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Pesticide Residue Contamination Study in Chilli in Karnataka by LC-MS/MS and GC-MS/MS Analyses

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Abstract: Pesticide residues in food and crops are a direct effect of use of pesticides to crops growing in the field. Large quantity of pesticides is being used to control various pests, which can enter into food cycle affecting other living beings other than pests. The present study aims to evaluate the level of pesticide residue contamination affected in chilli crop during the cultivation in Karnataka. Pesticide Residue quantification was done using validated LC-MS/MS and GC-MS/MS Techniques. The method was applied for the analysis of the chilli samples and results showed that most of the samples have detectable pesticide residues. The residues of Acetamiprid and Fenzaquin were detected in more number of samples. Out of the 40 chili samples, 33 samples were found to be contaminated with pesticide residues but not more than the MRL values.

Keywords: Pesticide residues, Chilli, LC-MS/MS, GC-MS/MS, APEDA

I. INTRODUCTION

Chilli (*Capsicum annum L.)* which belongs to the "*Solanaceae*" family is most widely used and universal spice of India. The nutritive value of chilli is excellent. They are rich in vitamins, especially in vitamin A and C. Every 100 gms of dried pods yield about 160 calories energy through 36 gms carbohydrates, 18 gms proteins, 16 gms fat, 480 mg calcium, 3.1 mg. phosphorous, 31 mg iron, 2.5 mg nicotinic acid, 640 I.U. vitamin 'A' and 40 mg vitamin 'C' (11). Chilli has high medicinal value due to the abundance of availability of carotenoids, capsaicinoids , oleoresins, and mineral content . Most of the studies have demonstrated that consumption of chilli rich diets, increases in energy expenditure and oxidation of fat, and also it helps in the curing of many diseases (3).

Chilli is the universal spice of India. India is one of the largest producers of chilli followed by China, Thailand, Ethiopia and Indonesia and Indian chilli is considered to be famous for two important commercial qualities of color and pungency levels. Indian chilli is mainly exported to Asian countries like Vietnam, Thailand, Sri Lanka, Bangladesh and U.A.E. (14). India accounts for 1.2 million tonnes of production annually, and is the largest producer in terms of international trade, exporting 20 per cent of its total production.(11) India is the world's largest producer, consumer and exporter of chilli peppers. Guntur in Andhra Pradesh produces 30% of all the chilies produced in India and the state of Andhra Pradesh as a whole contributes 75% of India's chili exports.(12). The other major states growing chilli in the country are Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal. The productivity is high in the states of Andhra Pradesh and Tamil Nadu, where chilli is grown under irrigation than in Maharashtra and Karnataka, where the crop is raised mainly under rain-fed situations.(13).

Population explosion has resulted in tremendous pressure on the increased need of agricultural productivity. Ever increasing demand for food resulted in using chemical pesticide across the world to improve the quality and yield and to extend the storage life of food crops 1. Reports indicated that 900 chemicals are used worldwide, legally and illegally, in various food products and for the treatment of crops and vegetables 2. Applied chemicals and degradation product may remain as residues in the agricultural products, which is a concern for humans and also for the export. (6) Aflatoxin and pesticide residues are the two major constraints in increasing our exports. Buyers expect a high degree of hygiene and sanitation in processing and preparation of chillies for export. (14).

Although modern polar pesticides like organophosphorus and carbamates that replaced classical organochlorine pesticides are less persistent. It is not possible to control the residues of pesticides in food commodities; hence, these compounds will accumulate in the human body after consumption through diets. Hence, to overcome the effects of pesticides on different groups, the uniform maximum residue limits (MRL's) was established as 0.01 mg/kg for any pesticides. (3)

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Analysis of pesticides is challenging as they are often present at low concentrations of pesticides in complex matrices. To achieve the low quantification of pesticide residues, the sample preparation method should be effective. For the rapid analysis of pesticide residues, traditional methods such as Soxhlet extraction, solvent extraction, Liquid-Liquid extraction etc are less commonly used due to laborious, more time and solvent consumption and tedious experimental procedure. Therefore, modern sample preparation methods and clean-up steps have been introducing in food analysis.

