CHAPTER 3

APPLICATION OF MULTIRESIDUE METHODS TO REAL SAMPLES

Spices are essential ingredients in Indian food, and nearly all aspects of the national cuisine incorporate spices in some form. As a result of this, food safety risks in spices becomes an important consideration. In this chapter, applying the multiresidue analysis methods developed in Part I to real samples, for establishing the ability of these methods to be used effectively for routine food safety evaluations, is documented. Characterization of food safety hazards due the presence of pesticide residues in spices were performed taking into account two aspects, *viz.* (a) compliance to the national maximum residue limits (MRLs) in spices, and (b) the consumption pattern of spices.

A survey was conducted by collecting samples (whole form) each for six spices, *viz.* cardamom, cumin, ginger, chillies, cinnamon and curry leaves from local markets in Kochi, Kerala. These samples were analysed in duplicate using the QuEChERS sample preparation and instrumentation methods developed and optimized in Part 1, Chapters 3 (LC-MS/MS) and 4 (GC-MS/MS), for 78 commonly used pesticides in India. The details of sample collection were explained in Part 2, Chapter 2. The average results obtained for each pesticide were then used to perform safety evaluations in the five spices studied.

Residue analysis results

Out of the total 78 residues tested in 60 samples of various spices, incidence of 30 compounds were observed across all the samples tested. Among the tested compounds, the highest percentage of pesticides were detected in cardamom (20.5%), followed by chillies (17.9%), cumin (14.1%), ginger (8.9%), cinnamon (5.1%) and curry leaves (7.6%). The total residue load, calculated as the sum of concentrations of all the incurred residues in all the samples of a particular spice, was taken as a measure of the extent of residue contamination in that spice. The highest residue load was observed in cumin (13.94 mg kg^{-1}), followed by cardamom (12.58 mg kg^{-1}, chillies (8.9 mg kg^{-1}, curry leaves (3.76 mg kg^{-1}), ginger (2.46 g kg^{-1}) and cinnamon (0.56 mg kg^{-1}). These results are summarized in Figure 2.2 below.

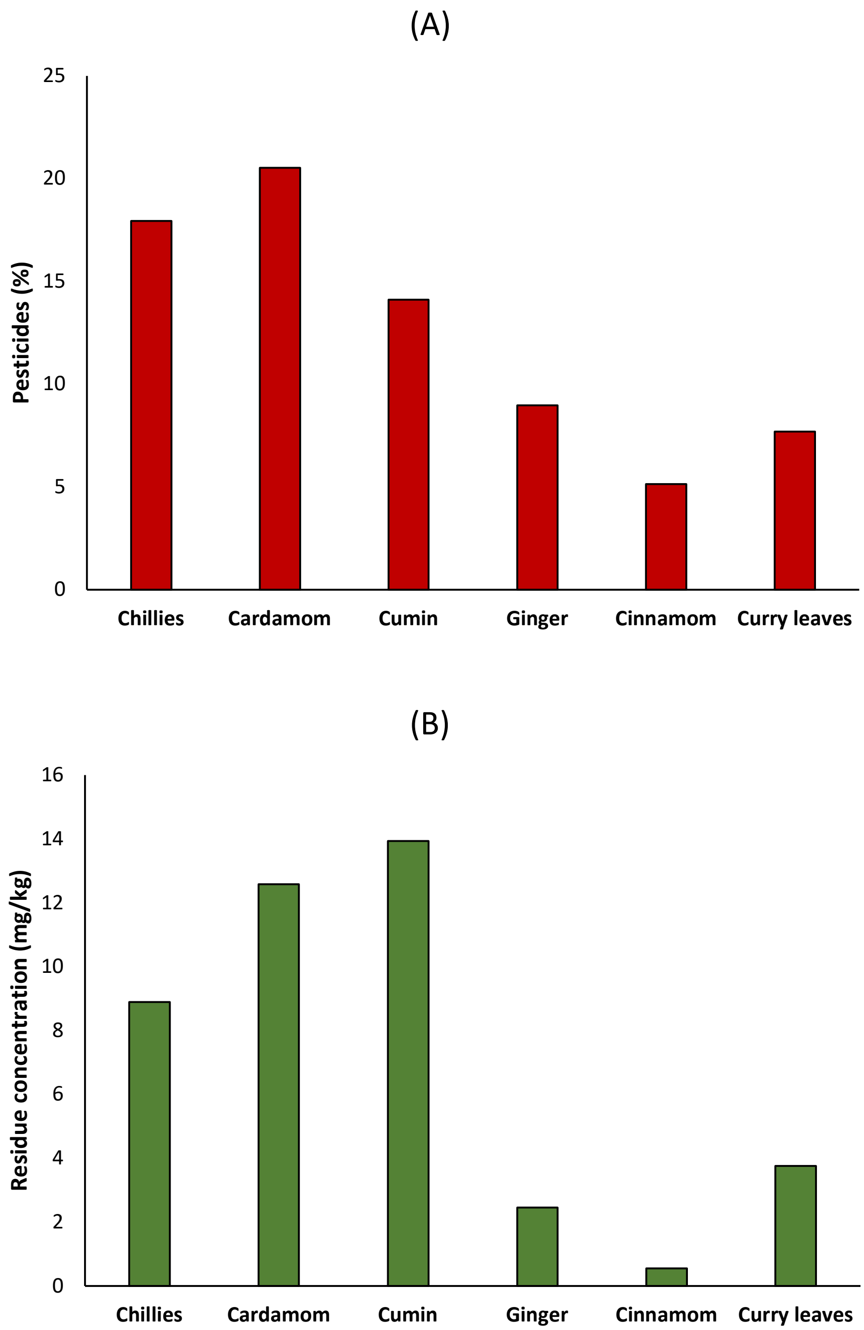

Figure 2.2 Incidence of pesticide residues: (A) residues detected in spices (%) out of the 78 compounds tested; (B) overall residue load in the samples of each spice tested

