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Studies on pesticide contaminants in moringa and neem floral honey by gas chromatography and mass spectrometry (GC & MS)

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Abstract
Apis cerana indica, a kind of Indian honey bee. For the production of commercial honey and beekeeping, unifulral honey, fab is utilized in south India. The presence of 27 organochlorine and organophosphorus pesticide residues was examined in moringa and neem floral honey samples taken from VINO Beekeeping Center, Virali Patti Village Vattalgundu, Dindigul, Tamil Nadu, India. Using a gas chromatography-mass spectrometry system and an analytical technique based on QUECHER extraction with acetonitrile, honey samples were analyzed. The statistical correlation coefficient for all compounds was 0.994 (Shimadzu TQ 8037). The average extraction recoveries for the pesticides examined varied from 65% to 115% for lower concentration levels (0.005 mg kg^{-1}) and from 70% to 120% for higher concentration ranges (0.10 mg kg^{-1}).
The three pesticide Dimethoate, Methyl parathion and α-Endosulfan were found in moringa floral honey samples with the detection limit of 0.001 mg kg^{-1}, In contrast, neem floral honey samples included levels of methyl parathion famphur (organophosphate insecticides) and Eldrin aldehyde that were over the sample's detection limit.
According to the study, from neem (*Azadirachata indica)* and moringa (*Moringa oleifera Lam*). floral honey made by VINO Beekeeping Center, Virali Patti Village Vattalgundu, Dindigul, Tamil Nadu, India. Doesn't contain any pesticide residue and is safe to eat.

Keywords: honey, moringa, neem, GC, MS, pesticides residue, organochlorine, organophosphorus

Introduction
Insecticides are showing up in honey samples from all across the world, exposing bees and other pollinators to the harmful compounds on a large scale. In order to generate food, pesticides are frequently utilized in agricultural techniques. Fruits, vegetables, cereals, and other foods may still contain very minute amounts of their residues. The way that pesticides are used varies from nation to nation. The scientific studies shows that there are numerous indications that pesticides or their residues are harmful to the environment, human health, and animal health.
Most nations restrict the maximum level of each authorized pesticide residue to maintain food safety (MRLs). These MRL levels also change depending on the consumption of various food types in various nations [1].
The organochlorine category of insecticides is regarded as being extremely dangerous (air, water, soil). While organophosphate chemicals are not bioconcentrated, they are not persistent in the environment, and they have only seldom been found in small amounts in beehive products [2].
Honey may contain pesticide residues as a result of direct treatments given to bees to combat Varroa [3] brought about by the application of pesticides in the agricultural field or environmental contamination [4]. Since more pesticides have been applied in now days to fulfil the food demand production, it is crucial to determine the concentration of pesticide in honey.
Honey bees fly great distances to gather nectar and pollen, which exposes them to these chemicals [5]. To ensure consumer protection, it is crucial to assess whether pesticide residues are present in honey. The residues of the pesticide detection has been done using a variety of extraction techniques, including solid-phase extraction (SPE) [6-8] and solid-phase microextraction (SPME) [9]. These methods are excellent substitutes for the conventional liquid-liquid extraction (LLE) [10]. These methods have many benefits, including a reduction in extraction time and solvent volume, and they have opened up new opportunities for sample processing [11].
This studies objective was to identify 27 pesticide residues in 10 samples of moringa and neem floral honey that were produced in Tamil Nadu, India using the QuEChERS extraction method.

Materials and Methods
Sample Collection
Beekeepers in the VINO Beekeeping Center, Virali Patti Village Vattalgundu, Dindigul, Tamil Nadu, India. Provided samples of their unifloral honey (Apis cerana indica) made from neem (Azadirachata indica) moringa (Moringa oleifera Lam). Five samples of each floral origin totaled ten in total. Prior to analysis, all samples were kept in the dark at -20° C.

Sample Preparation
Five grammes of the honey samples were homogenized in ten milliliters of HPLC-grade water, and then five grammes of the homogenate were transferred in a fifty milliliter centrifuge tube. HPLC Grade water10 mL was then added to the tube, and it was then vigorously shaken by hand for 5 minutes.
The QuEChERS salt kit was then added after 10 ml of acetonitrile had been introduced and stirred for 2 minutes. The materials were immediately shaken by hand for 2 minutes and then centrifuged for 5 minutes at 3500 rpm.