The objectives of the present study are to identify and assess the pesticide levels in the chilli in Karnataka from the point view of safety to consumers, comparing the observed residue levels with APEDA standards, enumerate the most prevalent pesticides to facilitate extension workers to adopt safe pesticide usage in crop cultivation.

II. EXPERIMENTAL DETAILS

2.1 Reagents and chemicals: All pesticide standards were obtained from Fluka (Buchs,Switzerland), Sigma Aldrich and Dr. Ehrenstorfer GmbH (Augsburg,Germany). Stock standard solution of each reference standard were prepared (100 ppm) by dissolving in methanol. Prepared stock solutions were kept at -20 ±2 °C and were stable for one year. A single composite standard solution was prepared by combining aliquots of each stock solutions and diluting with a methanol to obtain a final concentration of 1ppm. This is stable for six months when stored in a refrigerator at 2 ±2 °C. A mixed external calibration standard solution set of 5 ppb to 100 ppb was prepared in 100 mL methanol (LC-MS/MS grade) and in n-Hexane (LC-MS/MS grade) for GC-MS/MS and kept in a refrigerator at 2 ±2 °C. These solutions are stable for three months. Organic solvents were acquired from Baker (Griesheim, Germany). Vaterx Water was procured. Other chemicals were of LC - MS grade and were used without any further purification. When not in use, all standard solutions were stored in the dark at 4^{o}C.

2.2 Sample preparation and extraction: Chilli Samples were collected randomly from the different fields of Karnataka Region. The selection of potential active ingredients to be screened was based on the list as per APEDA for the export of chilli. For different chemical classes of pesticides, LC-MS/MS and GC-MS were separately used. Chilli samples were reduced to a mash with mixer in order to obtain a very homogeneous sample. To a small portion of crushed and homogenized sample water was added followed by ethyl acetate and sodium sulphate and was homogenized followed by centrifugation. For LC MS Analysis, organic supernatant was collected and evaporated to dryness under gentle stream of nitrogen. The dried extracts were then dissolved in methanol and filtered on 0.45micron nylon membrane before injection into the LC–MS/MS system. For GC-MS system, the dried organic supernatant was dissolved in n-Hexane.

2.3 LC–MS/MS Conditions

Instrument	LC-MS/MS 6460 Agilent Triple Quad
Column	Hypersil Gold
Mobile Phase A	5 mM Ammonium Formate in 20:80 (Methanol:Water)
Mobile Phase B	5 mM Ammonium Formate in 90:10 (Methanol:Water)
Injection volume	5 µl

2.4 GC–MS Conditions

Instrument	Shimadzu GC-MS Triple Quad
Column	Zebron ZB-XLB
Injection mode	Splitless
Split Ratio	5.0
Column Flow	1.5ml/min

2.5 Method Validation: The method validation was done for both the instruments for Specificity, LOQ, Linearity, Precision and Accuracy. For specificity the reference compound was injected at LOQ in six replicates separately and six replicates of sample blank solution, negative for residual pesticides in fruits and vegetables were injected. The solvent is injected to the column to check the interference from the solvent blank at the retention time of the peak. The LOQ was determined from linearity of residual pesticides experiment wherein lower concentrations of each Residual pesticide are analyzed. The solutions containing Residual pesticides at LOQ concentration are prepared and injected in six replicates separately and reported average S/N ratio and % RSD of response. For Linearity, solutions

