovery percentage.

The method's precision was ascertained with regards to the relative standard deviation (RSD) of the three exactly similar extractions of blank samples spiked with pesticides at the same as well as at different fortification

levels. The ruggedness of the method was tested by following minor changes in the protocol. The method's selectivity was evaluated by analysing blank honey matrix (n = 10) and reagent blank to determine any interference from endogenous compounds around the retention time window of target analytes.

Human health risk assessment

The human health risk assessment was carried out by calculating the estimated daily intakes (EDIs) using HI (Hazard Index) model[28]. The EDI, which is a realistic toxicological criterion for the pesticides exposure, was determined for each pesticide residue according to international guidelines as

$$\text{EDI} = \frac{C \times F}{C \times W},$$

wherein C is the mean residual concentrations of pesticides in honey (μg/kg), F the mean intake of total dietary honey per person in a year (70 g)[29], D the number of days in one year (i.e. 365 days) and W is the mean human body weight (adult 60 kg and child 15 kg).

The EDIs of all the detected pesticides were then compared with their corresponding acceptable daily intakes (ADIs) established by WHO/FAO[30], to calculate the hazard index (EDI/ADI) and percentage contribution to ADI (% ADI).

Results and discussion

Method validation and quality control

The optimized analytical conditions of GC-ECD and GC-FTD resulted in identification and effective separation of all the investigated pesticides with good peak resolutions (Figures 1 and 2). The method validation parameters are summarized in Table 2.

Matrix matched calibrations in triplicates showed that the method was linear with R^2 value >0.99 for all the investigated compounds. The detection and quantitation limits for each targeted pesticide residue were found comparable to the maximum residue levels (MRLs) established by European Union and Export Inspection Council of India[31,32]. The recovery percentage for all the pesticide residues was found to be 86.0–107.7% with relative standard deviation values <15% (Table 2). This complies with the European Commission guidance document SANTE 11945/2015 (ref. 26). Analysis of blank honey samples showed that the method was selective (Figures 1 and 2). The overall result revealed that the method was efficacious, reliable and sensitive, enabling multi-residue determination of all the targeted pesticides in honey that may have toxicological pertinence at trace levels.

Positive honey samples containing targeted residues were further analysed by GC-MS to qualitatively reconfirm the GC-ECD and GC-FTD results. GC-MS-SIM analyses were performed using three or more qualifier ions (Q). The selection of ions and their abundance for each targeted analyte was established by injecting identical pesticide standard under similar operating conditions in full-scan mode with their mass/charge ratio in the range 50–500 m/z. The retention time for all the positively confirmed pesticides was confined to ± 0.3 min of expected retention time. The peak heights of specific masses in sample peak were also confined to ±20% of the relative intensity of their corresponding masses in the mass spectra of the pesticide standards examined under the full scan mode in the GC-MS system. GC-MS-SIM was deemed as a valuable technique for confirmation of pesticide residues in the complex matrices and for ruling out false positives.

Pesticide residues in market honey samples

Out of 150 market honey samples, 18 (12.0%) were found to contain one or more targeted pesticide residues (Table 3). About 72% of the positive market honey samples (13/18) were found to be contaminated with organophosphates followed by organochlorines including fipronil (22.2%, 4/18) and synthetic pyretheroids (5.6%, 1/18). The frequently detected pesticide residues were monocrotophos in 4 samples (up to 306.9 μg/kg) followed by dichlorvos in 3 samples (up to 69.2 μg/kg) trailed by chlorpyrifos, profenofos, ethion and lindane in 2 samples each, with maximum quantified concentrations of 31.2, 15.7, 72.2 and 32.2 μg/kg respectively. Phorate, malathion, quinalphos, fipronil, endrin and cypermethrin were determined in one sample each with quantified residual concentrations of 61.1, 19.5, 57.1, 29.7, 12.1 and 25.4 μg/kg respectively (Table 4). Monocrotophos and dichlorvos were detected more frequently with relatively higher levels compared to other targeted pesticide residues. The occurrence of organophosphorus pesticides in honey samples points towards the change in tendency of Indian farmers towards pesticide's applications during the last few years. The analytical results suggest a shift in the pattern of pesticide usage from organochlorines to organophosphates. Various studies conducted in India on agricultural soils[33], surface and ground water[34], vegetables[35], fruits[36], milk and butter[37] and fish[38] have also revealed their contamination by various types of organophosphorus pesticides. Further, to substantiate these findings, the critical review of data available from India also revealed that during the last six years, i.e. 2010–11 to 2015–16, the overall indigenous chemical pesticide demand was remarkably higher for insecticides with organophosphates accounting for the major share of the insecticides (65%) followed by carbamates (13%),