Incidence of residues in spices

In chillies, out of the 10 samples tested, residues were detected in all except 2 samples. The number of residues detected were 14, and for these the concentrations ranged from 0.02 - 3.00 mg kg^{-1}. The pesticide which showed highest incurred concentration was profenofos, which occurred in 4 samples. Acetamiprid, triazophos, azoxystrobin, fipronil were detected in 3 samples each, and quinalphos, chlorpyrifos, Imidacloprid, ethion, metalaxyl, γ cyhalothrin, cypermethrin, pyraclostrobin and spirotetramat were detected in 2 samples each. The cumulative pesticide load in chillies was the third highest among all samples tested, at 8.9 mg kg^{-1}. The details are summarized in Figure 2.3.

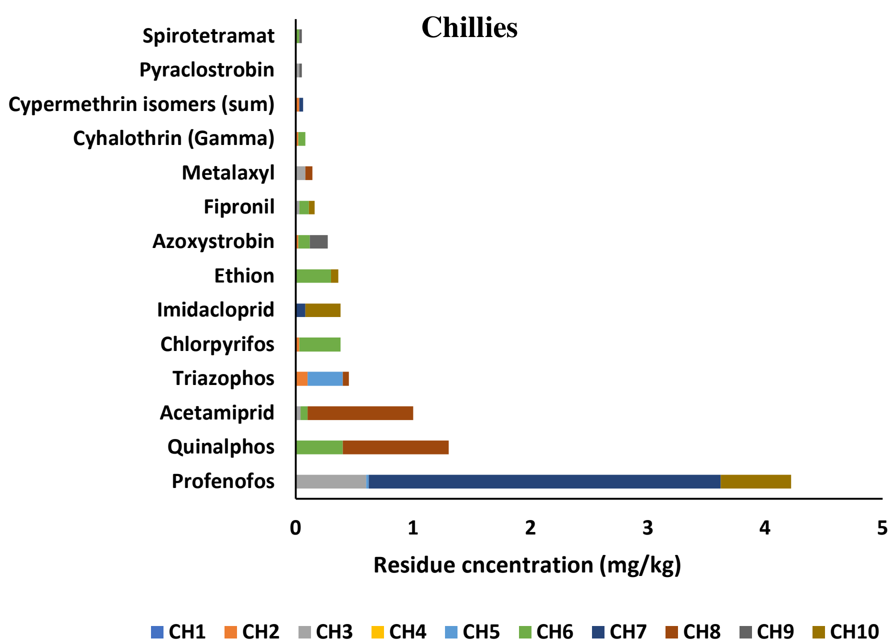

Figure 2.3 Pesticide residues detected in chillies

In cardamom also, residues were detected in all except 2 samples. The number of residues detected were 16, and for these the concentrations ranged from 0.02 - 1 mg kg^{-1}. The highest incurred value was for acetamiprid, at 1 mg kg^{-1}. Incidence of the pesticides were as follows: quinalphos - 6 samples; metalaxyl, γ cyhalothrin - 5 samples; phorate, chlorpyrifos, imidacloprid and triazophos - 4 samples; acetamiprid, hexaconazole and profenofos - 3 samples; methamidophos and carbofuran - 2 samples; buprofezin, parathion-methyl, azinphos-methyl and deltamethrin - 1 sample each. The cumulative pesticide load in all cardamom samples was the second highest among all spices tested, at 12.6 mg kg^{-1}. The details are summarized in Figure 2.4.

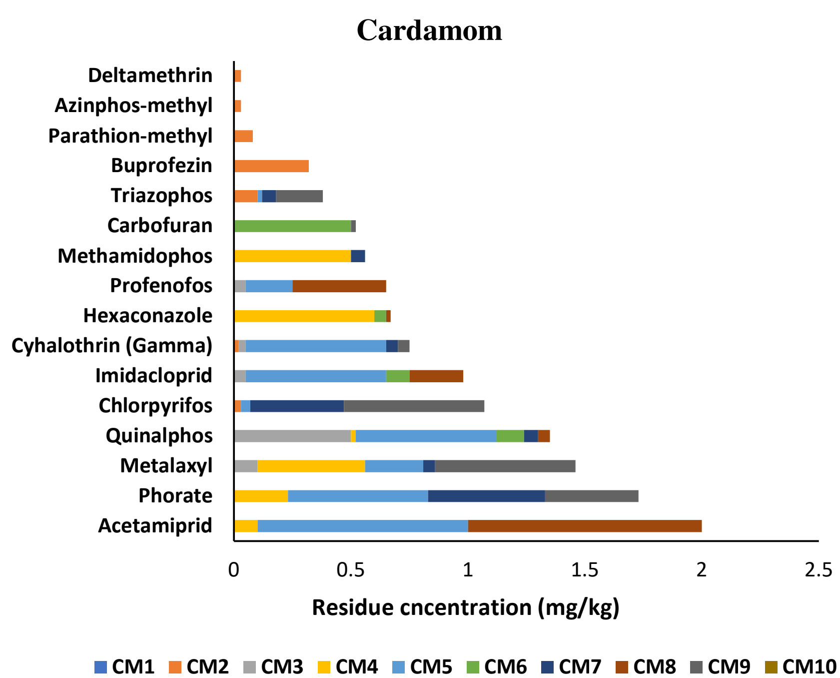

Figure 2.4 Pesticide residues detected in cardamom

In cumin, residues were detected in all except 3 samples. The number of residues detected were 11, and for these the concentrations ranged from 0.03 - 3 mg kg^{-1}. The highest incurred value was for hexaconazole, at 3 mg kg^{-1}. Incidence of the pesticides were as follows: Imidacloprid - 5 samples; profenofos - 4 samples; carbaryl, quinalphos, chlorpyrifos, spirotetramat and triazophos - 3 samples; hexaconazole and boscalid - 2 samples; iprobenfos and azoxystrobin - 1 sample. The cumulative pesticide load in cumin was the highest among all samples tested, at 13.94 mg kg^{-1}. The details are summarized in Figure 2.5.