15 mldSPE polypropylene centrifuge tube was then filled with 6 ml of supernatant. After shaking hands with the tube for 30 seconds, it was centrifuged at 3500 rpm for 5 minutes, then 2 ml of the supernatant was removed and transferred to a GC vial for analysis after being filtered with an Axiva 0.2 m nylon syringe filter. [12, 13].

In figure: 1, a schematic illustration of the modified QuEChеRS/d-SPE for detecting pesticides in honey is displayed.

Instrumentation

A GCMS/MS Triple Quadrupole System was used for the GC analysis (Shimadzu TQ 8037). It was 280°C within the injector. In the split mode, the samples were injected. 1:60 was the split ratio. It contained 1 l of injection. A capillary column measuring 30 m by 0.25 mm by 0.25 m and made of 5% diphenyl and 95% dimethyl polysiloxane was employed. A continuous flow of 1.90 mL min-1 was used for career gas.

The ovens temperature was as follows: 70°C at first, held for two minutes; 25°C per minute up to 150°C; 3 ° C per minute up to 200 °C; and then increased to 280°C at 8°C per minute, held for eight minutes. With same temperature and the MS ionization potential was 70 eV: Ion source 230 °C, interface 280 °C [14].

As stated in Table: 1, analysis was carried out in MRM mode while monitoring the specifics of each analyte.

Results and Discussion

Twenty seven residues of organochlorine and organochlorous pesticides were examined in five samples of moringa and samples of neem floral honey. Over the range of 0.005-0.025 gml-1, the calibration curves that were created were linear. The correlation coefficient for all pesticides was 0.995.

For concentrations between 0.010 mg kg-1 and for the pesticides studied, the mean extraction recoveries ranged from 65 to 115%. Figure: 3 shows the Sample MRM chromatograms of a honey sample that was injected with pesticides at a dosage of 0.005 mg kg-1.

Dimethoate, methyl parathion, and alpha-endosulfan were three different pesticides that were detected in samples of moringa flower honey in amounts more than 0.001 mg kg-1. Organophosphate pesticides Methyl parathion, Famphur, and Eldrin Aldehyde were found at concentrations over 0.001 mg kg-1; the other pesticides were either not found at all or were found at concentrations below 0.001 mg kg-1. The experimental results were within the allowed limits with respect to the MRL values.

Table 2 displays the findings of the analysis of the honey samples. All of the examined samples GC-MS chromatograms are shown in Figures: 4 to 12.

Parathion-methyl is the important pesticide found in three samples of honey.

A show the insecticide used on crops that is an organophosphate pesticide.

Insect populations dramatically rise during the flowering season of moringa and neem crops. Farmers use a variety of insecticides to get rid of insects, which could be why honeybees nectar is contaminated.

Maximum residual limits of 0.05 mg kg-1 for parathion-methyl in honey were determined by the European Commission. As a result, we can state that honey made in India from moringa and neem flowers is safe for ingestion. In other parts of the world, according to several investigations, honey may have been contaminated with pesticides. 10 samples of Bosnian honey were examined for the presence of 26 organochlorine, carbamate, and orgaphosphorus pesticide residues using the QuEChERS method by Kurtagic and Copra-Janicijevic1. They found six different pesticides, including methyl parathion, dichlorvos sulfotep, and malathion (organophosphate pesticides), as well as propham and carbofuran (carbamate pesticides)

Khan *et al*., [15] tested the presence of organochlorine residues in samples of honey from the Pannonian region of the Republic of Serbia. All samples of honey were confirmed to contain organochlorine pesticides. However, none of the pesticide concentrations that were found were higher than what was permitted. de Souza *et al*., [16]. Analysis of the miticide Amitraz residue in 70 samples of Iranian honey and beeswax revealed that the residue was below MRL in every sample. According to a study by Al Naggar *et al*., [17] on 18 samples of honey from Egypt, contaminants from organochlorine and organophosphorous pesticides were found in 55.6% of the samples. In the Una-Sana Canton region in the northwest of Bosnia and Herzegovina, Alibabić *et al*., [18] assessed the health safety criteria of 18 various places production of honey. They examined 46 samples of honey for the presence of pesticide, heavy metal, radioactive, and antibiotic residues. They didn't find any pesticides, according to their findings.