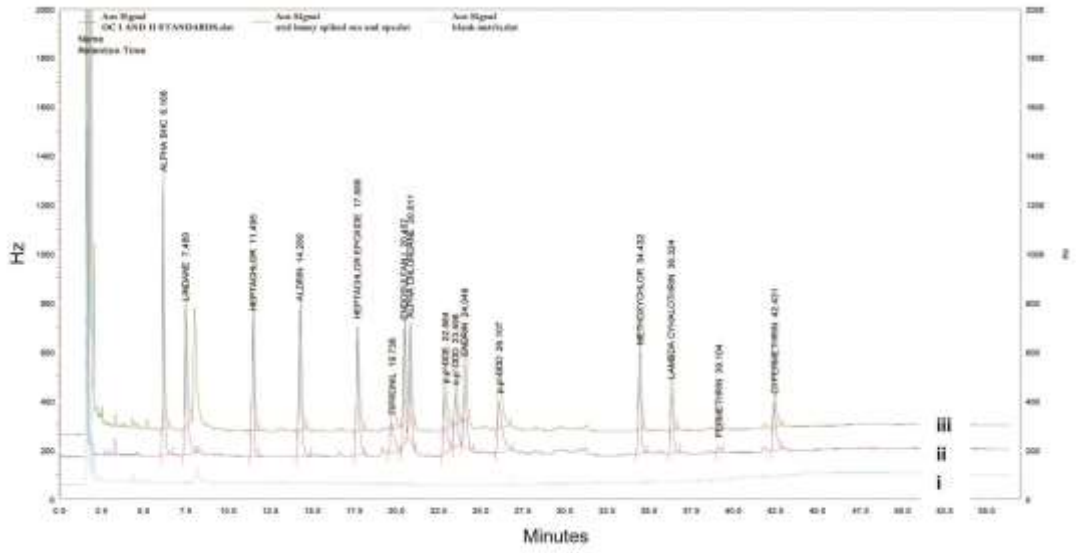

Figure 1. Overlaid GC-ECD chromatograms of (i) blank honey sample, (ii) organochlorines, fipronil and synthetic pyrethroids standard mixture, and (iii) honey sample spiked with pesticide standards.

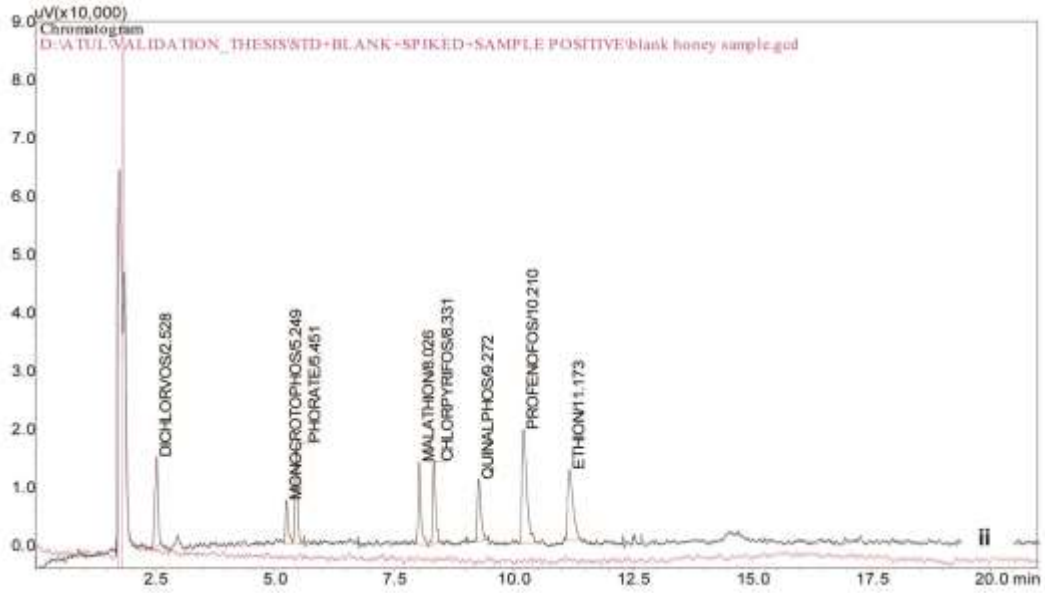

Figure 2. Overlaid GC-FTD chromatograms of (i) blank honey sample, and (ii) honey sample spiked with organophosphate pesticides