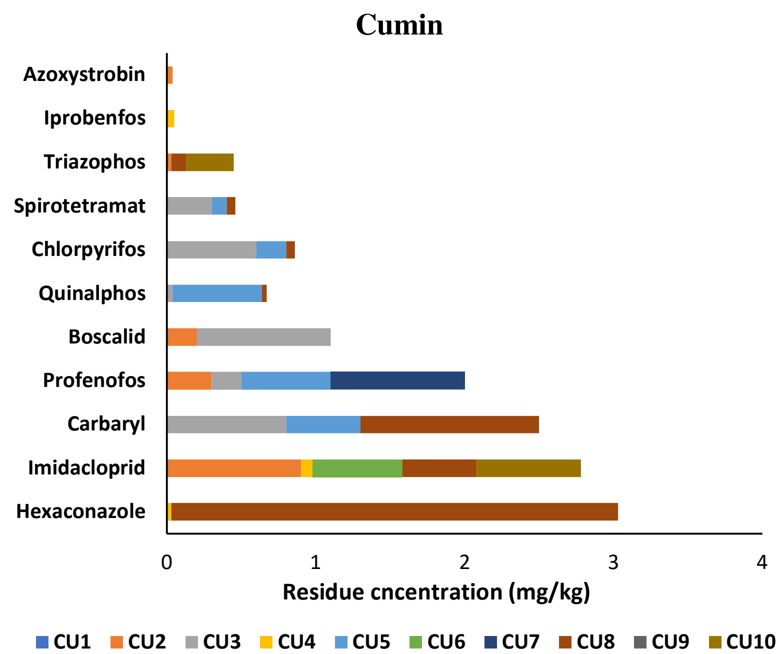

Figure 2.5 Pesticide residues detected in cumin

Ginger, cinnamon and curry leaves in general showed lower pesticide incidence. In ginger, residues were detected in 6 samples. The number of residues detected were 7, and for these the concentrations ranged from 0.02 – 0.6 mg kg^{-1}. The highest incurred value was for triazophos, at 0.6 mg kg^{-1}. Incidence of the pesticides were as follows: triazophos, hexaconazole, imidacloprid and fenbuconazole in 3 samples; chlorpyrifos in 2 samples; g cyhalothrin and phorate in 1 sample each. The cumulative pesticide load in ginger was 2.46 mg kg^{-1}. The details are summarized in Figure 2.6.

In cinnamon, residues were detected only in 4 samples, which made it the cleanest spice in the survey. The number of residues detected were 4, and for these the concentrations ranged from 0.02 – 0.2 mg kg^{-1}, again the lowest among the tested spices.

The highest incurred value was for fipronil, at 0.2 mg kg^{-1}. Incidence of the pesticides were as follows: Imidacloprid in 3 samples, fipronil, acephate and malathion in 2 samples. The cumulative pesticide load in cinnamon was only 0.56 mg kg^{-1}. The details are summarized in Figure 2.7.