In all 109 samples of honey from Turkey that Mahmoudi *et al*., [19] tested, they discovered Aldrin, cis-chlordane, trans-chlordane, oxy-chlordane, 2,4′ -DDE, and 4,4′ -DDE were among the organochlorine pesticides they found in small amounts. Oxychlordane residue levels were found to be greater than Turkish Alimentarius Codex maximum residual limits in 55 out of 109 samples (MRLs). Fifty samples of honey were obtained from markets in Portugal and Spain by Khan *et al*., [20] who then tested them for organochlorine, carbamate, and organophosphorus pesticide levels. Gamma-HCH residue was found in 50% of the samples, followed by HCB in 32% and the other isomers of HCH (α-HCH and β-HCH), which were found in 28% and 26% of the samples, respectively. They came to the conclusion that Spanish honeys were less polluted than Portuguese ones.

Conclusion

We can draw the conclusion that Indian made moringa and neem floral honey is safe for eating based on the findings of this study. Pesticides were found in a honey sample in particular, however they are all within acceptable limits. The findings show that Indian honey from the unifloral plant, which is obtained in VINO Beekeeping Center, Virali Patti Village Vattalgundu, Tamil Nadu, India. is not as polluted as honey from other geographical origins,
which may include some pesticide residue.

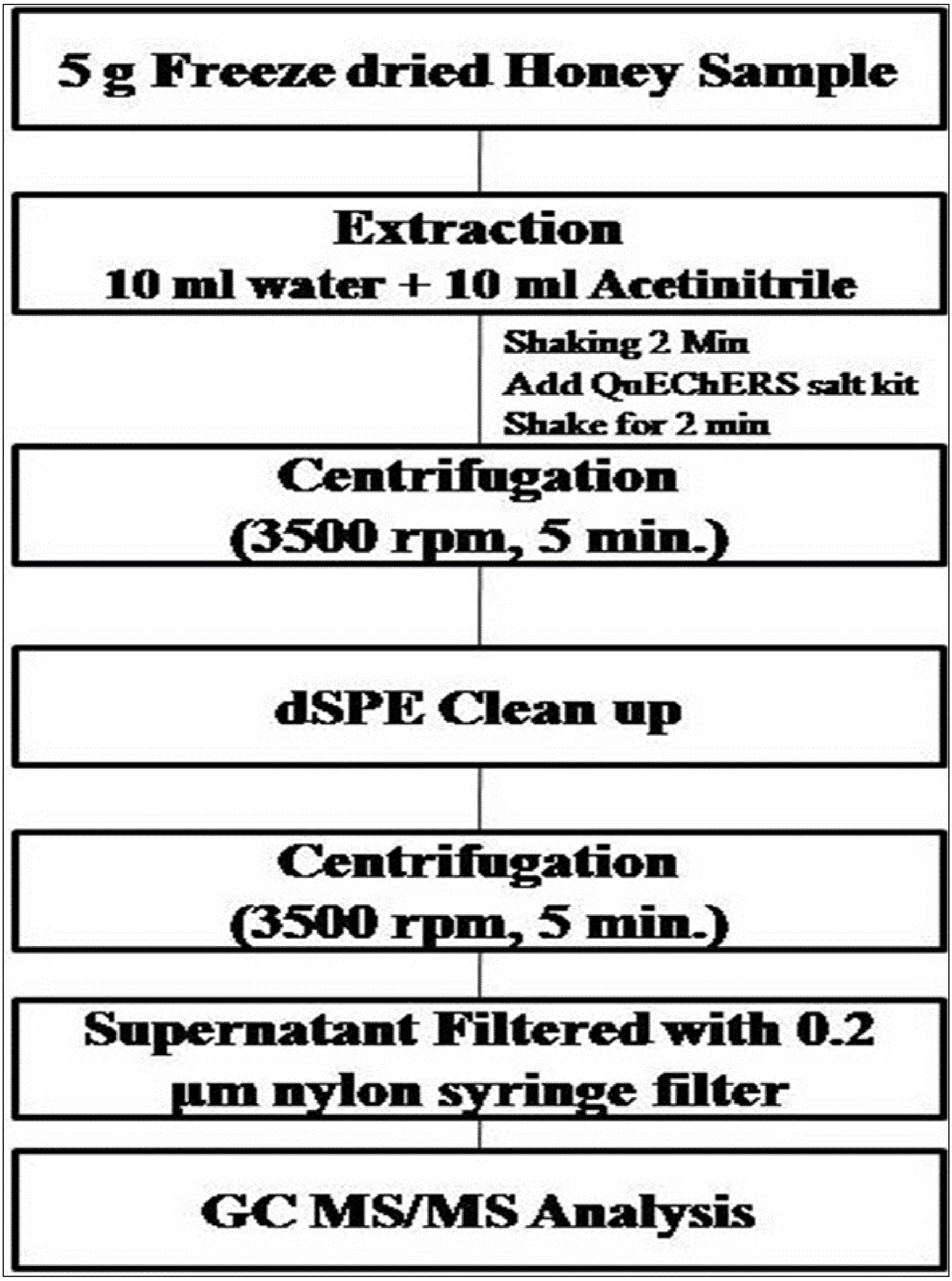

Fig 1: The method for determining the presence of pesticides in honey using modified QuECheRS/d-SPE

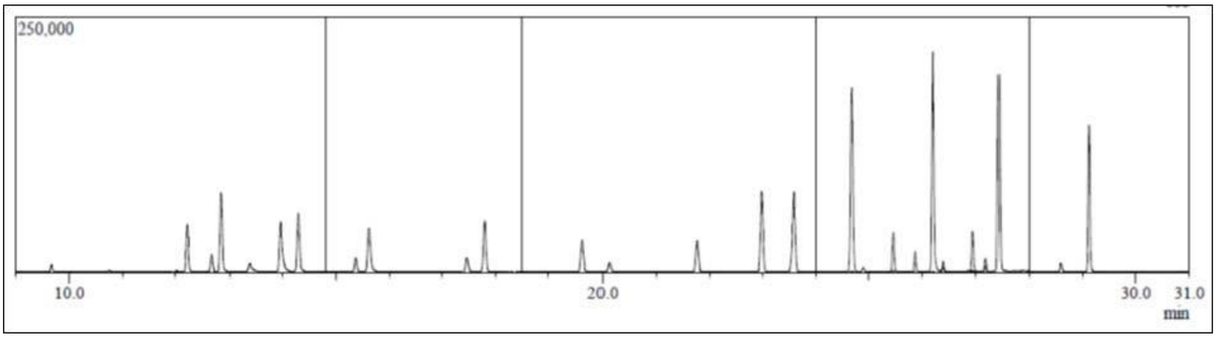

Fig 2: Pesticides were added to a honey sample at a concentration level of 0.005 mg/kg in the sample MRM chromatograms.

