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Assessment of organochlorine and organophosphate pesticide residues in coriander and mustard floral honey by gas chromatography mass spectroscopy (GC MS/MS)

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Abstract
Coriander and mustard floral honey samples collected form Rajasthan State of India were analyses for 27 organochlorine and organophosphorus pesticides residues. An analytical procedure based on QuEChERS extraction with acetonitrile followed by gas chromatography-mass spectrometry was used and samples were analysed on GC MS/MS Triple quadrupole System (Shimadzu TQ 8037). The calibration curves constructed were linear over the range from 0.005-0.025 µg ml^{-1}. The correlation coefficient was ≥ 0.995 for all pesticides standard. The mean recoveries for extractions were 65- 115% for lower concentration range (0.005 mg kg^{-1}) and 70-120% for the higher concentration range (0.10 mg kg^{-1}) for pesticides analysed.
Three different pesticide, Dimethoate, Methyl parathion (organophosphate pesticides) and alpha-Endosulfan (organochlorine pesticides) were detected more than 0.001 mg kg^{-1} in coriander floral honey samples while in mustard floral honey samples Methyl parathion Famphur (organophosphate pesticides) and Eldrin Aldehyde were detected more than 0.001 mg kg^{-1} rest of pesticides were either not detected or detect below than 0.001 mg kg^{-1}.
The study reveals that coriander and mustard floral honey produced from Rajasthan, India do not contain pesticide residue and safe for human consumption.

Keywords: Honey, Coriander, Mustard, GC MS/MS, Pesticides residue, organochlorine, organophosphorus

Introduction
Pesticides are widely used in agriculture practices to produce food. Their residues may remain in very small amounts in fruits, vegetables, grains, and other foods. Pesticides uses way are vary from country to country. There are several evidences that pesticides or their residues have a negative impact on the human, animal health and environment.
To ensure food safety, most countries regulate the maximum level of each permitted pesticide residue (MRLs). These MRLs values also vary according to different food type's intake in different countries [1].
Organochlorine group of insecticides are considered very hazardous because of its ability to bioaccumulate into the food chain, to remain stable for many years and to move into the environment in every potential way (air, water, soil). While organophosphate compounds are not stable in the environment and are not bio-concentrated and they were detected rarely and at lower concentrations into beehive products [2].
The presence of pesticide residues in honey comes from the direct treatments of bees against the Varroa [3] or from indirect treatments, either through the use of pesticides in agriculture or as a result of environmental contamination [4]. Determination of pesticides residue in honey is essential, since the application of pesticides has increased significantly in recent decades to meet the demand for food production.
Honey bees travel long distances to collect nectar and pollen which will lead to exposure in contact to such pesticides [5]. For these reasons it is important to evaluate the presence of pesticide residues in honey to assure consumer protection.
There are many extraction methods have been reported in literature for the pesticide residues determination in honey based on solid-phase extraction (SPE) [6-9], solid-phase micro-extraction (SPME) [10]. These techniques are very good alternatives to the traditional liquid-liquid extraction (LLE) [11]. These techniques have results in new possibilities in sample processing and a lot of advantages such as reduction of extraction time and solvents volume.

The aim of this study was to analyse 27 pesticides residue in 10 samples of coriander and mustard floral honey originated from India, using QuEChERS extraction method and analyzed on GC-MS/MS for their detection.

Materials and Methods

Chemicals and Reagents: The CRMs standard of all pesticides was purchased from Dr Ehrenstorfer GmBH (Augsburg, Germany). The standard stock solutions were prepared in the concentration of 100 mg L^{-1} and were stored at -20 degree C. The calibration standards and working standards were prepared by diluting stock solution with acetonitrile on the day of analysis. HPLC grade acetonitrile, acetone and *n*-hexane (pro analysis) were obtained from sd Fine-Chem Ltd. HPLC grade Water was procured from LOBA Chemie Pvt. Ltd.

The QuEChERS kits containing 4 g of anhydrous magnesium sulphate, 1 g trisodium citrate dihydrate, 1 g of sodium chloride, and 0.5 g, trisodium citrate hemihydrate) and tubes with 900 mg anhydrous magnesium sulphate and 150 mg primary-secondary amine (PSA) for dispersive solid phase extraction (dSPE) were purchased from Restek (USA).

Sample Collection: The unifloral honey samples of coriander (*Coriandrum Sativum* L) and mustard from *Apis melifera* was collected from beekeepers of Kota and Baran district of Rajasthan state of India (Fig-1). Total ten samples were collected, five of each floral origin. All samples were stored at -20 degree C in the dark until analysis.