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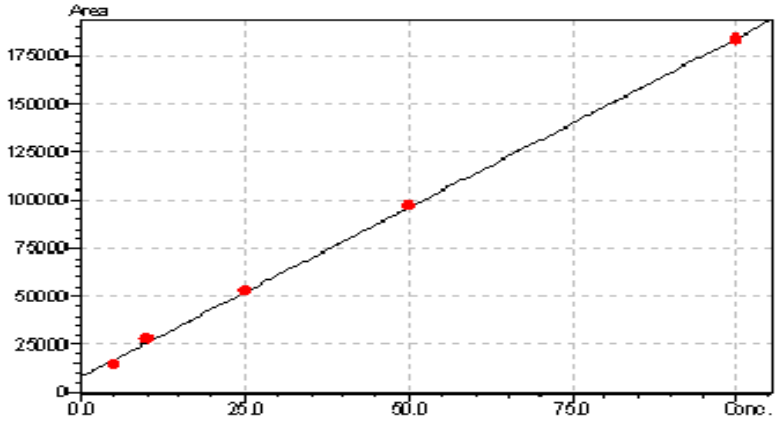
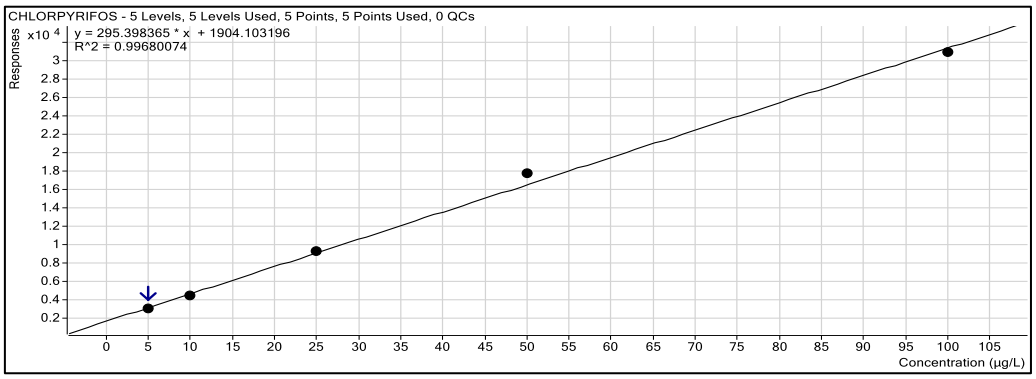
of Lower concentrations of mixed Residual Pesticides standard are prepared and each concentration is injected on the same day. The data generated is analyzed by linear regression analysis to calculate the slope, intercept and the correlation coefficient. Repeatability is carried out during experiment by injecting the six numbers of replicate injections of mixed residual pesticides standard at LOQ and 10 LOQ. Reproducibility is carried out during experiment by injecting the six number of replicate injections of the mixed residual pesticides standard at LOQ and 10 LOQ for day 1,day-2 and day-3.The RSD for six replicated injections of residual pesticides at LOQ level and 10 LOQ level for different pesticides are established for three different pesticides. The accuracy of the method is determined by spiking the known impurities at LOQ (10µg/l, 50µg/l, and 100 µg/l).

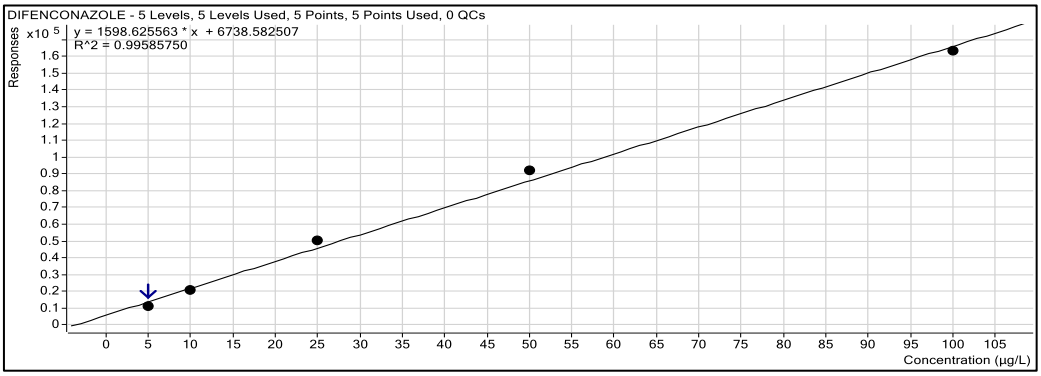
III. RESULTS AND DISCUSSION

3.1 Method Validation: The method was found specific for residual pesticides. No interference was observed by analyte or compound at the retention time of the same analyte in the blank.LOQ was found to be 10µg/l of RS concentration and the method is linear over the range of 0.5 LOQ, LOQ, 2 LOQ,5 LOQ and 10 LOQ with correlation coefficient greater than 0.99.Recovery also was within the prescribed limit (70% to 120%) at LOQ,5 LOQ and 10 LOQ. Linearity graphs of the pesticide residues detected in the samples are as given in Fig 1.