pyrethroids (9.5%), organochlorines (7.5%) and other newer formulations (5%)[39].

Except for presence of lindane (2) and endrin (1) individually in three honey samples, none of the targeted organochlorines pesticides could be detected in any of the tested market honey samples. Considering the 430 metric tonnes of fipronil's demand during the year 2015–16 in India[39], and its subsequent registered applications to control pests of cotton, rice, sugarcane and vegetables, fipronil's detection to the tune of 29.7 μg/kg in one honey sample should not be confounding and therefore irrefutable. Detection of these banned pesticides in market honey samples could also be attributed to their extensive applications in the past. Since organochlorines were massively used in agricultural applications before being finally banned for use, they may still continue to persist in the environment. There could be various routes like polluted water, soil and air from where these pesticides bio-accumulate in plants and finally show their presence in honey through contaminated pollens and nectars. This in consistent with the study by Panseri *et al.*[40] who suggested that organochlorines bio-accumulate in the contaminated

Table 2. Validation parameters for determination of pesticide residues in honey

Compounds	t_R (min)	Data calibration: Regression equation $y = mx + c$	Data calibration: R^2	Data calibration: Linear range	LOD	LOQ	Recovery ± RSD%: Fortification levels (µg/kg): 25	50	100	200	Average recovery %	RSD_{Pooled}
Organophosphorus (n = 3)												
Dichlorvos	2.53	$y = 127.87x + 397.26$	0.9994	10–200	7.3	21.9	87.1 ± 5.2	76.9 ± 13.5	89.7 ± 7.1	91.0 ± 1.3	86.2	8.1
Monocrotophos	5.25	$y = 35.31x + 318.15$	0.9947	10–200	21.3	63.9	BQL	90.8 ± 7.3	84.6 ± 4.7	86.1 ± 10.5	87.2	7.9
Phorate	5.45	$y = 109.78x + 11.492$	0.9992	10–200	8.1	24.3	85.2 ± 9.6	91.7 ± 4.8	84.4 ± 4.6	82.7 ± 2.6	86.0	6.0
Malathion	8.03	$y = 114.72x + 407.4$	0.9996	10–200	5.9	17.8	91.9 ± 1.6	80.9 ± 8.1	85.7 ± 8.6	89.4 ± 3.4	87.0	6.2
Chlorpyrifos	8.33	$y = 145.47x - 271.02$	0.9995	10–200	6.4	19.3	105.3 ± 4.8	102.0 ± 3.2	111.1 ± 6.9	105.4 ± 1.9	106.0	4.6
Quinalphos	9.27	$y = 120.62x - 192.91$	0.9997	10–200	5.3	15.8	107.5 ± 8.2	112.2 ± 5.9	110.1 ± 11.2	100.9 ± 6.8	107.7	8.3
Profenofos	10.21	$y = 300.46x - 39.137$	0.9998	10–200	4.2	12.7	92.9 ± 5.2	95.6 ± 12.2	100.2 ± 11.4	83.2 ± 5.3	93.0	9.1