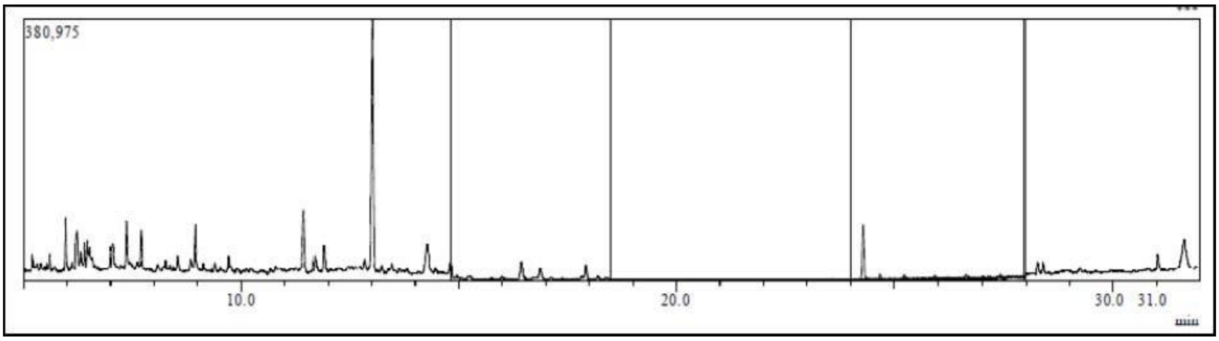

Fig 3: Honey sample 1 from the moringa flower chromatogram

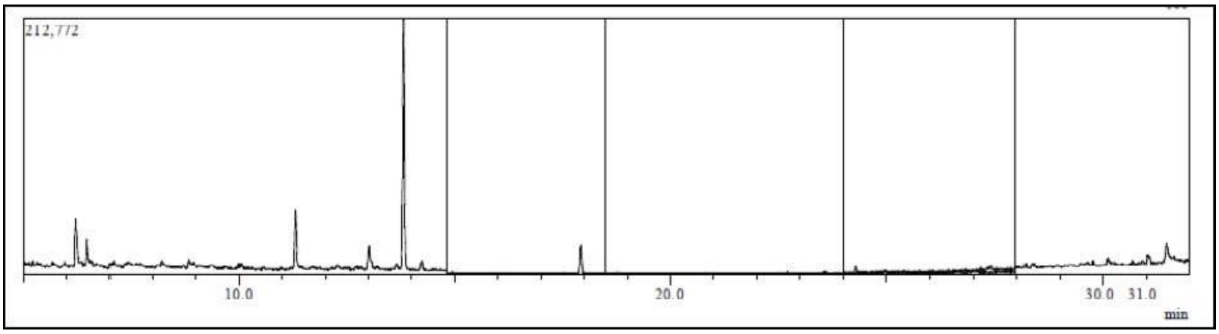

Fig4: Honey Sample 2 from the moringa flower's chromatogram

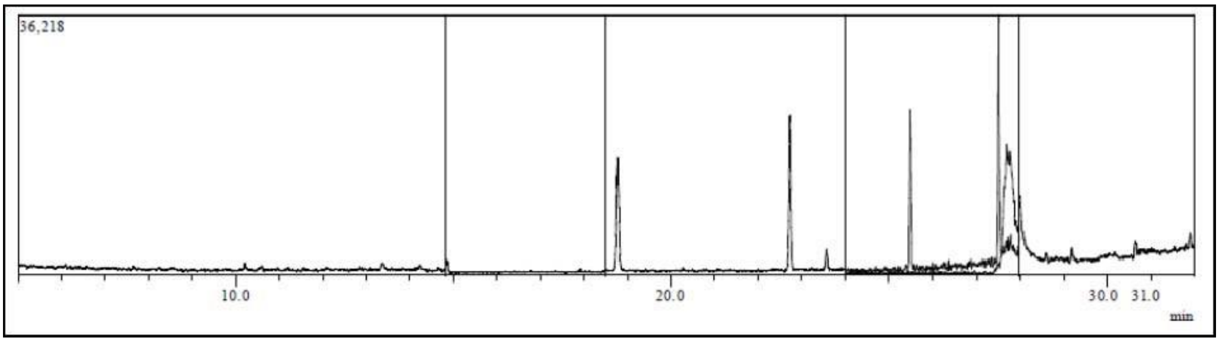

Fig 5: Sample 3 of the honey from the moringa flower's chromatogram

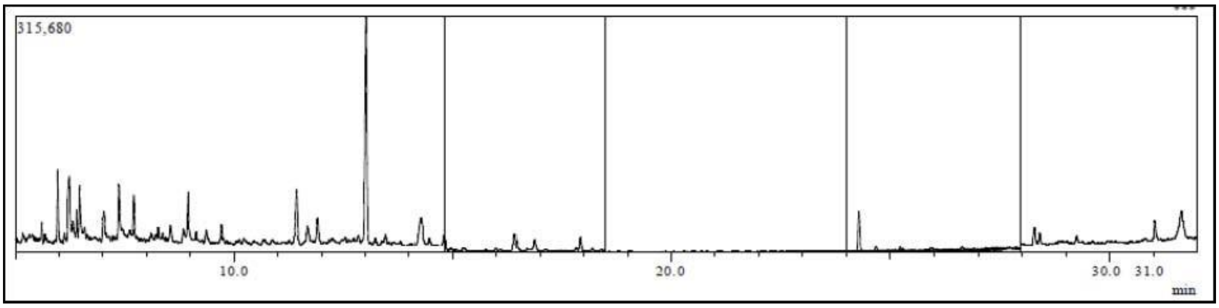

Fig 6: Honey sample 4 from the neem flower chromatogram

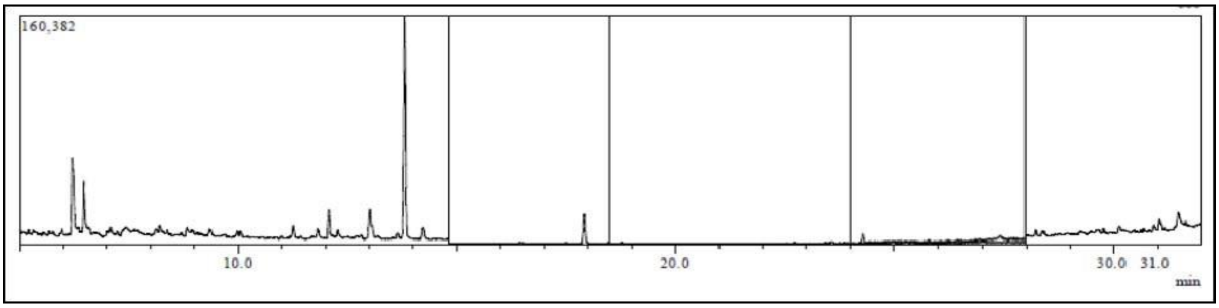