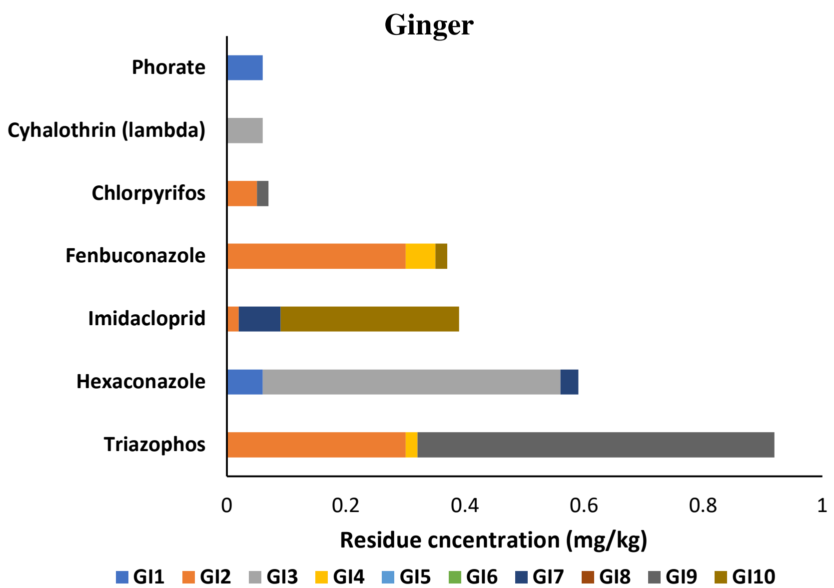

Figure 2.6 Pesticide residues detected in ginger

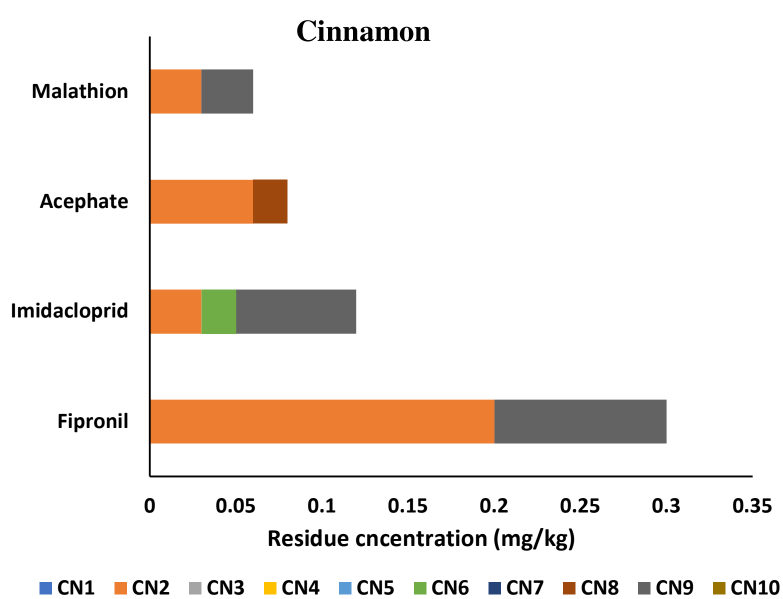

Figure 2.7 Pesticide residues detected in cinnamon

In curry leaves, residues were detected in 6 samples. The number of pesticides detected were 6, and for these the concentrations ranged from 0.03 – 0.5 mg kg^{-1}. The highest incurred value was for chlopryrifos, at 0.5 mg kg^{-1}. Incidence of the pesticides were as follows: chlorpyrifos and profenofos in 4 samples; cypermethrin, triazophos and bifenthrin in 3 samples; and fipronil in 2 samples. The cumulative pesticide load in ginger was 4.03 mg kg^{-1}. The details are summarized in Figure 2.8.

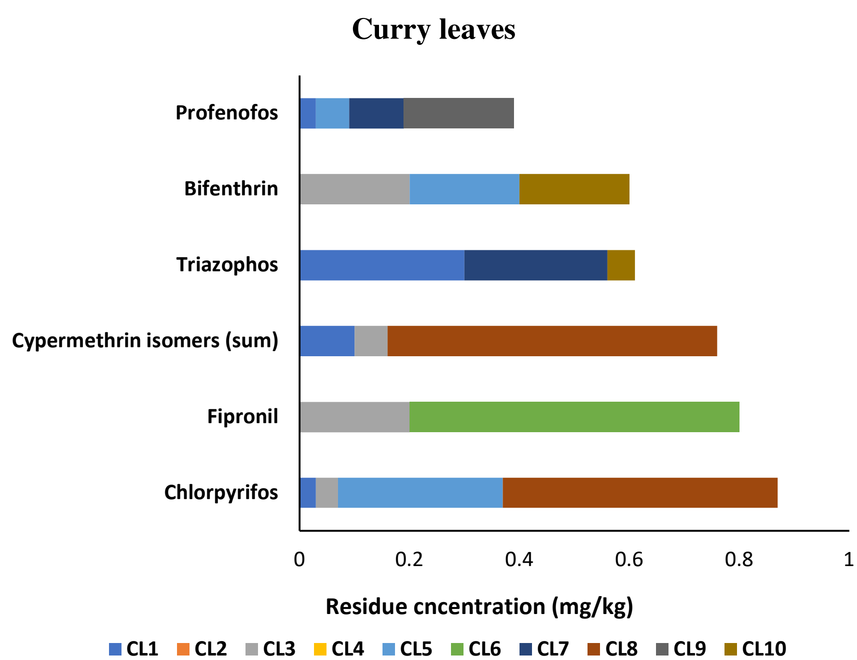

Figure 2.8 Pesticide residues detected in curry leaves

Compliance with national MRLs in spices

In India, the FSSAI sets national MRLs for foods, including spices. The MRLs in spices for the pesticides included in this study were compiled in Table 2.x. For spices, MRLs are available for only 23 out of the 78 pesticides covered in this study. As per the extant regulations, where an MRL is not specified for a commodity / pesticide combination, then the default MRL of 0.01 mg kg^{-1} is considered to apply. Although this scenario occurs mostly because the studies required for fixing MRLs as per FSSAI guidelines[2] have not been carried out for these combinations, it is adopted in order to ensure consumer safety. Also, since 0.01 mg kg^{-1} is near the quantification limit for current level of instrumentation techniques, this rule meant that for the 55 pesticides without MRLs, any reported value was tantamount to non-compliance as per FSSAI regulations.

Chillies have liberal MRLs under FSSAI, as a distinction is made between chilli and dried chilli, and a dehydration factor of 10 is applied for dried chilli[6]. Thus, in all cases where MRLs have been fixed for chilli, the MRL of dried chilli is fixed 10 times the MRL of chilli. So, even though 14 compounds had been detected in this spice, the number of pesticides above MRLs were only 6, as shown in Figure 2.9 below. Among these, the pesticide detected at concentrations above the MRL highest number of times was profenofos, in 40% of the samples. The other four residues were detected at concentrations above MRL in 20% samples.