Sample Preparation: The honey samples (5 g) were thoroughly homogenized with 10 ml HPLC Grade water and 5 g of the homogenate was transferred into 50 ml polypropylene centrifuge tube, 10 ml HPLC Grade water was added to it and thoroughly shakes by hand for 5 minutes. Thereafter 10 ml of acetonitrile was added and mixed for 2 minutes, and the QuEChERS salt kit was added. Immediately the samples were shaken by hand for 2 minutes and subsequently centrifuged at 3500 rpm for 5 minutes. Thereafter, 6 ml of supernatant was transferred in a 15 ml dSPE polypropilene centrifuge tube. The tube was hand-shaken for 30 seconds and subsequently centrifuged at 3500 rpm for 5 minutes. Finally, 2 ml of supernatant was taken and filtered through Axiva 0.2 µm nylon syringe filter and transfer to GC vial for analysis [1, 12, 13].

The schematic diagram of modified QuECheRS/d-SPE for the determination of pesticides in honey was shown in fig-2.

Instrumentation: GC analysis was performed on a GC MS/MS Triple quadrupole System (Shimadzu TQ 8037). The injector temperature was 280 degree C. The samples were injected in the split mode. Split ratio was 1:60. Injection volume was 1 µl. A capillary column RTX-5sil MS (5% Diphenyl-95% Dimethyl Polysiloxane), 30 m x 0.25 mm x 0.25µm, was used. Career gas was He with constant flow of 1.90 mL min^{-1}.

The oven temperature was as follows: initial temperature of 70 degree C, held for 2 min, increased to 25 degree C min^{-1} up to 150 degree C, at 3 degree C min^{-1} up to 200 degree C, and then increased to 280 degree C at 8 degree C min^{-1} and held for 8 min. The MS ionization potential was 70 eV, and the temperatures were as follows: interface 280 degree C, Ion source 230 degree C [1, 12, 13].

Analysis was performed in MRM mode monitoring specific ions of each analyte as it is shown in Table 1.

Results and Discussion

Ten samples of coriander and mustard floral honey (five of each) were analyses for 27 organochlorine, and organochlorous pesticides residues. The calibration curves constructed were linear over the range from 0.005-0.025 µg ml^{-1}. The correlation coefficient was ≥ 0.995 for all pesticides standard.

The mean recoveries for extractions were 65- 115% for concentration range 0.010 mg kg^{-1} and for pesticides analysed. Fig-3 represents the Sample MRM chromatograms of honey sample spiked with pesticides at concentration level of 0.005 mg kg^{-1}.

Three different pesticide, Dimethoate, (0.001 mg kg^{-1}), Methyl parathion (0.001 mg kg^{-1}) (organophosphate pesticides) and alpha- Endosulfan (0.001 mg kg^{-1}) (organochlorine pesticides) were detected more than 0.001 mg kg^{-1} in coriander floral honey samples while in mustard floral honey samples Methyl parathion (0.002 mg kg^{-1}, 0.001 mg kg^{-1}) Famphur (0.001 mg kg^{-1}, 0.002 mg kg^{-1}) (organophosphate pesticides) and Eldrin Aldehyde (0.001 mg kg^{-1}) were detected more than 0.001 mg kg^{-1} rest of pesticides were either not detected or detect below than 0.001 mg kg^{-1}. The experimental results towards the MRL values were within the permissible limits Results of analyzed honey samples are shown in Table 2. GC-MS Chromatogram of all samples analyzed were presented from Fig-4 to fig-13

The most common pesticide detected in even three samples of honey is Parathion-methyl. It is an organophosphate pesticide used as an insecticide on crops. During flowering season of coriander and mustard crops insect population drastically increase. To reduce insects, farmers uses several types of insecticide and is possible the reason for the contamination of nectar collected by honeybees.

European commission set 0.05 mg kg^{-1} maximum residue limit (MRLs) for Parathion-methyl in honey. So we can say that honey produced from coriander and mustard floral origin in India is safe for human consumption. Several studies reported pesticides contamination in honey in other geographical location of world. Kurtagic and Copra-Janicijevic[1] evaluated 26 organochlorine, carbamate and orgaphosphorus pesticides residues in 10 samples of honey originate from Bosnia using QuEChERS method and detect Six different pesticides, propham and carbofuran (carbamate pesticides), methyl parathion, dichlorvos sulfotep and malathion (organophosphate pesticides) in analyzed honey samples.

Kartalovic *et al.* [12] investigated organochlorine residues in honey samples from Pannonian region in the Republic of Serbia. They were found presence of organochlorine pesticides in all samples of honey. But all of the detected concentrations of pesticides were below the maximum allowed value. Amoli *et al*. [14] analyzed miticide Amitraz residue in 70 honey and beeswax samples from Iran and found residue were below MRL in all sample.