Fig 7: Honey sample 5 from the neem flower chromatogram

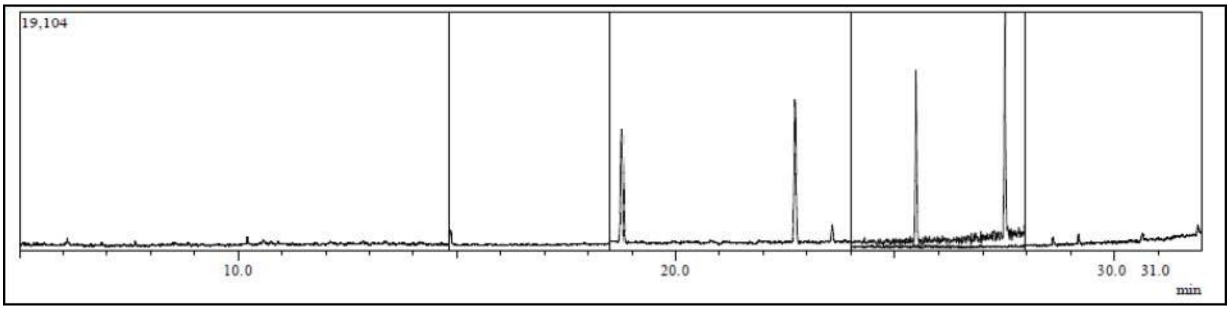

Fig 8: Honey sample 6 from the moringa flower chromatogram

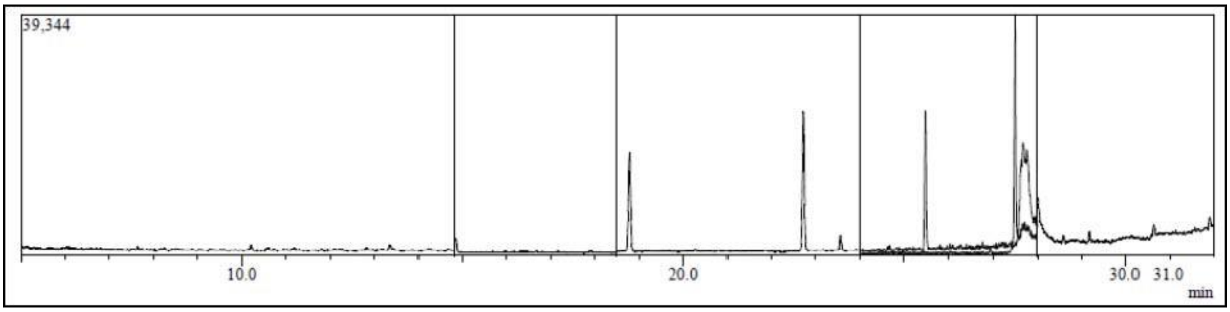

Fig 9: Honey sample 7 from the moringa flower chromatogram

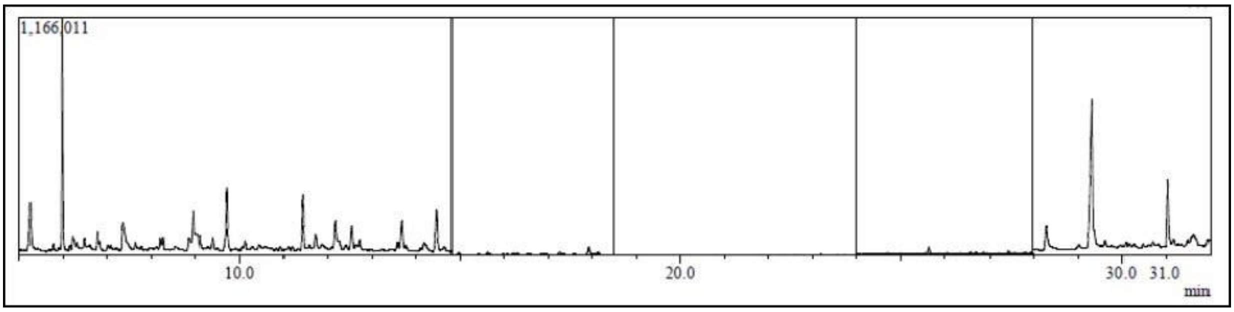

Fig 10: Honey sample 8 from the neem floral chromatogram

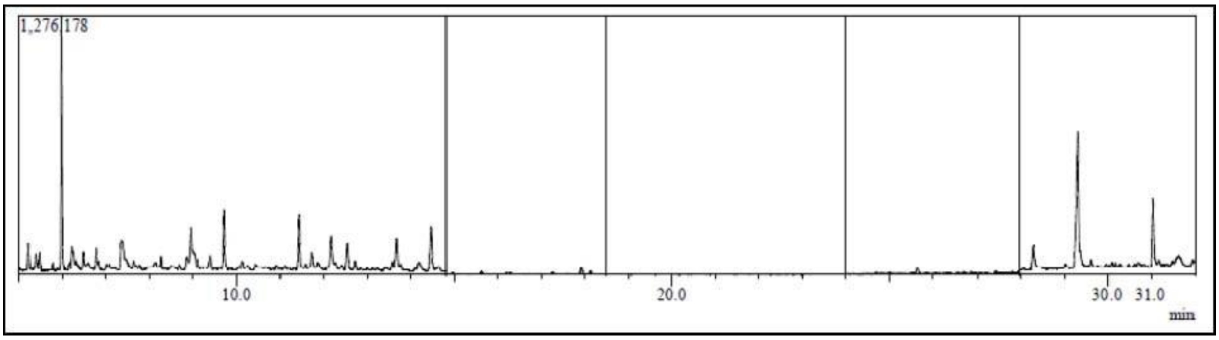

Fig 11: Honey sample 9 from the neem floral chromatogram