Ethion	11.17	$y = 209.29x - 441.88$	0.9997	10–200	5.3	16.0	91.0 ± 3.5	91.3 ± 2.9	98.0 ± 7.4	90.7 ± 2.1	92.7	4.5
Organochlorines (n = 3)												
α-BHC	6.17	$y = 12976x + 10814$	0.9996	5–100	2.8	8.4	92.7 ± 2.0	117.1 ± 7.1	87.1 ± 1.0	–	99.0	4.3
Lindane	7.49	$y = 13070x + 20084$	0.9987	5–100	5.3	15.8	93.7 ± 7.0	99.3 ± 11.5	98.7 ± 5.3	–	97.2	8.3
Heptachlor	11.50	$y = 28858x + 20409$	0.9987	5–100	5.3	15.8	86.9 ± 3.4	116.0 ± 10.8	101.9 ± 5.1	–	101.6	7.2
Aldrin	14.28	$y = 22340x + 8437.9$	0.9988	5–100	5.1	15.2	79.4 ± 3.2	100.6 ± 7.6	101.9 ± 0.5	–	94.0	4.8
Heptachlor epoxide	17.69	$y = 15497x + 17822$	0.9992	5–100	4.3	12.9	106.8 ± 7.4	99.5 ± 1.3	101.3 ± 13.2	–	102.5	8.8
Fipronil (phenylpyrazole)	19.74	$y = 22960x - 45561$	0.9962	5–100	9.1	27.3	99.7 ± 1.4	98.6 ± 4.6	92.4 ± 15.5	–	96.9	9.4
Endosulfan I	20.46	$y = 34195x - 17987$	0.9981	5–100	6.4	19.1	100.2 ± 0.7	96.6 ± 9.3	108.5 ± 9.6	–	101.8	7.8
α-Chlordane	20.81	$y = 21482x - 2006.6$	0.9987	5–100	5.3	15.8	88.6 ± 8.3	89.6 ± 2.0	93.7 ± 3.3	–	90.6	5.2
p,p'-DDE	22.88	$y = 28171x + 122539$	0.9990	5–100	4.7	14.1	73.4 ± 5.5	102.8 ± 1.5	93.1 ± 1.7	–	89.8	3.4
o,p'–DDD	23.50	$y = 17828x - 22317$	0.9986	5–100	5.6	16.8	80.9 ± 7.3	101.1 ± 6.2	95.0 ± 2.5	–	92.3	5.7
Endrin	24.05	$y = 15175x - 49004$	0.9994	5–100	3.6	10.8	101.6 ± 10.7	92.6 ± 9.2	93.6 ± 2.1	–	95.9	8.2
p,p'-DDD	26.11	$y = 13352x + 22591$	0.9990	5–100	4.6	13.7	105.8 ± 6.8	97.4 ± 6.2	97.2 ± 4.8	–	100.1	6.0
Methoxychlor	34.43	$y = 11845x - 29758$	0.9992	5–100	4.1	12.4	95.8 ± 10.8	96.1 ± 5.8	100.7 ± 6.6	–	97.5	8.0
Synthetic pyrethroids (n = 3)												
λ-Cyhalothrin	36.32	$y = 12663x + 20406$	0.9989	5–100	4.9	14.8	94.1 ± 9.6	101.2 ± 8.6	98.5 ± 6.1	–	97.9	8.2
Permethrin	39.10	$y = 2164.9x + 11556$	0.9969	5–100	8.2	24.6	109.1 ± 0.9	76.0 ± 3.7	97.5 ± 9.9	–	94.2	6.1
Cypermethrin	42.43	$y = 14960x - 42959$	0.9982	5–100	6.3	18.8	92.1 ± 13.0	93.4 ± 9.5	91.6 ± 13.4	–	92.4	12.1

*BQL: Below quantification level, Pooled RSD was calculated as:

$$RSD_{Pooled} = \sqrt{\frac{RSD_1^2(n_1 - 1) + RSD_2^2(n_2 - 1) + \ldots}{(n_1 - 1) + (n_2 - 1) + \ldots}}.$$

Table 3. Market honey samples containing pesticide residues

Samples	No. of samples	No. of positive samples (%)	Samples containing pesticide above MRLs (%)
Certified branded	59	01 (1.7)	1 (1.7)
Uncertified branded	56	11 (19.6)	9 (16.1)
Unbranded	35	06 (17.1)	5 (14.3)
Total	150	18 (12.0%)	15 (10.0%)

Table 4. Levels of pesticide residues detected in market honey samples (N = 150)

