Evaluation of curryleaf Farmgate Samples for Pesticide Residues

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Abstract— *Seventy five curry leaf samples from curry leaf grown farms in Guntur, Ananthapur and Medak districts of Andhra Pradesh and Telangana, India were analysed for pesticide residues by LCMS/MS. Recovery studies were performed at 0.025 0.05 and 0.5 mg kg -1 fortification levels of each compound and the recoveries obtained ranged from 81.0 % to 119.0 % with relative standard deviations lower than 19.1 %.The method showed good linearity over the assessed range 0.01–1.0 mg kg-1with correlation coefficient of 0.999 and the detection and quantification limits for the pesticides studied varied from 0.001mg Kg-1and 0.05 mg Kg-1, respectively. The samples were detected with number of compounds of different groups dominated by Organo Phosphates (29.6%) followed by Synthetic Pyrethroids (20.69%), Fungicides (13.79%), Neo nicotinoids (10.34%), Herbicides(6.90%) and Carbamates(3.45%).The residue levels ranged from 37.78 mg Kg-1 to 0.05 mg Kg-1.It is therefore necessary to conduct extensive monitoring studies on pesticide usage pattern on curry leaf in different agro-climatic regions of Andhra Pradesh and Telangana to know the exact status of pesticide contamination at farm gate level.*

Keywords— Curryleaf, farmgate, pesticide residues, quantification.

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, b-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 (Sharmila 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 more frequently and at high doses. The use of these chemicals leaves residues in the plant parts consumed as food (Agnihotri 1999), enters food chain directly or indirectly. The residues present in high quantities will affect the health of the consumers. There is a change in usage pattern from Organochlorine (OC) to other group of pesticides like organo phosphates (OP) and synthetic pyrethroids (SP), Fungicides etc., studied by various workers by analyzing farm gate samples in India (Madan et al. 1996; Parihar et al. 1997). 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 at farm gate and to suggest them the proper dosages, waiting periods etc. these studies were conducted to know the type of pesticides used by the farmers and their residues in samples at farmgat.

II. MATERIAL AND METHOD

Farm gate samples of curry leaf were collected in villages of three selected districts i.e., Medak, Guntur and Anantapur. A total of 75 samples were collected from Medak (10), Guntur (25) and Anantapur (40).Curry leaf sample of 1 kg (1/4 kg each, randomly from four different locations in the field was collected from each farmer in Medak, Guntur and Anantapur districts.

Samples were extracted for pesticide residues following the validated QuEChERS method. These extracts were analyzed by LC-MS/MS Each sample was processed and analyzed for determination of pesticides. Samples were analyzed within 24 hrs after collection.

2.1 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 randomly from 5 locations of the farm in polythene bags. Each sample was homogenized separately with robot coupe blixer 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 2500-3000rpm. 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 95.10to 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 mg kg-1 were prepared by suitably diluting the stock solution in methanol and used as standard check in analysis, linearity and recovery studies

2.2 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 ie.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 levels of pesticides present in commodity was estimated using the formula: = (Peak area of sample × Volume of sample injected x Concentration of standard injected × Dilution Factor) / Peak area of standard x Volume of standard injected

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$$\frac{\text{Peak area of sample} \times \text{Volume of sample injected} \times \text{Concentration of standard injected} \times \text{Dilution Factor}}{\text{Peak area of standard} \times \text{Volume of standard injected}}$$

III. RESULTS AND DISCUSSIONS

A multi residue method was used to monitor the pesticide residues by LC MS-MS and the pesticides detected are shown in table - 1) and quantified..The results of the present study indicated that all 75 (100%) curry leaf samples were contaminated with more than one pesticide. It is found that 100% of farm gate curry leaf samples are contaminated with more than 1 pesticide and showed much higher levels of contamination than any other fruits and vegetables studied by several workers from different parts of India.

