Determination of Pesticide Residues in curry leaf in different markets of Andhra pradesh and Telangana, India

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Abstract—Studies were conducted for determining the residues of commonly used pesticides in curry leaf samples collected every month from different markets of AP and Telangana India during 2014 and 2015. Samples collected from eight selected markets every month were analysed using QuEChERS method on LC-MS/MS. During 2014, only in the December month all the market samples contained pesticide residues whereas in the November month there were no pesticide residues from all the eight markets. During 2015, in April and December months, all the market samples showed pesticide residues, whereas in November month only two market samples had pesticide residues. The most commonly detected pesticide residues were of Profenophos, Ethion, Cyfluthrin, Bifenthrin, Chlorpyriphos, Triazophos, Phorate, Methyl parathion, Cypermethrin, Fenpropathrin, Monocrotophos, Acetamiprid, Methamidophos, Acephate, Allethrin, alpha cypermethrin, Fipronil, Carbendazim, Deltamethrin, Malathion, quinalphos etc indicating that, curry leaf samples contained detectable levels of the pesticide residues for which Maximum Residue Limits (MRL) are not fixed. As there are no MRLs for curry leaves, it should be considered as most important to fix MRLs to ensure food safety and consumer health and to create awareness among the farmers about the application dose, method of application and Pre Harvest Intervals. The mismanagement or non-availability of proper information about the pesticide application can lead to contamination of pesticide residues in curry leaf. The findings of this study provided important data about contamination of pesticide residues in curry leaf sold in different local markets of AP and Telangana states and hence, it is essential to conduct monitoring studies in other curry leaf growing agro climatic regions, which may serve as basis for future policy about the standards and quality control of pesticides.

Keywords—Curry leaf, QuEChERS method, Chlorpyriphos, Cypermethrin, Monocrotophos.

I. INTRODUCTION

Curry leaf (Murraya koenigii) is a leafy spice, belongs to the Rutaceae family, is native to India, Sri Lanka, Bangladesh and the Andaman Islands. Its leaves are widely used in Indian cookery for flavouring foodstuffs. The major constituent responsible for the aroma and flavor is due to the presence of essential oils used in the soap industry (Salikutty and Peter, 2008), it has anti carcinogenic properties due to the presence of carbazole alkaloids (Khanum et al., 2000). Traditionally curry leaf is used in Ayurvedic medicines for treating many diseases. Oxidative stress related diseases are treated by extensive use of synthetic antioxidants which in turn causes unwanted side effects, hence there is increasing interest of using naturally occurring antioxidants (Maxwell, 1995). Curry leaves can be used as antioxidants as they contain the antioxidants tocopherol, l-carotene and lutein (Palaniswamy, 2001). As a rich source of antioxidants curry leaf showed highest antioxidant and free radical scavenging activity (Mylarappa et al., 2008). The phyto chemical constituents of Murraya koenigii are also useful in waste water treatment to reduce the effect of harmful compounds (Sharma et al., 2013). Curry leaf is now grown throughout India and attacked and damaged by number of pests and diseases at various stages of its growth. As a part of crop protection and for increasing crop yields, curry leaf farmers are using wide range of chemicals leaving residues in the plant parts consumed as food (Agnihotri 1999), which enters food chain directly or indirectly. European union, the major importers of curry leaf have sent a red alert message that the residues in curry leaves are much more than the permissible limits, which created a panic among the exporters. Since there is a need to analyse the pesticides used by farmers in the market samples at different locations and to create awareness among the consumers, farmers and extension workers and also to suggest them the proper dosages, waiting periods etc. these studies were taken up to
know the type of pesticides used by the farmers and their residues in samples at market.