Eissa *et al*. [15] study on 18 honey samples from Egypt indicated that organochlorine and organophosphorous pesticides residues were detected in 55.6% samples. Mujic, *et al*. [16] evaluated the health safety parameters of honey (meadow, chestnut, acacia, amphorae, and honeydew) produced from 18 different locations in the region of Una-Sana Canton in the north-western part of Bosnia and Herzegovina. They analyzed pesticides, heavy metals, radioactive elements, and antibiotic residues in 46 honey samples. Their result reveals that the content of pesticides was not found.

Yavuz *et al*. [17] analyzed 109 samples of honey from turkey and they were found residues of organochlorine pesticides Aldrin, cis-chlordane, trans-chlordane, oxy-chlordane, 2,4′-DDE, and 4,4′-DDE in all honey samples. In the 55 samples of 109, levels of organochlorine pesticide residues of oxy-chlordane were determined as higher than Turkish Alimentarius Codex maximum residual limits (MRLs).

Blasco *et al*. [13] collected fifty samples of honey from local markets of Portugal and Spain and were analyzed organochlorine, carbamate, and organophosphorus pesticide residues. Among them, 50% of the samples were contain gamma-HCH residue, followed by HCB in 32% samples and the other isomers of HCH (alpha-HCH and beta-HCH) in 28 and 26% of the samples, respectively. They were concluded that Portuguese honeys were more contaminated than Spanish ones.

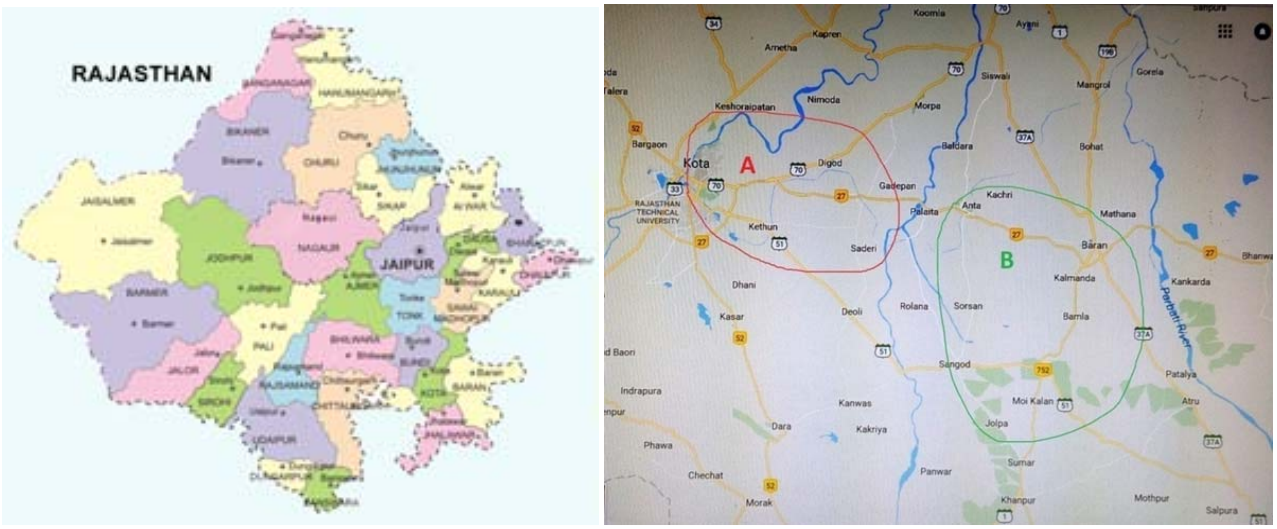

Fig 1: Location of the sample collection area in the Rajasthan State of India A (Kota) Sample 1-5, B (Baran) Sample 6-10