Cypermethrin





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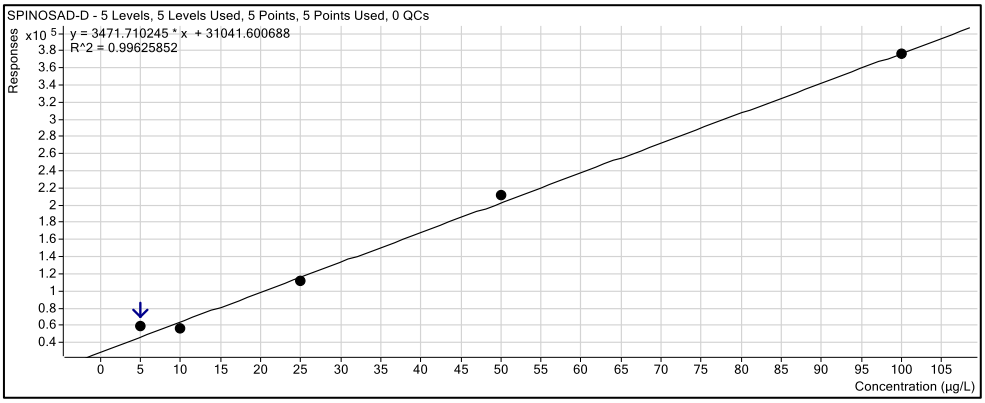