II. MATERIALS AND METHODS
Market samples of curry leaf were collected from different markets in AP and Telangana every month from 2014 to 2015. Curry leaf sample of 1 kg (1/4 kg each, randomly from four different vendors in the market were collected from these eight markets. Samples were extracted for pesticide residues following the validated QuEChERS method utilizing LC-MS/MS

Sample extraction procedure: Curry leaf samples were analyzed for pesticide residues following the AOAC official method 2007.01 (QuEChERS) after validation of the method in the laboratory. The samples were collected from different markets. Each sample was homogenized separately with robot couple bliser and homogenized 15 ±0.1g sample was taken in 50 ml centrifuge tube and 30±0.1 ml acetonitrile was added to sample tube. The sample was homogenized at 14000-15000 rpm for 2-3 min using Heidolph silent crusher. 3±0.1 g sodium chloride was added to sample, mixed thoroughly by shaking gently followed by centrifugation for 3 min at 2500-3000 rpm to separate the organic layer. The top organic layer of about 16 ml was taken into the 50 ml centrifuge tube and added with 9±0.1 g anhydrous sodium sulphate to remove the moisture content. 8 ml of extract was taken in to 15 ml tube, containing 0.4±0.01 g PSA sorbent (for dispersive solid phase d-SPE cleanup),1.2±0.01 g anhydrous magnesium sulphate and 0.05 g of GCB (Graphatised Carbon Black), AOAC official method 2007.01 suggests that, it is desirable to add 50 mg of GCB per milliliter of extract for any commodities with higher pigments such as green leafy vegetables. The sample tube was vortexed for 30 sec then followed by centrifugation for 5 min at 2500-3000 rpm. The extract of about 1 ml (0.5 g sample) was taken for analysis on LCMS/MS under standard operational conditions (Table-1). Certified Reference Materials (CRM) of different pesticides having purity ranging from 95.10 to 99.99 per cent were stored in a freezer at low temperature, with light and moisture excluded. Solvents used in the study were all glass distilled before use. Sodium sulphate, sodium chloride and magnesium sulphate were activated in hot air oven at 450 °C for 5 h. A weighed amount of analytical grade material of each pesticide was dissolved in a minimum quantity of distilled acetone and diluted with methanol to obtain a stock solution of 1000 mg kg⁻¹. The intermediate standards and working standards of 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 kg kg⁻¹ were prepared by suitably diluting the stock solution in methanol and used as standard check in analysis, linearity and recovery studies (Table-2).

METHOD VALIDATION
The analytical method for estimation of residues of pesticides in curry leaves has been validated by conducting recovery studies using control samples. 15g of sample was taken in 50 ml centrifuge tubes in three replicates, each were spiked with pesticide mixture at the required fortification levels i.e. LOQ, 5x LOQ and 10x LOQ, adding an appropriate volume of working standard. This mixture was then shaken to attain a proper homogeneity of pesticides in the samples. The tubes containing fortified samples were left open for a while, just to allow the evaporation of excess solvent. Sample extraction procedure was followed as given above.

III. RESULTS AND DISCUSSION
Samples collected from eight different markets every month during 2014 and 2015 were analysed and the results are presented hereunder. It is observed that in some samples there were no pesticide residues. During 2014, only in the December month all the market samples contained pesticide residues whereas in the November month there were no pesticide residues from all the eight markets. During 2015, in April and December months, all the market samples showed pesticide residues, whereas in November month only two market samples had pesticide residues. Number of markets detected with pesticide residues month-wise in curry leaf samples during 2014-15 are depicted in figures (1-9).