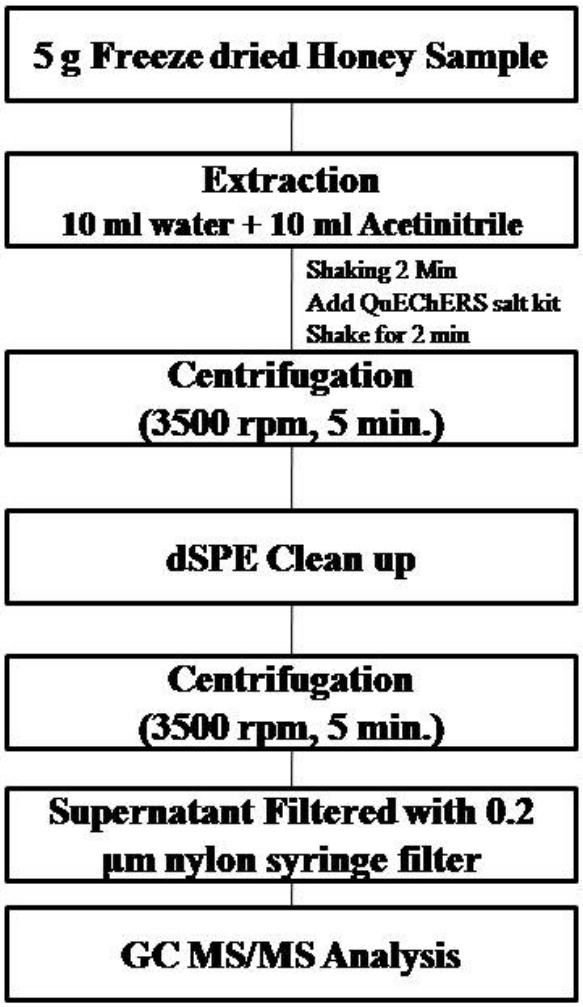

Fig 2: The procedure of modified QuECheRS/d-SPE for the determination of pesticides in honey

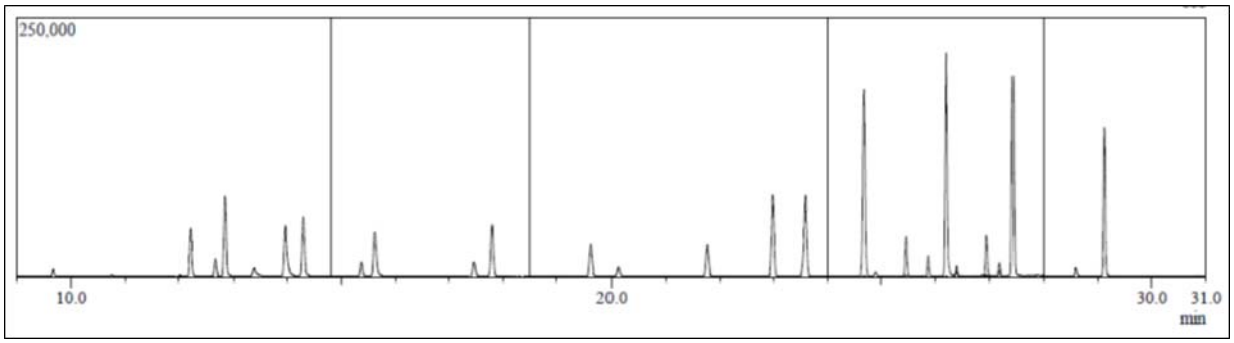

Fig 3: Sample MRM chromatograms of honey sample spiked with pesticides at concentration level of 0.005 mg/kg