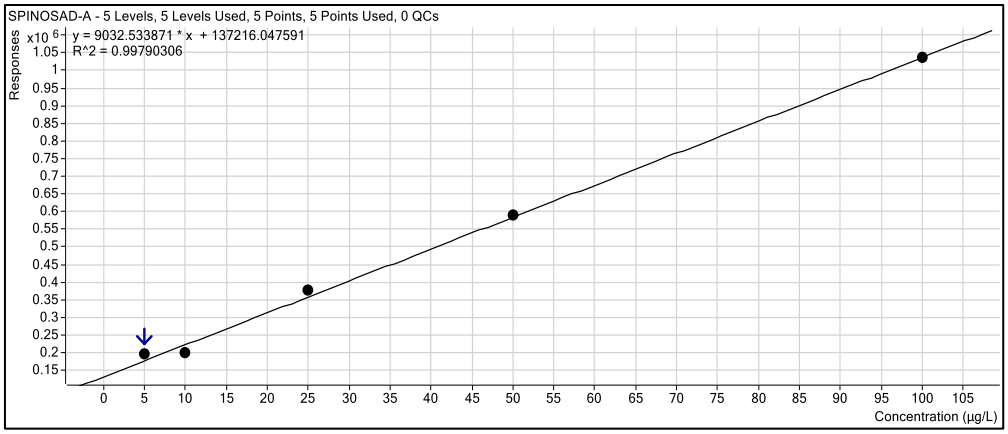

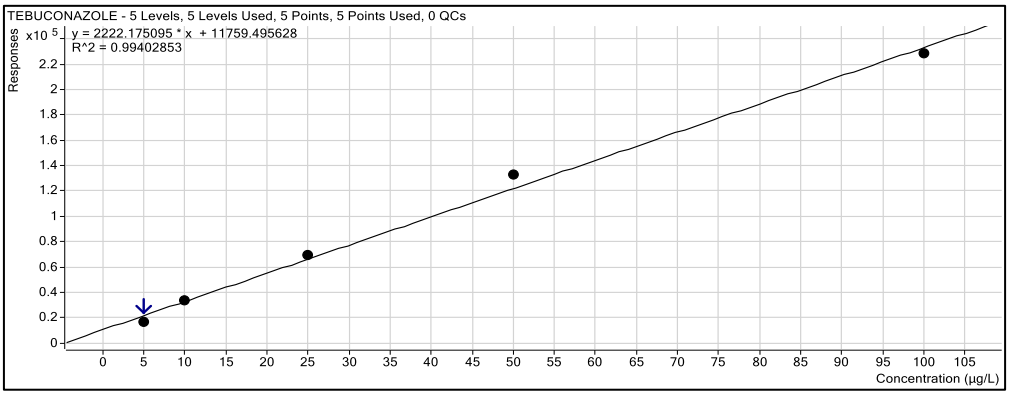

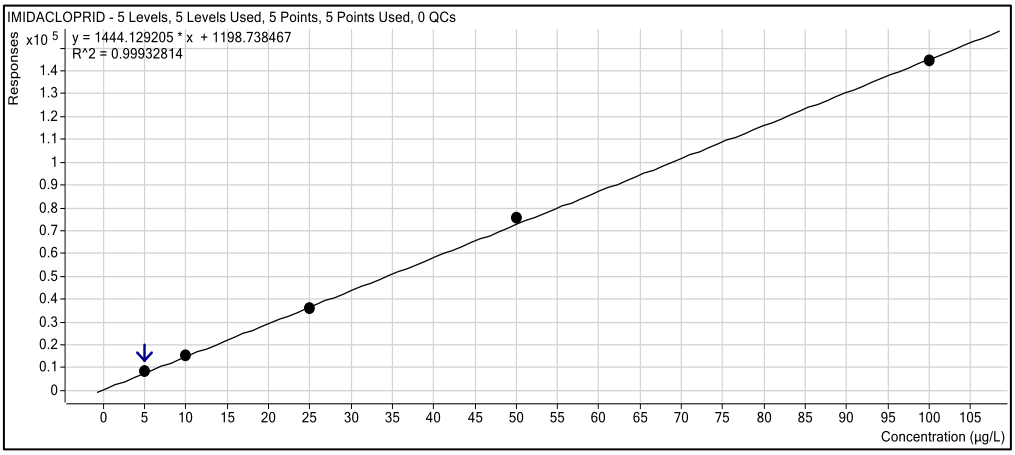

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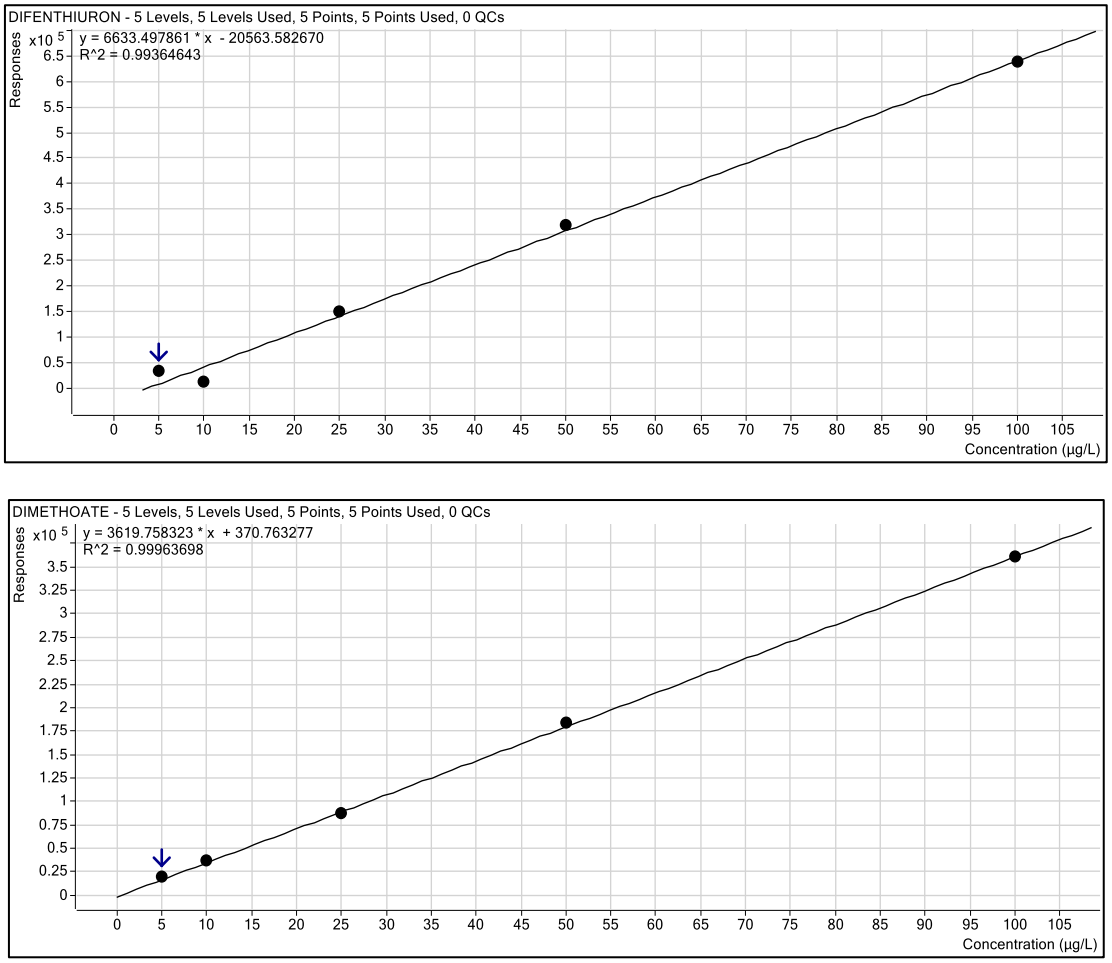