Mehidipatnam rythubazar: In the year 2014, pesticides were not detected during the months of February, May, July, August and November; whereas in the other months notable number of pesticides i.e.,15 insecticides were detected. Of the 12 curry leaf samples analysed, four (33.33%) samples were highly contaminated with ethion while one (8.33%) sample was least contaminated with acephate, dimethoate, methamidophos, methyl parathion, phorate, quinalphos, triazophos, fenpropatrin, permethrin and fipronil. Residue levels of triazophos was high (4.330 mg kg⁻¹) followed by profenophos (3.352 mg kg⁻¹) while methyl parathion was least (0.050 mg kg⁻¹). Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 and number of pesticides detected month wise during 2014-15 presented in figure 1. During 2015, pesticides were not detected during the months of July and August; whereas in the other months notable number of pesticides i.e.,20 insecticides and three fungicides were detected. Of the 12 curry leaf samples analysed, seven (58.33%) samples were highly...
contaminated with cypermethrin and acetamiprid while one (8.33%) samples were least contaminated with anilophos, phorate, phosphamidon, quinalphos, lambda cyhalothrin, spiromesifen and spirotetramat. Residue levels of acetamiprid was high (5.468 mg kg\(^{-1}\)) followed by profenophos (4.728 mg kg\(^{-1}\)) while spirotetramat was least (0.056 mg kg\(^{-1}\)). Besides insecticides, among the 12 curry leaf samples analysed during 2015, three (25%) samples were highly contaminated with tebuconazole while one (8.33%) sample was least contaminated with carbendazim and trifloxystrobin. Residue levels of tebuconazole was high (2.722 mg kg\(^{-1}\)) followed by carbendazim (0.365 mg kg\(^{-1}\)) while trifloxystrobin was least (0.183 mg kg\(^{-1}\)).

Erragadda rythubazar
In the year 2014, pesticides were not detected during the months of, September, October and November; whereas in the other months notable number of pesticides i.e., 11 insecticides and one fungicide were detected. Among the 12 curry leaf samples analysed, residue levels of profenophos was high (5.728 mg kg\(^{-1}\)) followed by acetamiprid (4.468 mg kg\(^{-1}\)) while thiamethoxam was least (0.068 mg kg\(^{-1}\)). Five (41.67%) samples were highly contaminated with cypermethrin while one (8.33%) sample was least contaminated with triazophos, cyfluthrin and thiamethoxam. Of the 12 curry leaf samples analysed during 2014, 8.33% per cent samples were contaminated with fungicide tebuconazole with residue concentration of 3.293 mg kg\(^{-1}\). Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 and number of pesticides detected month wise during 2014-15 is presented in figure 2. During 2015, pesticides were not detected during the months of, September and October; whereas in the other months notable number of pesticides i.e., 24 insecticides, one fungicide and one herbicide were detected. Of the 12 curry leaf samples analysed, six (50.00%) samples were highly contaminated with chlorpyriphos while one (8.33%) sample was least contaminated with chlorpyriphos-methyl, methyl parathion, phorate, triazophos, allylthrin, alpha-cypermethrin, cyfluthrin, cypermethrin, permethrin, spiromesifen, spirotetramat, acetamiprid and abamectin. Residue levels of profenophos was high (25.690 mg kg\(^{-1}\)) followed by acetamiprid (11.98 mg kg\(^{-1}\)) while allethrin was least (0.046 mg kg\(^{-1}\)). Of the 12 curry leaf samples analysed during 2015, 8.33 per cent samples were contaminated with herbicide pendimethalin with residue concentration of 0.28 mg kg\(^{-1}\). 16.67 per cent samples were contaminated with fungicide tebuconazole with residue range of 0.38-10.29 mg kg\(^{-1}\).

Nalogonda rythubazar
In the year 2014, pesticides were not detected during the months of, January, February, March, May, August, September, October and November; whereas in the other months notable number of insecticides i.e., five were detected. Of the 12 curry leaf samples analysed, one (8.33%) sample was contaminated with chlorpyriphos, methamidophos, alpha-cypermethrin, cyfluthrin and deltamethrin. Residue levels of cyfluthrin was high (0.479 mg kg\(^{-1}\)) followed by methamidophos (0.256 mg kg\(^{-1}\)) while chlorpyriphos was least (0.036 mg kg\(^{-1}\)). Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014, and number of pesticides detected month wise during 2014-15 are presented in figure 4. During 2015, pesticides were not detected during the months of, March, May, August, September, October and November; whereas in the other months notable number of pesticides i.e., 11 insecticides and one herbicide were detected. Of the 12 curry leaf samples analysed, three (25%) samples were highly contaminated with profenophos...
and bifenthrin while one (8.33%) sample was least contaminated with chlorpyrifos, ethion, monocrotophos, phosphamidon, deltamethrin and fenpropathrin. Residue levels of acetamiprid was high (1.136 mg kg\(^{-1}\)) followed by profenophos (1.088 mg kg\(^{-1}\)) while phosphamidon was least (0.064 mg kg\(^{-1}\)). Besides insecticides, of the 12 curry leaf samples analysed during 2015, 16.67 per cent samples were contaminated with herbicide atrazine with residue range of 0.048-0.628 mg kg\(^{-1}\).