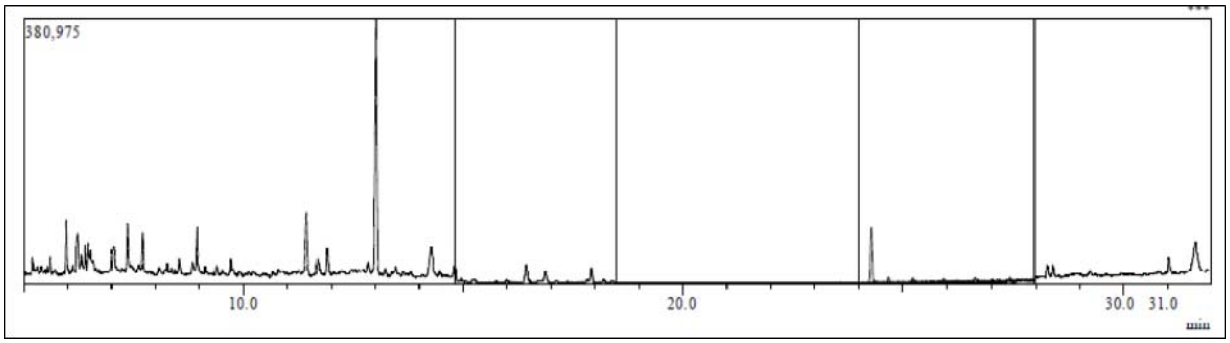

Fig 4: Chromatogram of Coriander floral Honey sample 1

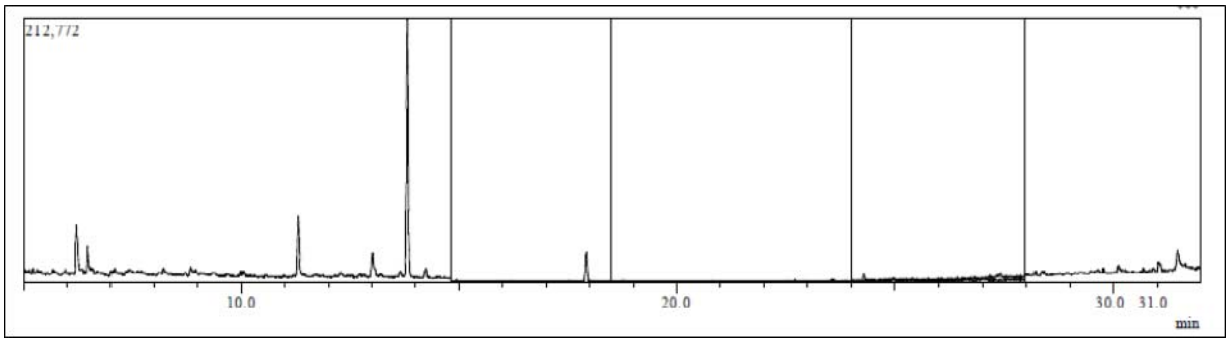

Fig 5: Chromatogram of Coriander floral Honey sample 2

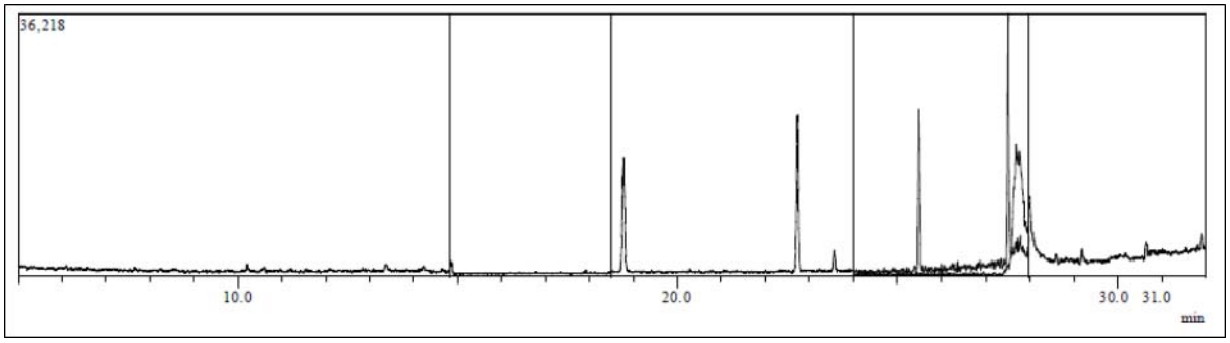

Fig 6: Chromatogram of Coriander floral Honey sample 3

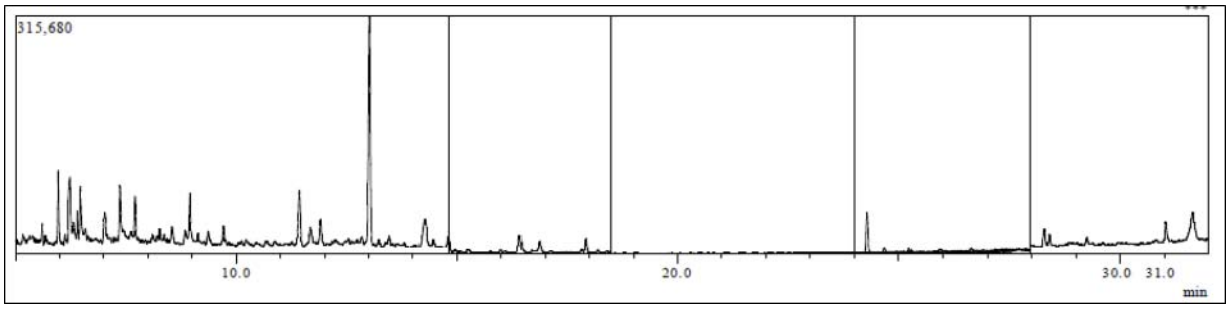

Fig 7: Chromatogram of Mustard floral Honey sample 4

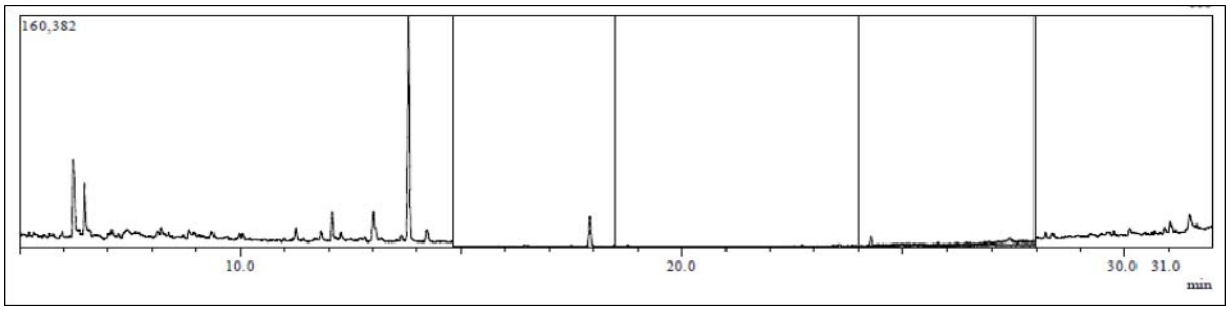

Fig 8: Chromatogram of Mustard floral Honey sample 5

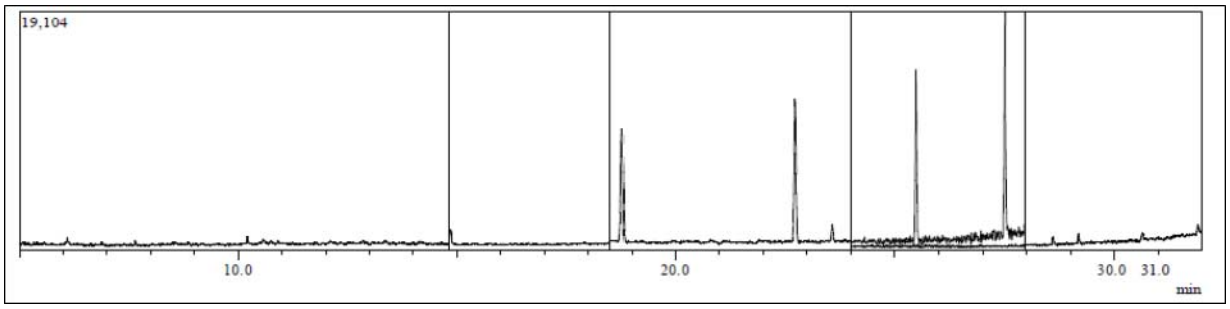

Fig 9: Chromatogram of Coriander floral Honey sample 6

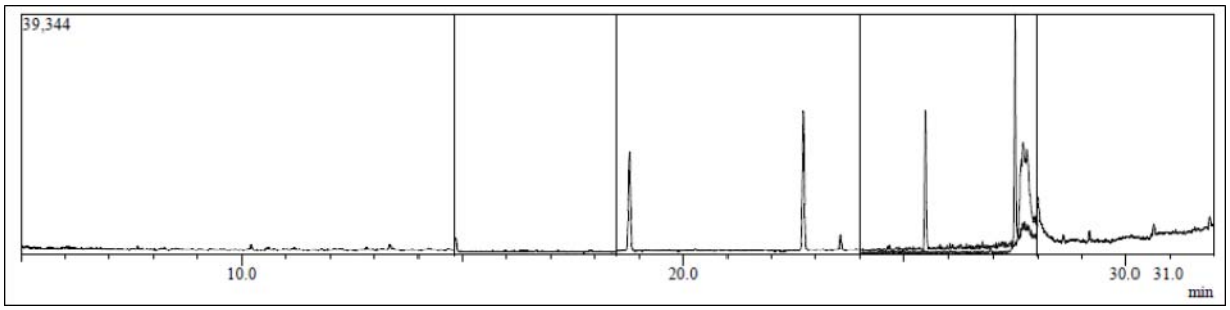

Fig 10: Chromatogram of Coriander floral Honey sample 7

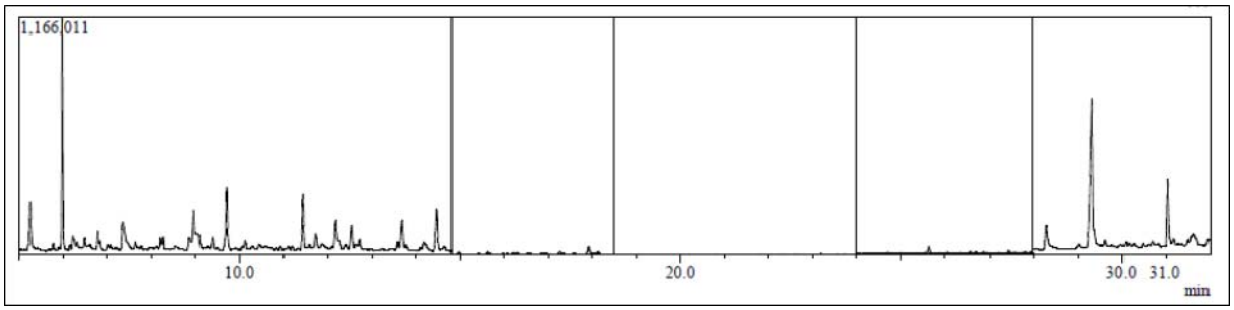

Fig 11: Chromatogram of Mustard floral Honey sample 8

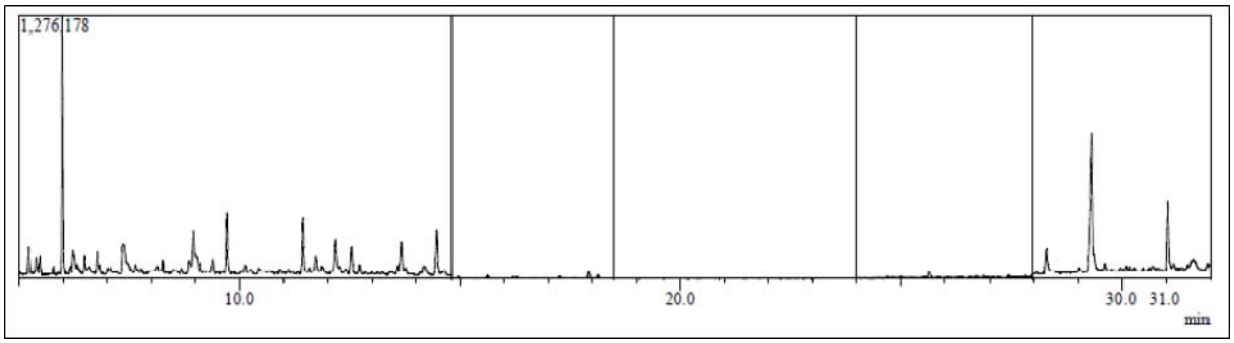

Fig 12: Chromatogram of Mustard floral Honey sample 9

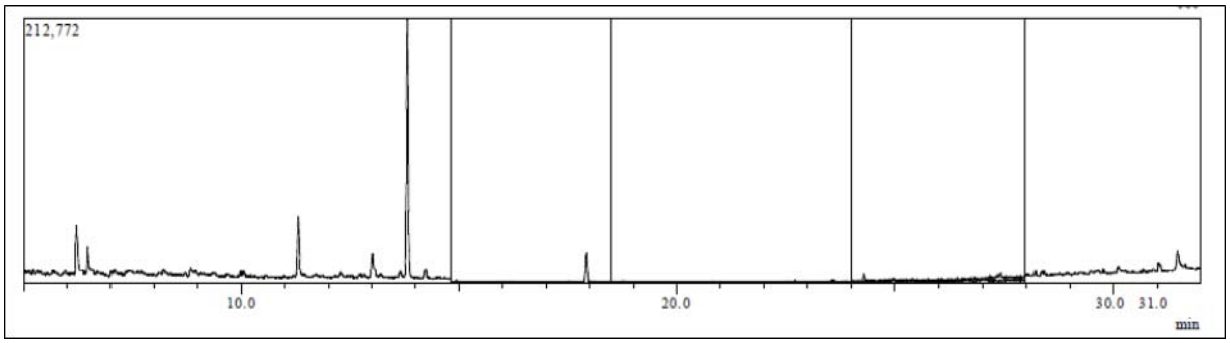