Fig 1. Linearity graphs of the pesticide residues

3.2 Pesticide residues: The optimized method was used for analysis of 40 samples for the complete pesticide residues as per APEDA for exporting. Results showed that most of the chili samples contained detectable pesticide residues (Tables II). The residues of acetamiprid and fenazaquin were detected in more number of samples. Cypermethrin, were not found in any of the samples. Out of the 40 chilli samples, only 7 samples did not contain any pesticide residues and 33 samples were found to be contaminated majorly with 15 residues as indicated in Table 1.

Table I. Incidence of pesticide residues in 33 chilli samples

Pesticide No.	Name of Pesticides	Application	No. of Positive Chilli Samples
1.	Difenthiuron	Insecticide	2
2.	Cypermethrin	Insecticide	0
3.	Spinosad-D	Pesticide	1
4.	Spinosad-A	Pesticide	1
5.	Imidacloprid	Insecticide	2
6.	Tebuconazole	Fungicide	1
7.	Dimethoate (including Omethoate)	Insecticide	1
8.	Spinosad (A+D)	Pesticide	10
9.	Chloropyrifos	Pesticide	1
10.	Difenconazole	Fungicide	3
11.	Fenazaquin	Pesticide	15
12.	Acetamprid	Insecticide	15
13.	Chlorantrinipole	Insecticide	3
14.	Metalaxyl	Fungicide	2
15.	Azoxystrobin	Fungicide	9

Three pesticides namely Fenazaquin, Acetamprid and Spinosad were found in more samples out of 40 samples. Fenazaquin and Acetamprid are found to not to pose threat for human health and is not likely to be carcinogenic.

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Spinosad has an exceptionally good toxicity profile, and the U.S. EPA has classified spinosad as a "reduced -risk pesticide." In addition, the amount applied for fruit -fly control, and the manner in which it is applied (i.e., in a bait), should not expose members of the public to a significant risk. The small amount of spinosad people could contact as it is applied, either from the ground or by air, would not be toxic for humans. All other pesticides were found in very less number of samples.

The MRM compound of reference pesticides standard by LC-MS /MS and GC-MS/MS is as indicated in Fig 2 and Fig 3.