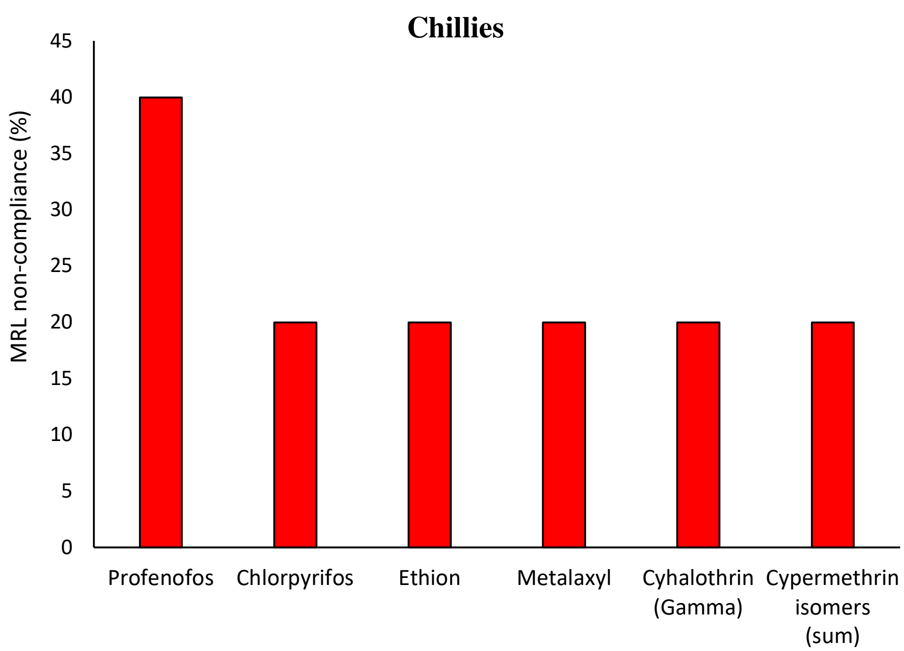

Figure 2.9 Pesticides detected above MRL levels in chillies

In cardamom, the situation was more complex, with all 16 compounds detected were at concentrations above MRL. The reason for this was that FSSAI has established specific MRL for cardamom in only one compound, *viz*. quinalphos, at 0.01 mg kg^{-1}, and dehydration factor is not seen to be applied in the FSSAI regulations for any spice other that chilli. The MRL for quinalphos in cardamom can be contrasted with the MRL for the same compound in chillies which is 2 mg kg^{-1} (see Table 2.1). As per FSSAI rules, the default MRL to be used in cases where specific MRL is not fixed for a commodity / pesticide combination is also 0.01 mg kg^{-1}, and thus all the pesticide detected in cardamom were found to be at above-MRL concentrations. Among these, the most detected compound was quinalphos, which occurred in 60% of the samples. Metalaxyl and γ cyhalothrin were detected at above-MRL concentrations in 50% of the samples. The pesticides which were found to be above MRLs in the least number of samples were buprofezin, parathion methyl, azinphos methyl and deltamethrin, which were detected in one sample each. Figure 2.10 summarizes the incidence of pesticide residues above MRL levels in cardamom.

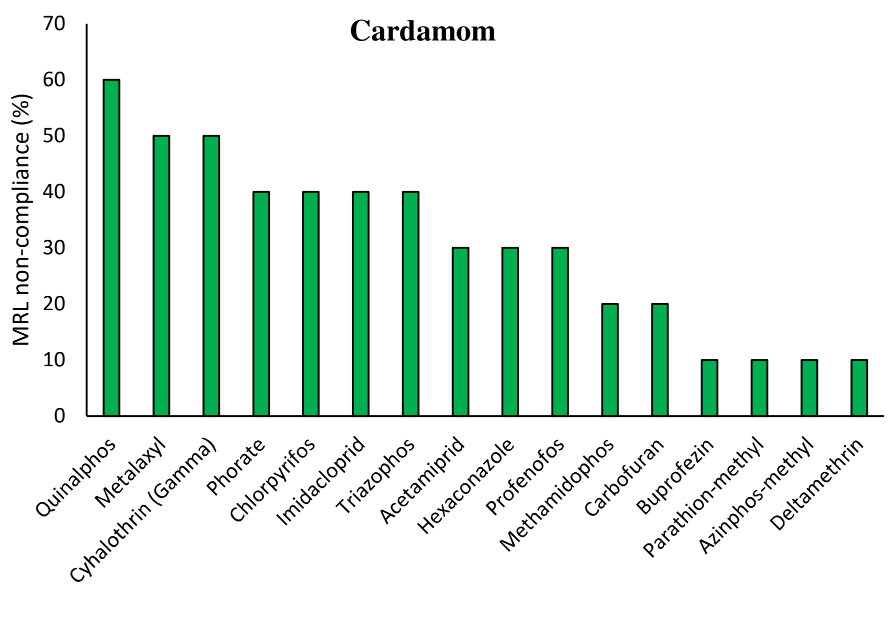

Figure 2.10 Pesticide residues detected above MRL levels in cardamom

In cumin also, all the 11 pesticide residues detected were found to be above MRL levels. As in the case of cardamom, the reason for this is the lack of MRLs in cumin. Only pyraclostrobin has MRL in cumin (Table 2.1), which was not detected in the present study. Thus, the default MRL of 0.01 mg kg^{-1} had to be applied for all the detected pesticides.

Imidacloprid was the pesticide with the greatest number of incidences above MRL, in 50% of the samples studied. Azoxystrobin and iprobenfos were the pesticides with least number of incidences above MRL in cumin. Figure 2.11 summarizes the incidence of pesticide residues above MRL levels in cumin.