Fig 13: Chromatogram of Mustard floral Honey sample 10

Table 1: MRM Conditions of Pesticides Detected by GC-MS/MS

S. No.	Pesticide	RT	m/z	Ref. Ions
1	Sulfotep	12.227	322.00>202.00	322.00>294.00-322.00>174.00
2	Phorate	12.662	260.00>75.00	260.00>231.00-260.00>47.00
3	alpha-Hexachlorocyclohexane	12.842	218.90>182.90	218.90>144.90-218.90>109.00
4	Dimethoate	13.368	125.00>47.00	125.00>79.00-125.00>62.00
5	beta- Hexachlorocyclohexane	13.958	218.90>182.90	218.90>144.90-218.90>109.00
6	gamma-Hexachlorocyclohexane	14.294	218.90>182.90	218.90>144.90-218.90>109.00
7	Disulfoton	15.369	186.00>97.00	186.00>153.00-186.00>125.00
8	delta- Hexachlorocyclohexane	15.618	218.90>182.90	218.90>144.90-218.90>109.00
9	Parathion-methyl	17.456	263.00>109.00	263.00>136.00-263.00>246.00
10	Heptachlor	17.799	271.80>236.90	271.80>117.00-271.80>201.90
11	Aldrin	19.632	262.90>193.00	262.90>191.00-262.90>203.00
12	Parathion	20.115	291.10>109.00	291.10>137.00-291.10>81.00