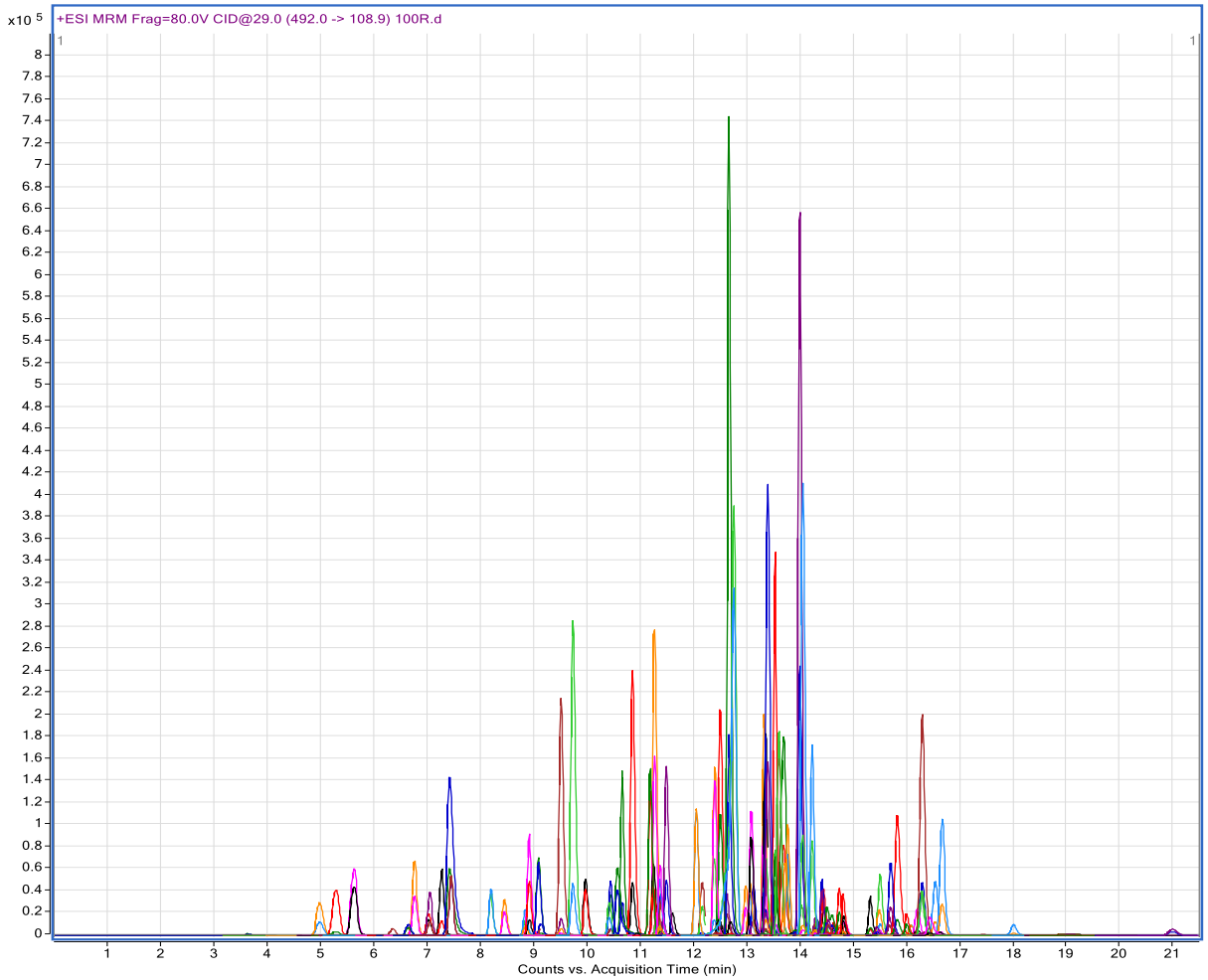

Fig 2: MRM Compounds of reference pesticides standard by LC-MS/MS

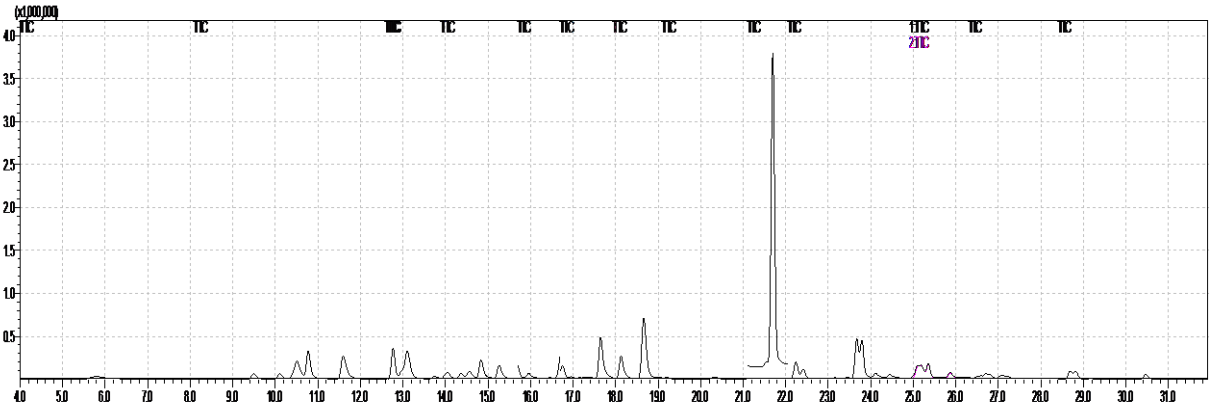

Fig 3: MRM Compounds of reference pesticides standard by GC-MS/MS

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Table II – Pesticide residue level in the chilli samples

Pesticides No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
EU-MRLS	0.01	0.5	2	2	0.01	0.6	0.02	2	0.01	0.8	0.5	0.01	1.00	0.5	3
S1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02
S4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	0.01	ND
S5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	0.01	ND
S6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.03	0.01	ND	ND	ND
S7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.03	0.01	ND	ND	ND
S8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.04	ND	ND	ND
S10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.01	ND	ND	ND
S11	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.04	ND	0.1	ND	ND	ND
S12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S13	ND	ND	ND	ND	ND	ND	ND	ND	0.01	ND	ND	ND	ND	ND	ND
S14	ND	ND	ND	ND	ND	ND	ND	0.05	ND	ND	ND	0.02	ND	ND	0.02
S15	ND	ND	ND	ND	ND	ND	0.01	ND	ND	ND	ND	ND	ND	ND	ND
S16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.08	ND	ND	0.09
S17	ND	ND	ND	ND	ND	ND	ND	0.07	ND	ND	0.06	ND	ND	ND	ND
S18	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.04	ND	ND	ND
S20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.05

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Pesticides No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
EU-MRLS	0.01	0.5	2	2	0.01	0.6	0.02	2	0.01	0.8	0.5	0.01	1.00	0.5	3
S21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	0.04	ND	ND	ND
S23	ND	ND	ND	ND	ND	0.04	ND	ND	ND	0.04	ND	ND	ND	ND	ND
S24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	0.04	ND	ND	ND