**Warangal rythubazar**

In the year 2014, pesticides were not detected during the months of, January, February, March, May, June, August and November; whereas in the other months notable number of insecticides i.e., eight were detected. Of the 12 curry leaf samples analysed, three (25%) samples were highly contaminated with cyfluthrin, while one (8.33%) was least contaminated with methyl parathion, profenophos, triazophos and fenpropathrin. Residue levels of cyfluthrin was high (3.934 mg kg\(^{-1}\)) followed by phorate (0.548 mg kg\(^{-1}\)) while methyl parathion was least (0.053 mg kg\(^{-1}\)). Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 are presented in table 4.16, and number of pesticides detected month wise during 2014-15 is presented in figure 4.6. During 2015, pesticides were not detected during the months of, March, August and November; whereas in the other months notable number of pesticides i.e., 21 insecticides and three fungicides were detected. Of the 12 curry leaf samples analysed, six (50%) samples were highly contaminated with acetamiprid while one (8.33%) sample was least contaminated with chlorpyrifos-methyl, diazinon, dichlorvos, dimethoate, ethion, methamidophos, triazophos, cyfluthrin, cypermethrin, lamda-cyhalothrin, imidacloprid, carbofuran and abamectin. Residue levels of acephate and were high (8.179 mg kg\(^{-1}\)) followed by acetamiprid (8.159 mg kg\(^{-1}\)) while imidacloprid was least (0.056 mg kg\(^{-1}\)). Besides insecticides, of the 12 curry leaf samples analysed during 2015, 8.33 per cent samples were contaminated with fungicides like myclobutanil (0.459 mg kg\(^{-1}\)), tebuconazole (1.824 mg kg\(^{-1}\)) and trifloxystrobin (0.332 mg kg\(^{-1}\)). Number of samples contaminated, per cent contamination and residue range of all pesticides during 2015 are presented in fig.6.

**Nellore rythubazar**

In the year 2014, pesticides were not detected during the months of, March, April, May, June, August, September, October and November; whereas in the other months notable number of insecticides i.e., ten insecticides and one fungicide were detected. Of the 12 curry leaf samples analysed, six (50%) samples were highly contaminated with chlorpyrifos while one (8.33%) sample was least contaminated with acephate, chlorpyrifos-methyl, dimethoate, monocrotophos, bifenthrin and cypermethrin. Residue levels of ethion was high (2.136 mg kg\(^{-1}\)) followed by chlorpyrifos (0.918 mg kg\(^{-1}\)) while cypermethrin was least (0.052 mg kg\(^{-1}\)). Besides insecticides, of the 12 curry leaf samples analysed during 2015, 8.33 per cent samples were contaminated with fungicide carbendazim with residue concentration of 0.05 mg kg\(^{-1}\). Number of samples contaminated, per cent contamination and residue range of all pesticides during 2015 are presented in fig.5.