13	Heptachlor epoxide	21.777	352.80>262.90	352.80>281.90-352.80>316.90
14	trans-Chlordane	22.988	372.80>263.90	372.80>265.90-372.80>336.80
15	cis-Chlordane	23.596	372.80>263.90	372.80>265.90-372.80>336.80
16	alpha-Endosulfan	23.554	338.90>160.00	338.90>266.90-338.90>195.90
17	p,p'-DDE	24.674	246.00>176.00	246.00>211.00-246.00>220.00
18	Dieldrin	24.701	276.90>241.00	276.90>170.00-276.90>172.00
19	Endrin	25.465	262.90>191.00	262.90>193.00-262.90>228.00
20	beta-Endosulfan	25.878	338.90>160.00	338.90>266.90-338.90>195.90
21	p,p'-DDD	26.205	235.00>165.00	235.00>199.00-235.00>99.00
22	Endrin Aldehyde	26.399	249.90>214.90	249.90>141.90
23	Famphur	26.949	218.00>109.00	218.00>79.00-218.00>186.00
24	Endosulfan sulfate	27.196	386.80>252.90	386.80>288.80-386.80>240.90
25	p,p'-DDT	27.436	235.00>165.00	235.00>199.00-235.00>99.00
26	Endrin Ketone	28.593	316.60>245.00	316.60>101.00
27	Methoxychlor	29.120	227.10>169.10	227.10>212.10-227.10>141.10