Pesticides	Detection frequency (% sample contaminated)	Mean ± SD (μg/kg)[a]	Minimum quantified (μg/kg)	Maximum quantified (μg/kg)	MRL (μg/kg) (as per EC)	% above MRL
Dichlorvos	3 (2.0)	53.4 ± 20.7	30.0	69.2	10*	2.0
Monocrotophos	4 (2.7)	247.7 ± 55.0	173.9	306.9	10*	2.7
Phorate	1 (0.7)	61.1 ± 0.0	61.1	61.1	10*	0.7
Malathion	1(0.7)	19.5 ± 0.0	19.5	19.5	20	0
Chlorpyrifos	2 (1.3)	26.9 ± 6.0	22.7	31.2	10	1.3
Quinalphos	1 (0.7)	57.1 ± 0.0	57.1	57.1	10*	0.7
Profenofos	2 (1.3)	14.6 ± 1.6	13.5	15.7	50	0
Ethion	2 (1.3)	45.8 ± 37.3	19.5	72.2	5	1.3
Lindane	2 (1.3)	24.0 ± 11.6	15.9	32.2	10	1.3
Fipronil	1 (0.7)	29.7 ± 0.0	29.7	29.7	10*	0.7
Endrin	1 (0.7)	12.1 ± 0.0	12.1	12.1	10*	0.7
Cypermethrin	1 (0.7)	25.4 ± 0.0	25.4	25.4	17	0.7
Overall Mean	18 (12%)					10%

α-BHC, heptachlor, aldrin, heptachlor epoxide, endosulfan I, α-chlordane, p,p'-DDE, o,p'-DDD, p,p'-DDD, methoxychlor, λ-cyhalothrin, permethrin – not detected. [a]Mean ± standard deviation of positive samples only; *No established MRL, default MRL set at 10 μg/kg (ref. 31).

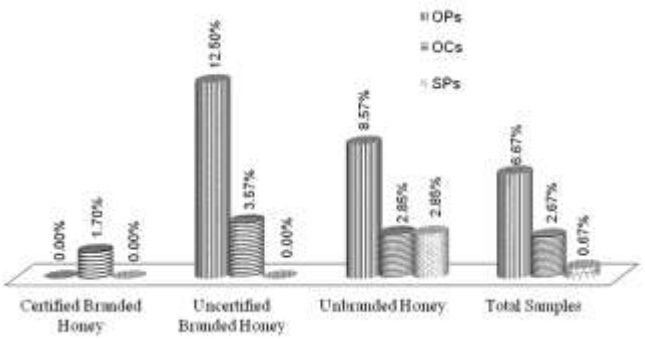

Figure 3. Proportions of market honey samples containing organophosphates (OPs), organochlorines including fipronil (OCs) and synthetic pyrethroids (SPs) above MRLs.

soil and may possibly enter into the human food web through non-fatty food products like honey.

Fifteen positive samples contained at least one pesticide residue above MRLs. However, a large proportion of samples above MRLs belonging to uncertified branded (9/56) and unbranded honey (5/35) were found contaminated with organophosphorus pesticides (Figure 3). Only one certified branded sample of honey out of 59 analysed samples was found to contain phenylpyrazole insecticide, fipronil. The obvious reasons for this type of result could be attributed to the practices of selling locally produced honey by beekeepers/traders without obtaining certification from food safety organizations. This is in line with the observations made in the present study, where most of the traders and beekeepers acknowledged that beehives are usually kept in proximity to the agricultural areas of extensive pesticide usage and major share of honey in India is being marketed without certification from FSSAI (The Food Safety and Standards Authority of India) and AGMARK (Certification mark employed under Agricultural Produce (Grading and Marking) Act, 1937).

Although FSSAI, the agency responsible for quality control of food items sold in Indian markets, has not yet established the MRLs for pesticides in honey, their significant absence in certified branded honey samples reflects the overall quality of such honey sold in Indian domestic markets. However, the relatively high mean concentrations of some organophosphorus pesticides is a matter of concern in terms of risks associated with consumer health and environmental contamination.