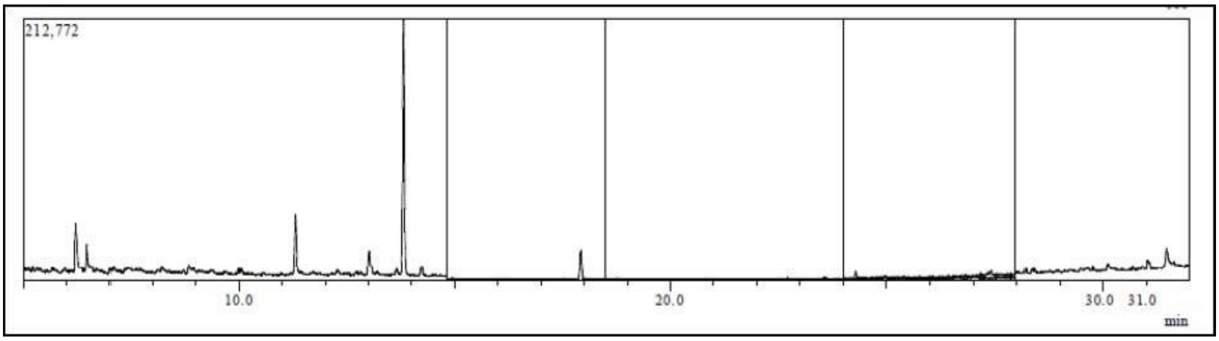

Fig 12: Honey sample 10 from the neem flower chromatogram

Table 1: Pesticides found by GC-MS/MS in MRM Conditions

S. No.	Pesticide	Retention Time	Mass (m)/ Charge (z)	Reference 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	α-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	β-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 residues found in honey samples