**Guntur rythubazar**

In the year 2014, pesticides were not detected during the months of, February, May, July, August and November; whereas in the other months notable number of insecticides i.e., 11 were detected. Of the 12 curry leaf samples analysed, three (25%) samples were highly contaminated with ethion, while one (8.33%) sample was least contaminated with acephate, chlorpyrifos, methamidophos and monocrotophos. Residue levels of cyfluthrin was high (12.654 mg kg\(^{-1}\)) followed by cypermethrin (5.510 mg kg\(^{-1}\)) while methamidophos was least (0.054 mg kg\(^{-1}\)). Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 are presented in table, and number of pesticides detected month wise during 2014-15 is presented in figure. During 2015, pesticides were not detected during the months of, March, July, August and November; whereas in the other months notable number of pesticides i.e., 21 insecticides and three fungicides were detected. Of the 12 curry leaf samples analysed, four (33.33%) samples were highly contaminated with monocrotophos and profenophos while one (8.33%) sample was least contaminated with anilophos, dichlorvas, dicofol, dimethoate, cypermethrin,
fenpropathrin, lambda-cyhalothrin and carboburan. Residue levels of acetamiprid was high (6.748 mg kg⁻¹) followed by monocrotophos (5.98 mg kg⁻¹) while chlorpyriphos was least (0.051 mg kg⁻¹). Besides insecticides, of the 12 curry leaf samples analysed during 2015, 8.33 per cent samples were contaminated with fungicides like carbendazim (0.08 mg kg⁻¹), tebuconazole (0.2 mg kg⁻¹) and trifloxystrobin (0.332 mg kg⁻¹). In addition to these 16.67 per cent of samples were contaminated with one herbicide namely, pendimethalin with residue range of 0.052-0.072 mg kg⁻¹. Number of samples contaminated, per cent contamination and residue range of all pesticides during 2015 are presented in fig 7

Vijayawada rythubazar

In the year 2014, pesticides were not detected during the months of May, August and November; whereas in the other months notable number of pesticides i.e., 16 insecticides and one fungicide were detected. Of the 12 curry leaf samples analysed, three (25%) samples were highly contaminated with ethion and cyfluthrin while one (8.33%) sample was least contaminated with acephate, chlorpyriphos, triazophos, allethin, alpha-cypermethrin, cypermethrin, deltamethrin, fenpropatrin, lambda-cyhalothrin, acetamiprid and imidaclorpid. Residue levels of profenophos was high (21.546 mg kg⁻¹) followed by cypermethrin (10.810 mg kg⁻¹) while chlorpyriphos was least (0.02 mg kg⁻¹).

Besides insecticides, of the 12 curry leaf samples analysed during 2014, 8.33 per cent samples were contaminated with fungicide metalaxyl with residue concentration of 0.137 mg kg⁻¹. Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 is presented in table 4.22, and number of pesticides detected month wise during 2014-15 is presented in figure 4.9. During 2015, pesticides were not detected during the months of, January, May, June, July and November; whereas in the other months notable number of pesticides i.e., 17 insecticides, two fungicides and one herbicide were detected. Of the 12 curry leaf samples analysed, five (41.67%) samples were highly contaminated with bifenthrin while one (8.33%) samples were least contaminated with anthophos, chlorpyriphos-methyl, phosphomidon, profenophos, cypermethrin, imidaclorpid and abamectin. Residue levels of acetamiprid was high (11.976 mg kg⁻¹) followed by monocrotophos (10.292 mg kg⁻¹) while thiamethoxam was least (0.068 mg kg⁻¹).

Besides insecticides, of the 12 curry leaf samples analysed during 2015, 8.33 per cent samples were contaminated with fungicides like carbendazim (0.256 mg kg⁻¹), and tebuconazole (0.056 mg kg⁻¹). Besides, 16.67 per cent of samples were contaminated with herbicide namely, pendimethalin with residue range of 0.072-0.280 mg kg⁻¹. Number of samples contaminated, per cent contamination and residue range of all pesticides during 2015 is presented in fig 8