Human health risk assessments

The EDIs of the detected pesticides for both adults and children were lower than the ADIs (Table 5). Therefore, calculated HI values were found to be less than unity, i.e. all the oral consumption of pesticides through honey remains distinctly under the safe limits. Hence, it is presumed that there are no acute effects on consumers from consumption of honey at current levels of contamination in India. Moreover, the per cent contribution of total dietary intake of detected pesticides through honey to the ADI was also found to be <1%. This clearly indicates that the consumption of honey has a minimum contribution to

Table 5. Estimated daily intakes and percentage contribution to acceptable daily intakes of pesticide residues found in market honey samples

Compound	ADI	Age group	EDI	HI	% ADI
Dichlorvos	4.0	Adult	1.71E-04	4.27E-05	4.27E-03
		Children	6.83E-04	1.71E-04	1.71E-02
Monocrotophos	0.6	Adult	7.92E-04	1.32E-03	1.32E-01
		Children	3.17E-03	5.28E-03	5.28E-01
Phorate	0.5	Adult	1.95E-04	3.91E-04	3.91E-02
		Children	7.81E-04	1.56E-03	1.56E-01
Malathion	30	Adult	6.23E-05	2.08E-06	2.08E-04
		Children	2.49E-04	8.31E-06	8.31E-04
Chlorpyrifos	10	Adult	8.60E-05	8.60E-06	8.60E-04
		Children	3.44E-04	3.44E-05	3.44E-03
Quinalphos	10	Adult	1.83E-04	1.83E-05	1.83E-03
		Children	7.30E-04	7.30E-05	7.30E-03
Profenofos	10	Adult	4.67E-05	4.67E-06	4.67E-04
		Children	1.87E-04	1.87E-05	1.87E-03
Ethion	2	Adult	1.46E-04	7.32E-05	7.32E-03
		Children	5.86E-04	2.93E-04	2.93E-02
Lindane	5	Adult	7.67E-05	1.53E-05	1.53E-03
		Children	3.07E-04	6.14E-05	6.14E-03
Fipronil	0.2	Adult	9.49E-05	4.75E-04	4.75E-02
		Children	3.80E-04	1.90E-03	1.90E-01
Endrin	0.2	Adult	3.87E-05	1.93E-04	1.93E-02
		Children	1.55E-04	7.74E-04	7.74E-02
Cypermethrin	50	Adult	8.12E-05	1.62E-06	1.62E-04
		Children	3.25E-04	6.49E-06	6.49E-04

ADI and EDI in μg/kg body weight/day; HI = EDI/ADI; E-0X = 10^{-X}; % ADI = Per cent contribution of total dietary intake of pesticides through honey to ADI.

the overall toxicological risks. However, considering the possible accumulative effects of substances with similar mode of action and customary (traditional and cultural) feeding of honey to the infants, old and ill people in India, precautionary measures should always be taken in the foreseeable future to safeguard consumers health.

Conclusion

The validated multi-residue method was utilized for determination of pesticide residues in market honey samples. The method proved simple, cost-effective and easily adaptable in the laboratory. The occurrence of residues of commonly used pesticides in honey samples suggested that the source of contamination could factually be the ambient environment. This affirms that honey can be employed as a sentinel to mirror the level of environmental pollution surrounding beehives. Toxicological risk associated with consumption of honey was found to be minimal, but precautionary measures should always be taken considering the customary feeding of honey to infants and possible overall exposures of other major food commodities to these chemicals in the foreseeable future. This could also present an opportunity for national and international food safety organizations and public health agencies to be proactive in preventing the risk associated with consumption of pesticide contaminated food and food products including honey.

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1. Kumar, A., Gill, J. P. S., Bedi, J. S., Kumar, A. and Chhuneja, P. K., Determination of antibiotic residues in honey by HPLC-DAD and assessment of potential risks to consumers. In Proceedings of the 15th Annual Conference of IAVPHS and National Symposium on Intersectoral Approaches to Combat Zoonoses: Strategies and Challenge, Tirupati, India, 2017, p. 266.
2. Tsipi, D., Triantafyllou, M. and Hiskia, A., Determination of organochlorine pesticide residues in honey, applying solid phase extraction with RP-C18 material. *Analyst*, 1999, **124**(4), 473–475.
3. Christodoulou, D. L., Kanari, P., Kourouzidou, O., Constantinou, M., Hadjiloizou, P., Kika, K. and Constantinou, P., Pesticide residues analysis in honey using ethyl acetate extraction method: validation and pilot survey in real samples. *Int. J. Environ. Anal. Chem.*, 2015, **95**(10), 894–910.
4. Bhardwaj, T. and Sharma, J. P., Impact of pesticides application in agricultural industry: an Indian scenario. *Int. J. Agric. Food Sci. Technol*., 2013, **4**(8), 817–822.
5. Aktar, M. W., Sengupta, D. and Chowdhury, A., Impact of pesticides use in agriculture: their benefits and hazards. *Interdis. Toxicol.*, 2009, **2**(1), 1–12.
6. Fernandez-Muino, M. A., Sancho, M. T., Muniategui, S., Huidobro, J. F. and Simal-Lozano, J., Nonacaricide pesticide residues in honey: analytical methods and levels found. *J. Food Protect.*, 1995, **58**(11), 1271–1274.
7. Kujawski, M. W., Barganska, Z., Marciniak, K., Miedzianowska, E., Kujawski, J. K., Slebioda, M. and Namiesnik, J., Determining pesticide contamination in honey by LC-ESI-MS/MS- Comparison of pesticide recoveries of two liquid-liquid extraction based approaches. *LWT – Food Sci. Technol*., 2014, **56**, 517–523.
8. Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., vanEngelsdorp, D. and Pettis, J. S., High levels of miticides

