

Risk Mitigation for Removal of Pesticide Residues in Curry Leaf for Food Safety

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Abstract The increased consumer awareness and legal issues on food safety with special reference to insecticide residues in foods, led to attempt for cheap and effective methods for removal of pesticide residues as the farmers are not following the Good Agricultural Practices. The most commonly used pesticides such as Profenofos, Triazophos, Ethion, Carbendazim, Chlorpyrifos and Cypermethrin were sprayed at recommended doses at vegetative stage and samples collected at 2 hours after treatment to quantify the deposits. The samples were subjected to various household treatments and analysed for residues using validated QuEChERS method, to estimate the percent removal and their effectiveness. For effective decontamination of all pesticides no single treatment was commonly effective, however the best household method for removal of pesticide residues were tap water wash and dipping in salt solution and also the method is effective in reducing the residues below MRL (Maximum Residue Limits).

Keywords Pesticide Residues, Curryleaf, Food Safety, Risk Mitigation, MRL

1. Introduction

Murraya koenigii L. (curry leaf) belonging to family Rutaceae is a leafy spice characterizing authentic Asian-Indian cuisine and it is used in small quantities for its distinct aroma as well as for preservation purposes., Curry leaf oil an volatile oil produced from the plant has uses in the soap industry (Salikutty and Peter, 2008). Recent studies have shown that carbazole alkaloids have several biological activities such as anti carcinogenic effects in dimethyl hydrazine (DMH) treated rats (Khanum *et al.*, 2000), anti platelet activity and vaso relaxing effects (Wu *et al.*, 1998). Chevalier (1996) also reported that curry leaf has medicinal value as traditionally used in Eastern Asia. Interest in greater use of curry leaf has been stimulated since its high antioxidant potency was reported and this antioxidant activity is attributed due to maha nimbine, murrayanol and mahanine from *M. koenigii* (Tachibana *et al.*, 2003; Ningappa *et al.*, 2008). Chowdhury *et al.* (2001) reported that these alkaloids have antimicrobial activity against gram positive and negative bacteria, and fungi. Lee *et al.* (2002) noted that enrichment of phenolic compounds within the plant extract is correlated with their enhanced antioxidant activity. It is reported to have antioxidant, anti-diabetic, anti carcinogenic, anti dysenteric stimulant, hypo glycaemic and anti microbial activities (Khanum *et al.*, 2000). Biologically

active carbazole alkaloids are reported to have anti microbial properties (Ramsewak *et al.*, 1999). Curry leaves have been reported to contain tocopherol, b-carotene, lutein and alkaloids (Khanum *et al.*, 2000). But it is observed that curry leaves have received red alert message from the European Union, the major importers, as the pesticide residue limits were found much beyond the permissible levels.

Maximum Residue Limits (MRLs) are set by Codex Alimentarius Commission (CAC) at international level and as on date, no MRLs are set for any pesticides on curryleaf, and by Food Safety and Standards Authority of India (FSSAI) of Ministry of Health and Family Welfare, Government of India, as per Food Safety and Standards Act, 2006 at national level based on the Good Agricultural Practices. To control the major insect pest of curry leaf farmers apply insecticides at almost weekly interval, and hence the risk of pesticide residues in foods need to be addressed as per FSSAI (Food Safety and Standards Authority of India) for the protection of consumer health and interests. In this context, household risk mitigation methods for removal of pesticide residues in curry leaf are to be recommended based on the scientific evaluation, as the food habits are changing enormously.

2. Materials and Methods

Field trial protocol

For the **Decontamination** of pesticide residues of commonly detected pesticides a field experiment was conducted utilizing **Chlorpyrifos** 20% EC @ 300 g a i/ha (1500 ml/ha), **cypermethrin** 10% EC @ 50 g a i/ha (550

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ml/ha), **ethion** 50% EC @ 500 g a i/ha (1250 ml/ha), **profenphos** 50% EC @ 500 g a i/ha (1250 ml/ha), **Carbendazim 50 WP and triazophos** 40% EC @ 500 g ai/ha 1250 ml/ha twice, first at Vegetative stage and second spray after 10 days. Zero '0' day samples were collected for estimation of deposits of pesticide within 2 hours of last spray.