3.1 Medak district

Of the ten farm gate curry leaf samples analyzed, insecticide residues representing three major chemical groups i.e. organophosphates and synthetic pyrethroids were detected. Thirty-nine pesticides were detected in various concentration ranges. Seven (70%) samples were highly contaminated with chlorpyriphos, Monocrotophos, ethion, quinalphos, triazophos, alpha-cypermethrin, bifenthrin and permethrin. Residue levels of monocrotophos was high (25.742 mg kg^{-1}) while those of dichlorvos were least (0.001 mg kg^{-1}). In addition to these insecticides; fungicides and herbicides were also detected. Among fungicides, seven (70%) samples were highly contaminated with metalaxyl while one (10%) sample was least contaminated with chlorothalonil, tebuconazole and trifloxystrobin. Residue levels of carbendazim was high (29.317 mg kg^{-1}) while trifloxystrobin was least (0.101 mg kg^{-1}). Among herbicides, six (60%) samples were highly contaminated with butachlor while one (10%) sample was least contaminated with alachlor. Residue levels of pendimethalin was high (29.588 mg kg^{-1}) while alachlor and butachlor were least (0.007 mg kg^{-1}). Number of samples contaminated, per cent contamination and residue range is presented in table -2

3.2 Guntur district

Curry leaf samples (25) were collected and analyzed for estimation of insecticide residues. Forty-five pesticides were detected in total, which included organophosphates and synthetic pyrethroids. Clorpyriphos, ethion, malathion, alpha-cypermethrin, bifenthrin and permethrin were found in the highest number of samples i.e., twenty-three (92%) while least contaminated with endosulfan sulfate, phenthoate, chlorfenvinphos and fenvalerate in one (4%) sample. Cypermethrin was found in high residue concentration of 25.94 mg kg^{-1} Fungicides and herbicides were also detected. Twenty three (92%) of samples were highly contaminated with metalaxyl while one (4%) sample was least contaminated with trifloxystrobin. Residue levels of carbendazim was least (0.001 mg kg^{-1}) and high (22.881 mg kg^{-1}). Butachlor and pendimethalin were found in more number of samples i.e., nineteen (76%) while alachlor in four (16%) samples. Residue levels of pendimethalin was high (17 mg kg^{-1}) while butachlor was least (0.022 mg kg^{-1}). Number of samples contaminated, per cent contamination and residue range is presented in table .3

3.3 Anantapur district

Forty farm gate curry leaf samples were analyzed for estimation of pesticide residues. Five major chemical groups i.e. organophosphates, synthetic pyrethroids, neonicotinoids, carbamates and avermectins were observed. High contamination with chlorpyriphos (25 - 62.5%) while least contamination with dimethoate, dichlorvos, carbofuran and abamectin (1 - 2.5%) was noticed. Residue levels of triazophos was high (7.356 mg kg^{-1}) while chlorpyriphos, acephate, chlorpyriphos-methyl, profenophos, anilophos, fenpropathrin and cypermethrin were least (0.012 mg kg^{-1}). Pendimethalin was the only herbicide detected in three (7.5%) of samples with residue range of 0.188-0.904 mg kg^{-1}. Eleven (27.5%) samples were highly contaminated with carbendazim while 1 (2.5%) samples were least contaminated with metalaxyl. Residue levels of carbendazim was high (1.816 mg kg^{-1}) while tebuconazole, hexaconazole and carbendazim were least (0.012 mg kg^{-1}). Number of samples contaminated, per cent contamination and residue range is presented in table .4 Shah et al.,2000) recorded that 68% of the samples were contaminated out of 214 vegetable samples in Gujrat, Chahal et al., (1997) found 68% contaminated among 96 farm gate vegetables in Punjab. Kole et al.,(2002) observed that 50% of the samples were contaminated among 149 farm gate samples in west Bengal. The contamination was mainly due to organophosphate insecticides, among the 28 pesticides detected 13(46.4%) were Organophosphates followed by 4 Synthetic pyrethroids (14.3%), 4 fungicides (14.3%), 3 Neo nicotinoids (10.7%), 2 herbicides (7.1%), 1 carbamate (3.6%) and 1(3.6%) belong to new group of pesticide called Oxadiazine. 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. 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.

TABLE 1
LC MS/MS OPERATING PARAMETERS

LC-MS/MS	SHIMADZU LCMS/MS - 8040.		
Detector	Mass Spectrophotometer		
Column	Kinetex, 2.6µ, C18 Column, 100 x3.0.		
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	**Time**	**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%
	21.00	B Conc	35%
	24.00	B Conc	35%
Total TimeProgramme	24 min		

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