and agrochemicals in North American apiaries: implications for honey bee health. *PLoS ONE*, 2010, **5**, e9754(3).
9. Naccari, C., Macaluso, A., Giangrosso, G., Naccari, F. and Ferrantelli, V., Risk assessment of heavy metals and pesticides in honey from Sicily (Italy). *J. Food Res*., 2014, **3**, 107.
10. Porrini, C. *et al.*, Honey bees and bee products as monitors of the environmental contamination. *Apiacta*, 2003, **38**, 63-70.
11. Aliferis, K. A., Tarantilis, P. A., Harizanis, P. C. and Alissandrakis, E., Botanical discrimination and classification of honey samples applying gas chromatography/mass spectrometry fingerprinting of headspace volatile compounds. *Food Chem*., 2010, **121**(3), 856–862.
12. Brouwer, A. *et al.*, Characterization of potential endocrine related health effects at low dose levels of exposure to PCBs. *Environ. Health Perspect*., 1999, **107**, 639.
13. Crisp, T. M. *et al*., Environmental endocrine disruption: an effects assessment and analysis. *Environ. Health Perspect*., 1999, **106**, 11.
14. Hurley, P. M., Hill, R. N. and Whiting, R. J., Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumours in rodents. *Environ. Health Perspect*., 1998, **106**, 437.
15. Fernandez, M., Pico, Y. and Manes, J., Analytical methods for pesticide residue determination in bee products. *J. Food. Prot*., 2002, **65**(9), 1502–1511.
16. Blasco, C., Fernández, M., Pena, A., Lino, C., Silveira, M. I., Font, G. and Picó, Y., Assessment of pesticide residues in honey samples from Portugal and Spain. *J. Agric. Food Chem*., 2003, **51**(27), 8132–8138.
17. Debayle, D., Dessalces, G. and Grenier-Loustalot, M. F., Multiresidue analysis of traces of pesticides and antibiotics in honey by HPLC-MS-MS. *Anal. Bioanal. Chem*., 2008, **391**(3), 1011–1020.
18. De Pinho, G. P., Neves, A. A., de Queiroz, M. E. L. R. and Silvério, F. O., Optimization of the liquid–liquid extraction method and low temperature purification (LLE–LTP) for pesticide residue analysis in honey samples by gas chromatography. *Food Control*, 2010, **21**(10), 1307–1311.
19. Rissato, S. R., Galhiane, M. S., de Almeida, M. V., Gerenutti, M. and Apon, B. M., Multiresidue determination of pesticides in honey samples by gas chromatography–mass spectrometry and application in environmental contamination. *Food Chem*., 2007, **101**(4), 1719–1726.
20. Anastassiades, M., Lehotay, S. J., Tajnbaher, D. and Schenck, F. J., Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *J. AOAC Int.*, 2003, **86**(2), 412–431.
21. Barakat, A., Badawy, H. M. A., Salama, E., Attallah, E. and Maatook, G., Simple and rapid method of analysis for determination of pesticide residues in honey using dispersive solid phase extraction and GC determination. *J. Food, Agric. Environment*, 2007, **5**(2), 97–100.
22. Barganska, Ż., Ślebioda, M. and Namieśnik, J., Determination of pesticide residues in honeybees using modified QUEChERS sample work-up and liquid chromatography-tandem mass spectrometry. *Molecules*, 2014, **19**(3), 2911–2924.
23. Malhat, F. M., Haggag, M. N., Loutfy, N. M., Osman, M. A. M. and Ahmed, M. T., Residues of organochlorine and synthetic pyrethroid pesticides in honey, an indicator of ambient environment, a pilot study. *Chemosphere*, 2015, **120**, 457–461.
24. Irungu, J., Raina, S. and Torto, B., Determination of pesticide residues in honey: a preliminary study from two of Africa's largest honey producers. *Int. J. Food Contam*., 2016, **3**(1), 14.