S. No	Honey Variety	Season of collection and Origin	Pesticides Found
1	Moringa Floral	Vattalgundu, Tamil Nadu, India (2020)	Parathion-methyl
2	Moringa Floral	Vattalgundu, Tamil Nadu, India, (2020)	-
3	Moringa Floral	Vattalgundu, Tamil Nadu, India, (2020)	Dimethoate, alpha-Endosulfan
4	Neem Floral	Vattalgundu, Tamil Nadu, India, (2020)	-
5	Neem Floral	Vattalgundu, Tamil Nadu, India, (2020)	-
6	Moringa Floral	Vattalgundu, Tamil Nadu, India, (2021)	-
7	Moringa Floral	Vattalgundu, Tamil Nadu, India, (2021)	-
8	Neem Floral	Vattalgundu, Tamil Nadu, India, (2021)	Parathion-methyl, Famphur
9	Neem Floral	Vattalgundu, Tamil Nadu, India, (2021)	Parathion-methyl, Endrin Aldehyde
10	Neem Floral	Vattalgundu, Tamil Nadu, India, (2021)	-

(- = Not Detected)

Table 3: Pesticide residues were found in samples of floral honey from moringa and neem trees (g kg^{-1})

S. No.	The pesticides' names	Sample of Moringa honey					Sample of Neem honey				
		1	2	3	4	5	1	2	3	4	5
1	Sulfotep	-	-	-	-	-	-	-	-	-	-
2	Phorate	-	-	-	-	-	-	-	-	-	-
3	α-Hexachlorocyclohexane	-	-	-	-	-	-	-	-	-	-
4	Dimethoate	-	1.07		-	-	-	-	-	-	-
5	β- Hexachlorocyclohexane	-	-	-	-	-	-	0.043	-	-	-
6	γ- Hexachlorocyclohexane (Lindane)		0.04	-	-	-	-	0.039	-	-	-
7	Disulfoton	-	-	-	-	-	-	-	-	-	-
8	δ- Hexachlorocyclohexane	-	-	-	-	-	-	-	-	-	-
9	Parathion-methyl	0.01	1.23	0.03	0.01	0.01	-	0.08	1.17	2.11	0.01
10	Heptachlor	-	-	-	-	-	-	-	-	-	-
11	Aldrin	-	-	-	-	-	-	-	-	-	-
12	Parathion	-	-	-	-	-	-	-	0.01	-	-
13	Heptachlor-exo-epoxide	-	-	-	-	-	-	-	-	-	-
14	trans-Chlordane	-	-	-	-	-	-	-	-	-	-
15	cis-Chlordane	-	-	-	-	-	-	-	-	-	-
16	α-Endosulfan	-	1.7	0.15	-	-	-	-	-	-	-
17	p,p'-DDE	0.01	0.01	0.39	0.44	0.03	-	-	0.60	0.461	0.10
18	Dieldrin	0.35	0.21	-	-	0.13	-	-	0.13	0.024	-
19	Endrin	-	-	-	-	-	-	-	-	-	-

20	β-Endosulfan	0.16	0.16	0.20	-	0.58	0.17	0.14	-	0.23	-
21	p,p'-DDD	-	-	0.04	0.01	0.10	0.01	0.01	0.10	0.04	-
22	Endrin Aldehyde	0.10	-	0.11	-	0.36	0.07	-	0.12	1.32	-
23	Famphur	-	0.05	0.06	-	-	-	0.02	2.02	1.44	-
24	Endosulfan sulfate	-	-	-	0.14	-	-	-	-	-	-
25	p,p'-DDT	0.11	-	0.19	0.12	-	-	-	-	-	0.70
26	Endrin Ketone	-	-	-	-	-	-	-	-	-	-
27	Methoxychlor	0.01	0.02	0.04	0.01	-	-	-	-	-	0.05

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