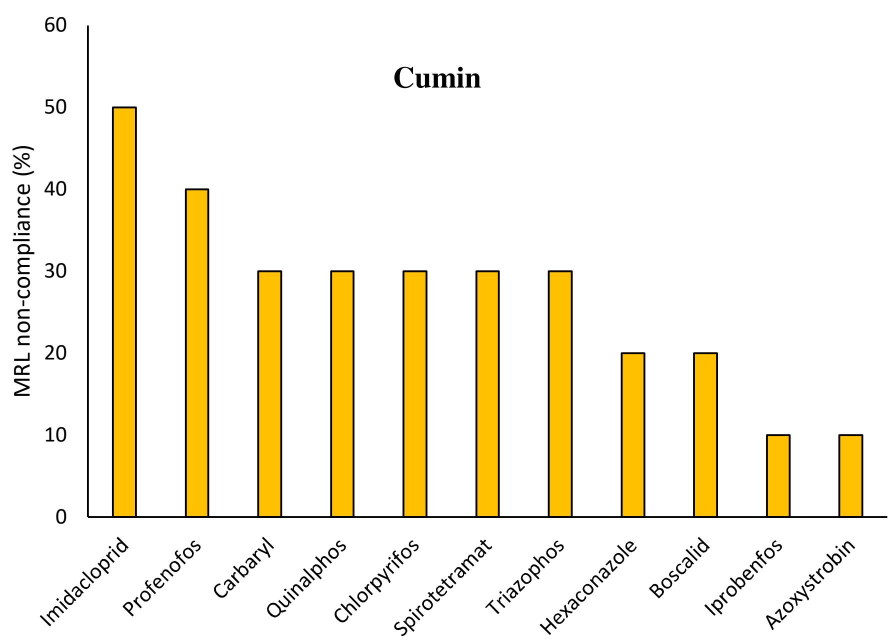

Figure 2.11 Pesticide residues detected above MRL levels in cumin

MRLs for ginger were not available as per FSSAI regulations, and thus the default MRL of 0.01 mg kg^{-1} had to be applied. Thus, all the 7 pesticides detected in ginger had to be considered as above MRL, with triazophos having the highest incidence above MRL and phorate having the lowest. Figure 2.12 summarizes the incidence of pesticide residues above MRL levels in ginger.

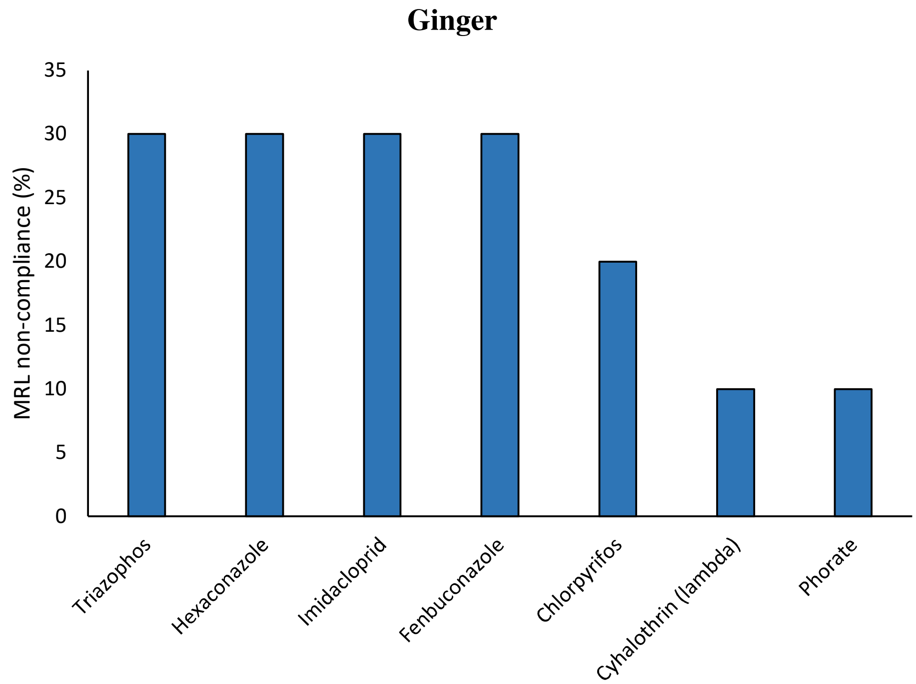

Figure 2.12 Pesticide residues detected above MRL levels in ginger

There were no MRLs available in the case of cinnamon also, and thus all detected pesticides had to be treated as above MRL. Among all the spices studied, cinnamon had the least number of incident pesticides as well as the least cumulative pesticide load. This is probably because cinnamon belong to the dried bark class of spices, and thus the extent of pest infestation in bark is less. The major pest related issues in cinnamon occur on leaves and shoots, thus foliar insecticides are often used in cultivation of cinnamon[8]. This is consistent with the type of pesticides detected in the study. Out of the four detected pesticides imidacloprid showed the highest incidence above MRL and malathion showed the least. Figure 2.13 summarizes the incidence of pesticide residues above MRL levels in cinnamon.