S25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S26	ND	ND	ND	ND	ND	ND	ND	0.02	ND	ND	0.03	ND	ND	ND	ND
S27	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.06	0.08	ND	ND	ND
S28	0.04	ND	ND	ND	ND	ND	ND	0.13	ND	ND	0.12	0.01	ND	ND	0.19
S29	ND	ND	ND	ND	ND	ND	ND	0.03	ND	ND	0.01	ND	ND	ND	ND
S30	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02
S31	ND	ND	ND	ND	0.04	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S32	ND	ND	ND	ND	ND	ND	ND	0.11	ND	ND	0.02	ND	ND	ND	0.02
S33	ND	ND	ND	ND	ND	ND	ND	0.03	ND	ND	0.04	ND	ND	ND	ND
S34	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	0.06	ND	0.01	ND	ND
S35	ND	ND	ND	ND	ND	ND	ND	0.05	ND	ND	ND	0.01	ND	ND	ND
S36	0.01	ND	ND	ND	ND	ND	ND	0.07	ND	ND	0.06	ND	ND	ND	0.08
S37	ND	ND	ND	ND	0.04	ND	ND	ND	ND	ND	0.01	0.01	ND	ND	ND
S38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.04	ND	ND	ND	ND
S39	ND	ND	ND	ND	ND	ND	ND	0.04	ND	ND	ND	0.02	ND	ND	0.08
S40	ND	ND	0.03	0.022	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

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IV. CONCLUSION

It is critical to understand the occurrence of agrochemical contaminants in foods for assessing the health risk and preserving consumer health. This study validated a multi-residue method for LC-MS/MS and GC- MS/MS, and used it to screen pesticides as per APEDA guidelines for export of chilli from India. These results pave the way for estimating the potential health risks associated with exposure to these pesticides. They also represent scientific evidence to create awareness on the necessity of good pesticide monitoring in Karnataka. Different strategies are being adopted for lowering the pesticide residues in food and by estimating the pesticide residues in a regular basis a proper control for the usage can be brought and also regulatory authorities can manage the quality.

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