Results of market samples are indicative of indiscriminate and over use of insecticides by the curry leaf growers Fig.9. It also indicates that farmers are neither adopting good agricultural practices (GAP) nor observing safe waiting period. Thus, constant monitoring from time-to-time is essential for maintaining up-to-date information on pesticide residues and guidelines for the manufacturers and users. The results are in line with the work done by Beena kumari (2007) who reported that, of the 60 market samples analysed 4-100 per cent contamination with low but measurable amounts of residues of four major chemical groups i.e., organochlorine, organophosphate, pyrethroid and carbamate pesticide was recorded. Residues of cypermethrin, chlorpyriphos and permethrin, each in two samples of brinjal, cabbage and cauliflower, exceeded their respective MRL values thereby showing 10 per cent samples with residues above maximum residue limits. Fifty vegetable samples in Kolar district of Kamataka were analysed and found that of all the samples contaminated, the organochlorines (97%) dominated followed by organophosphates (83%) and pyrethroids (60%). However, 58 per cent of the samples were found to contain the residues of these insecticides above their respective maximum residue limits (MRL). The results obtained in the present investigations are in agreement with earlier reports by Fytianos et al. (1985) Presence of organochlorine compounds in food commodities reported by many researchers like Kumari et al. (1996), Kumari et al. (2003), Kaphalia et al. (1990) revealed wide spread contamination due to these pesticides although their use has been either banned or restricted during the last one decade. Hence, the present research will not only serve as reference document but also be helpful in taking necessary and timely preventive measures to mitigate such problems. As reproted by Swarupa et al., (2016) the increase in frequency and magnitude of residues in the curry leaf could be attributed to indiscriminate and over use of pesticides by farmers despite efforts by various concerned agencies. It has been found that the farmers are neither following recommended waiting periods nor abide by good agricultural practices (GAP). (Bhanti et al.,2004). Therefore an effective way of educating the farmers via training and electronic media is advised particularly in view of the export potential of the crop. A periodical monitoring studies of pesticide residues may be extended.
to different agro climatic regions to know actual status of contamination and to strengthen the confidence of consumer in quality of food as well as food quality control authorities for future policies.

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Table.1: LC MS/MS Operating Parameters

| LC-MS/MS | SHIMADZU LCMS/MS - 8040. |
|----------|------------------------|
| Detector | Mass Spectrophotometer |
| Column   | Kinetex, 2.6μ, C18 Column, 100 x 3.0. |
| Column oven temperature | 40°C |
| Nebulizing gas | Nitrogen |
| Nebulizing gas flow | 2.0 litres/min |
| Pump mode/flow | Gradient / 0.4 ml/ min |
| Solvents | A: Ammonium Formate in Water (10Mm)  
B: Ammonium Formate in Methanol (10Mm) |

| LC programme | solvent | Conc |
|--------------|---------|------|
| 0.01         | B Conc  | 35%  |
| 2.00         | B Conc  | 35%  |
| 7.00         | B Conc  | 60%  |
| 9.00         | B Conc  | 60%  |
| 14.00        | B Conc  | 95%  |
| 17.00        | B Conc  | 85%  |
| 19.00        | B Conc  | 70%  |
|                | 2014 | 2015 |
|----------------|------|------|
|                | Total Time Programme | 24 min |

**MEHIDIPATNAM RYTHUBAZAR**

![Graph showing number of pesticide residues detected in Mehidipatnam rythubazar during 2014 and 2015](image1)

Name of the Month

**ERRAGADDA RYTHUBAZAR**

![Graph showing number of pesticide residues detected in Erragadda rythubazar during 2014 and 2015](image2)

Name of the Month

*Fig.1:* Number of pesticide residues detected in Mehidipatnam rythubazar during 2014 and 2015

*Fig.2:* Number of pesticide residues detected in Erragadda rythubazar during 2014 and 2015
Fig. 3: Number of pesticide residues detected in L. B. Nagar rythubazar during 2014 and 2015

Fig. 4: Number of pesticide residues detected in Nalgonda rythubazar during 2014 and 2015
Fig. 5: Number of pesticide residues detected in Warangal rythubazar during 2014 and 2015

Fig. 6: Number of pesticide residues detected in Guntur rythubazar during 2014 and 2015
Fig. 7: Number of pesticide residues detected in Nellore rythubazar during 2014 and 2015

Fig. 8: Number of pesticide residues detected in Vijayawada rythubazar during 2014 and 2015
Fig. 9: Number of markets detected with pesticide residues month-wise in curry leaf samples during 2014-15