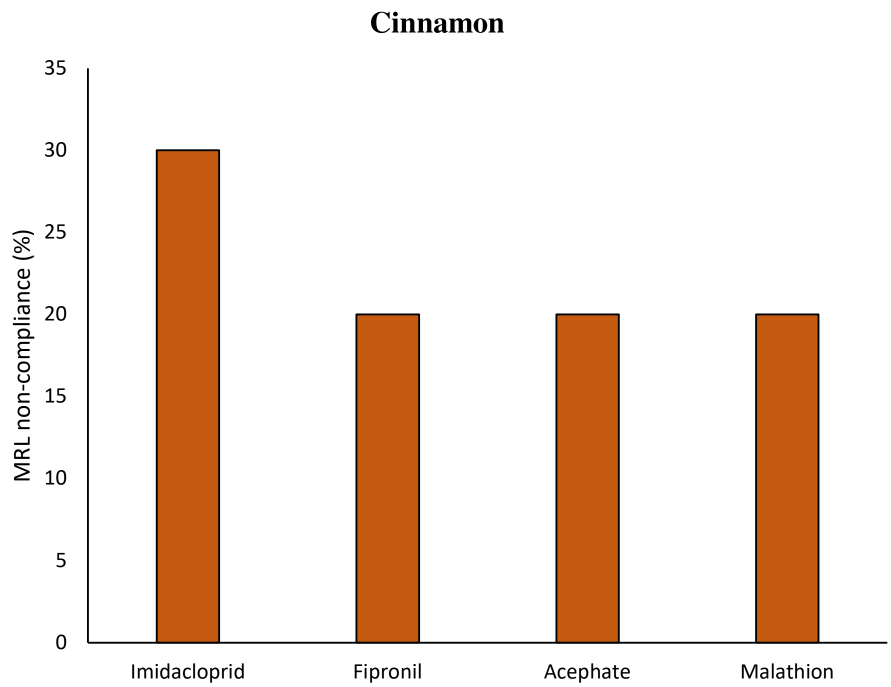

Figure 2.13 Pesticide residues detected above MRL levels in cinnamon

MRLs specific to curry leaves is not available under FSSAI, but the MRLs for leafy vegetables could be considered to apply for this spice. In the present study, dried curry leaves were tested and this affords the possibility of using a dehydration factor while evaluating compliance. However, for pesticides, MRL for leafy vegetables was available only for carbaryl, which was not detected in the curry leaf samples in the present study. Thus, the default MRL of 0.01 mg kg^{-1} had to be considered to apply in curry leaves also, necessitating that all the pesticides detected in curry eaves I the study had to be considered to be above the MRL level. Among the six pesticides detected in curry leaf, chlorpyrifos had the maximum incidence above MRL and fipronil had the least. Figure 2.14 summarizes the incidence of pesticide residues above MRL levels in curry leaves.

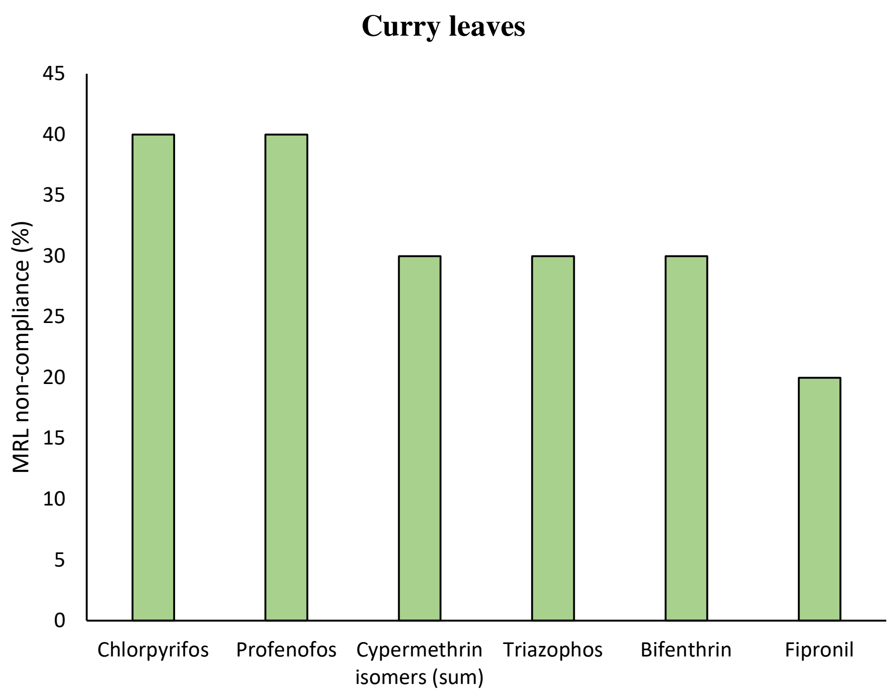

Figure 2.14 Pesticide residues detected above MRL levels in curry leaves

Food safety hazards in spices due to pesticide residues

Overall, 30 pesticides were detected among all the spice samples studied. These belonged to the following classes: organophosphates (12), pyrethroids (5), β-methoxyacrylates (2), carbamates (2), neonicotenoids (2), triazoles (2), acylalanines (1), cyclic ketoenols (1), oxathiines (1), phenyl pyrazoles (1) and thiadiazines (1). It can be noted that majority of compounds detected belonged to the classical and modern classes, while the detection of new generation pesticides was low (see Table 1.1). The pesticides chlorpyrifos, imidacloprid and triazophos were the most commonly detected pesticides occurring in 5 spices each, followed by cypermethrin, fipronil, hexaconazole and quinalphos which were detected in 3 spices each. The detected pesticides are commonly used for control of feed and storage pests, insects and fungal attacks. A summary of the different classes of pesticides detected in the various spices tested is shown in Table 2.4.

Table 2.4 Classes of pesticides detected in the spice samples studied

Pesticide	Class	Detected in	Usage
Acephate	Organophosphates	Cinnamon	Foliar and soil insecticide[9]
Acetamiprid	Neonicotenoids	Cardamom, chillies	Insecticide (aphids)[10]
Azinphos-methyl	Organophosphates	Cardamom	Foliar insecticide[11]
Azoxystrobin	β-methoxyacrylates	Chillies, cumin	Fungicide[12]
Bifenthrin	Pyrethroids	Curry leaves	Insecticide[13]
Boscalid	Oxathiines	Cumin	Fungicide[14]
Buprofezin	Thiadiazines	Cardamom	Insecticide[15]
Carbaryl	Carbamates	Cumin	Insecticide[16]
Carbofuran	Carbamates	Cardamom	Pesticide[17]
Chlorpyrifos	Organophosphates	Cardamom, curry leaves, cumin, Chillies, ginger	Pesticide[18]
Cyhalothrin γ and λ	Pyrethroids	Cardamom, chillies, ginger	Insecticide[19]
Cypermethrin	Pyrethroids	Curry leaves, chillies	Insecticide[20]
Deltamethrin	Pyrethroids	Cardamom	Insecticide[20]
Ethion	Organophosphates	Chillies	Insecticide[21]
Fenbuconazole	Triazoles	Ginger	Fungicide[22]
Fipronil	Phenyl pyrazoles	Curry leaves, cinnamon, Chillies	Insecticide[23]
Hexaconazole	Triazoles	cumin, cardamom, ginger	Fungicide[24]
Imidacloprid	Neonicotinoids	Cumin, cardamom, ginger, chillies, cinnamon	Insecticide[25]
Iprobenfos	Organophosphates	Cumin	Fungicide[26]
Malathion	Organophosphates	Cinnamon	Insecticide[27]
Metalaxyl	Acylalanines	Cardamom, chillies	Fungicide[28]
Methamidophos	Organophosphates	Cardamom	Insecticide[29]
Parathion-methyl	Organophosphates	Cardamom	Insecticide[30]
Phorate	Organophosphates	Cardamom, ginger	Acaricide[31] (mites, ticks)
Profenofos	Organophosphates	Chillies, cumin, cardamom, curry leaves	Insecticide[32]
Pyraclostrobin	β-methoxyacrylates	Chillies	Fungicide[33]
Quinalphos	Organophosphates	Cardamom, Chillies, cumin	Pesticide[34]
Spirotetramat	Cyclic ketoenols	Cumin, chillies	Insecticide[35]
Triazophos	Organophosphates	Ginger, curry leaves, chillies, cumin, cardamom	Acaricide[36] (mites, ticks)