25. AOAC (Association of Official Analytical Chemist). AOAC official method 2007.01: pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate. AOAC Int., Gaithersburg, USA, 2007.
26. EC (European Commission). Document number SANTE/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed, 2016.
27. ICH (International Conference on Harmonisation). Harmonised Tripartite Guideline Validation of Analytical Procedures: text and methodology Q2 (R1), 2005.
28. Eissa, F., El-Sawi, S. and Zidan, N. E., Determining pesticide residues in honey and their potential risk to consumers. *Pol. J. Environ. Stud*., 2014, **23**(5), 1573–1580.
29. Lalhmangaihi, R. and Laha, R. C., Beekeeping: a nature eco-friendly business. In *Science and Technology for Shaping Future of Mizoram* – Proceedings of the Mizoram Science Congress (ed. Lalchhandama, K.), Allied Publishers, New Delhi, 2017, pp. 35–39.
30. FAO/WHO. Pesticide residues in food. In *Report of the joint meeting of the FAO panel of experts on pesticide residues in food and the environment and the WHO core assessment group on pesticide residues*. FAO plant production and protection paper, 2016, p. 229; http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Report2016/JMPR_2016_Report_full.pdf. (accessed on 13 September 2017).
31. EC (European Commission). 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 J. Eur. Commun.*, 2005, pp. 1–16;. http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R0396 (accessed on 15 March 2016).
32. EIC (Export Inspection Council), India. Honey residue monitoring plan (RMP) for export to EU Year 2017-18. EIC/RMP/HN/2017-18; Rev-0. https://www.eicindia.gov.in/Services/Pre-Compliance/PDF/RMP%202017-18%20Honey.pdf (accessed on 25 November 2017).
33. Kumari, B., Madan, V. K. and Kathpal, T. S., Status of insecticide contamination of soil and water in Haryana, India. *Environ. Monit. Assess*., 2008, **136**(1), 239–244.
34. Lari, S. Z., Khan, N. A., Gandhi, K. N., Meshram, T. S. and Thacker, N. P., Comparison of pesticide residues in surface water and ground water of agriculture intensive areas. *J. Environ. Health Sci. Eng.*, 2014, **12**(1), 11.
35. Ananda G. S. R. and Somashekar, R. K., Evaluation of pesticide residues in farmgate samples of vegetables in Karnataka, India. *Bull. Environ. Contam. Toxicol*., 2012, **89**(3), 626–632.
36. Harinathareddy, A., Prasad, N. B. L. and Devi, L. K., Pesticide residues in vegetable and fruit samples from Andhra Pradesh, India. *J. Biol. Chem. Res.*, 2014, **31**, 1005–1015.
37. Bedi, J. S., Gill, J. P. S., Aulakh, R. S. and Kaur, P., Occurrence and spatial distribution of pesticide residues in butter and ghee (clarified butter fat) in Punjab (India). *Environ. Monit. Assess*., 2016, **188**(2), 100.
38. Ramesh, B. K. and Selvanayagam, M., Pesticides pollution in water, sediment and fishes of Kolavai lake in Chengalpet, Tamil Nadu, India. *Int. J. Chem. Concepts*, 2015, **1**, 9–14.
39. Directorate of plant protection, quarantine and storage, Government of India. State-wise estimated demand of chemical pesticides during 2011–12 to 2015–16 and projected demand for 2016–17, 2017; http://ppqs.gov.in/divisions/pesticides-monitoring-documentation. (accessed on 14 March 2017).
40. Panseri, S., Catalano, A., Giorgi, A., Arioli, F., Procopio, A., Britti, D. and Chiesa, L. M., Occurrence of pesticide residues in Italian honey from different areas in relation to its potential contamination sources. *Food Control*, 2014, **38**, 150–156.

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