Table 2: Pesticides residue detected in honey samples

S. No	Type of Honey	Geographical Origin and Season of collection	Detected Pesticides
1	Coriander Floral	Kota, Rajasthan, India (2014)	Parathion-methyl
2	Coriander Floral	Kota, Rajasthan, India, (2014)	ND
3	Coriander Floral	Kota, Rajasthan, India, (2014)	Dimethoate, alpha-Endosulfan
4	Mustard Floral	Kota, Rajasthan, India, (2014)	ND
5	Mustard Floral	Kota, Rajasthan, India, (2014)	ND
6	Coriander Floral	Baran, Rajasthan India, (2015)	ND
7	Coriander Floral	Baran, Rajasthan India, (2015)	ND
8	Mustard Floral	Baran, Rajasthan India, (2015)	Parathion-methyl, Famphur
9	Mustard Floral	Baran, Rajasthan India, (2015)	Parathion-methyl, Endrin Aldehyde
10	Mustard Floral	Baran, Rajasthan India, (2015)	ND

ND=Not Detected

Table 3: Pesticides residue detected in coriander and mustard floral honey samples (μg kg^{-1})

S.N.	Name of Pesticides	Coriander Honey Sample					Mustard Honey Sample				
		1	2	3	4	5	1	2	3	4	5
51	Sulfotep	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	Phorate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	alpha-HCH	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	Dimethoate	ND	ND	1.07	ND	ND	ND	ND	ND	ND	ND
5	beta-HCH	ND	ND	ND	ND	ND	ND	ND	0.043	ND	ND
6	gamma-HCH (Lindane)	0.04	ND	ND	ND	ND	ND	ND	0.039	ND	ND
7	Disulfoton	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	delta-HCH	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	Parathion-methyl	1.23	0.03	0.01	0.01	0.01	ND	0.01	2.11	1.17	0.08
10	Heptachlor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	Aldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	Parathion	ND	ND	ND	ND	ND	ND	ND	ND	0.01	ND
13	Heptachlor-exo-epoxide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	trans-Chlordane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	cis-Chlordane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
16	alpha-E-osulfan	ND	0.15	1.7	ND	ND	ND	ND	ND	ND	ND
17	p,p'-DDE	0.39	0.01	0.01	0.44	0.03	0.00	ND	0.461	0.60	0.10
18	Dieldrin	0.21	0.35	ND	0.13	ND	ND	ND	0.024	0.13	ND
19	Endrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	beta-Endosulfan	0.20	0.16	0.16	ND	0.58	0.14	0.17	0.23	ND	ND
21	p,p'-DDD	ND	ND	0.04	0.01	0.10	0.01	0.01	0.10	0.04	ND
22	Endrin Aldehyde	0.11	ND	0.10	ND	0.36	0.07	ND	0.12	1.32	ND
23	Famphur	ND	0.06	0.05	ND	ND	0.002	ND	1.44	2.02	ND
24	Endosulfan sulfate	ND	ND	ND	ND	0.14	ND	ND	ND	ND	ND
25	p,p'-DDT	0.10	0.19	ND	ND	0.12	ND	ND	ND	0.70	ND
26	Endrin Ketone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
27	Methoxychlor	0.02	0.01	0.01	0.04	ND	ND	ND	ND	0.05	ND

ND=Not Detected

Conclusion

Based on results obtained in this work we can conclude that the coriander and mustard floral honey of Indian origin are safe for human consumption. In particular honey sample, pesticides were detected but all are within permissible limit. The results indicate that Indian honey is unpolluted as worlds other geographical origin honey contain some pesticides residue.

Conflicts of Interest: None

Acknowledgement

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