Although there are a number of pesticides detected in the spices studied, in assessing MRL compliance, two factors need to be considered: (a) low number of MRLs in FSSAI regulations in spices, and (b) the comparatively low intake of spices as compared to other staple foods. The first factor necessitates that for majority of spice – pesticide combinations, the default MRL of 0.01 mg kg^{-1} needs to be considered for compliance assessment. Since this concentration level is very close to the quantification level of mass spectrometric instruments used for analysis of pesticide residues, any quantified value will result in non-compliance with MRL regulations. This is, however, a legal requirement and does not necessarily mean there is high food safety hazard due to pesticide residues in spices. The second factor of extent of consumption of spices as compared to staple foods directly illustrates this point. A comparison of dietary consumption data for staple foods like cereals and millets, as compared to spices was shown in Table 2.1. It can be seen that spices are consumed at very low quantities as compared to staple foods. Thus, a spice in which incidence of a pesticide occurs at a particular concentration will pose much lower food safety risk as compared to a staple food with the same incidence concentration. For example, the incidence of the pesticide propiconazole at 0.06 mg kg^{-1} in the spice cumin (default MRL 0.01 mg kg^{-1}, dietary consumption 2 g $person^{-1}$ day^{-1}) will pose much less food safety risk than the same concentration in rice (MRL 0.05 mg kg^{-1}, dietary consumption 257 g $person^{-1}$ day^{-1}) even though the MRL is exceeded in both cases.

Hazard characterization of residues in spices

For the hazard characterization, the residue which showed highest incidence in each spice was considered. The theoretical maximum daily intake (TMDI, mg $person^{-1}$ day^{-1}) was calculated by multiplying the residue concentration (mg kg^{-1}) by the average consumption of the spice (kg $person^{-1}$ day^{-1}). The maximum permissible intake (MPI, mg $person^{-1}$ day^{-1}) was calculated by multiplying the acceptable daily intake (ADI, mg kg body $weight^{-1}$ day^{-1}) by the average body weight of a child, taken as 16 kg[7]. The results of the evaluation are shown in Table 2.5.

Table 2.5 Hazard characterization in spices

Spice	Highest residue concentration		ADI (mg kg^{-1} day^{-1})[a]	Average Consumption (kg person^{-1} day^{-1})[b]	TMDI (mg person^{-1} day^{-1})	MPI (mg person^{-1} day^{-1})
	Pesticide	Conc. (mg kg^{-1})				
Chillies	Profenofos	3	0.03	0.003	0.009	0.48
Cardamom	Acetamiprid	1	0.07	0.002	0.002	1.12
Cumin	Hexaconazole	3	0.005	0.002	0.006	0.08
Ginger	Triazophos	0.6	0.001	0.003	0.0018	0.016
Cinnamon	Fipronila	0.2	0.0002	0.002	0.0004	0.0032
Curry leaves	Chlorpyrifos	0.5	0.01	0.002	0.001	0.16

[a] ADI values are from the online Codex Pesticide MRL Database[37]
[b] Average consumption values for spices are taken from FSSAI Guidance Manual 2021[2]

It is seen that in all the spices studied, the theoretical maximum daily intake values are considerably less than the maximum permissible intake values, thereby showing that the incident residue levels in spices obtained in the study does not indicate any food safety hazard to consumers.

Conclusions

The residue analysis methods developed in Part I were applied to real samples of the spices chillies, cardamom, cumin, ginger, cinnamon and curry leaves collected from local markets for assessing the compliance against the national pesticide MRL regulations issued by FSSAI. The methods used QuEChERS sample preparation followed by GC-MS/MS and LC-MS/MS instrumentation as optimized in Part I, Chapter 3 and 4. In the study, analysis of 78 pesticides in 60 samples were covered.

The maximum number of pesticides were detected in cardamom, whereas the total residue load was seen to be maximum in cumin. The least number of pesticides and residue load was observed in cinnamon. Among the samples tested, 30 pesticides were seen to occur at levels above MRL levels in the spices tested. Maximum pesticide incidences as levels above MRLs were observed in cardamom, followed by cumin ad chillies. Cinnamon showed the least number of pesticides with concentrations above MRLs. Majority of the pesticides detected in the spices belonged to organophosphates, carbamates and pyrethroid classes, with only few occurrences of new generation pesticides. Although 30 compounds were found in the study to be above MRL levels, it was noted for majority of these compounds, specific MRLs for spices were not available and so the default MRL of 0.01 mg kg-1 had to be used, which caused any quantified value to be above the MRL level. It was also observed that the consumption levels of spices were very small when compared to those for staple foods, which minimized the risks associated with pesticide residues in spices, and this was confirmed by risk characterization calculations.

The capability of the sample preparation and instrumental methods developed for multicomponent pesticide residue analysis in spices, for assessing compliance with national regulations and for assessing food safety risks associated with presence of pesticide residues, was thus effectively demonstrated.