Evaluation of decontamination methods for removal of pesticide residues:

The zero day samples which are free from pests and damage were collected from various treatments separately in large quantities and made into thirteen sets, each in four replications. One set of the sample from each treatment (in four replications) was analyzed for deposits of the pesticide. The remaining sets of samples of zero day from each treatment samples were subjected to various decontamination methods separately and the residues were calculated to know the efficiency of the various decontamination methods in the removal of pesticide residues from the curry leaf samples. The decontamination methods used in the study / risk mitigation methods are given (Table 1).

Per cent removal of pesticide:

$$\text{Per cent removal} = \frac{\text{Initial deposit} - \text{Residues after treatment}}{\text{Initial deposit}} \times 100$$

Sample extraction procedure

Curry leaf samples were collected from the fields sprayed with insecticides and brought to the laboratory and analyzed for pesticide residues following the AOAC official method 2007.01 (QuEChERS) after validation of the method in the laboratory. Each sample was homogenized separately with robot coupe blixer and homogenized 15 ± 0.1 g 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 (graphitised carbon black). 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 2).

Table 1. Decontamination methods used in the study or risk mitigation methods

Treatment Number	Treatment Name	Method	
T ₁	Tap water	Four litres of tap water was taken	in the plastic tub of 7 litres capacity 2 kg of curry leaves were dipped for 10 min, followed by washing with tap water for 30 sec. Further the leaves were kept for air drying on tissue paper for 5 min, followed by analysis.
T ₂	Tamarind solution	80 g of tamarind was added to 4 litres of water	
T ₃	Lemon water	Juice of 4 lemons was added to 4 litres of water	
T ₄	Salt solution	Four litres of 2 per cent salt solution was prepared by mixing 80 g of table salt in 4 litres of water	
T ₅	Butter milk	Four litres of butter milk was prepared by mixing 80 g of curd in 4 litres of water	
T ₆	Bio wash	Four litres of bio wash was prepared by mixing 8 ml of commercial formula bio wash to 4 litres of water	
T ₇	Acetic acid	Four litres of 4 per cent acetic acid solution was prepared by mixing 160 ml of 100 per cent glacial acetic acid in 4 litres of water	
T ₈	Baking soda (NaHCO ₃)	Four litres of 0.1 per cent baking soda solution was prepared by mixing 4 g of baking soda in 4 litres of water	
T ₉	Formula 1 (4% Acetic acid + 0.1% NaHCO ₃ + 1 Lemon l ⁻¹)	Four litres of formula 1 was prepared by mixing 160 ml of acetic acid, 4 g of sodium bicarbonate and lemon juice of 4 lemons added to 4 litres of water	
T ₁₀	Cooking	2 kg of curry leaf sample was cooked in pressure cooker for 5 min, further the leaves were kept for air drying on tissue paper for 5 min	followed by analysis
T ₁₁	Frying	2 kg of curry leaf sample was fried in oil by repeated stirring until they become brittle by loosing entire moisture approximately for 5 min	
T ₁₂	Drying	2 kg of curry leaf sample spread over on a tissue paper and shade dried for approximately 5 days until entire moisture was lost in leaves	

Table 2. LCMS/MS standard operating parameters

LC-MS/MS	SHIMADZU LC-MS/MS - 8040.		
Detector	Mass Spectrophotometer		
Column	Kinetex, 2.6 μ , C18 Column, 100 x 3.0.		
Column oven temperature	40°C		
Retention time	Profenophos -16.15 min, Triazophos -13.21 min, Chlorpyrifos -16.75 min, Cypermethrin -17.4 min, Ethion -16.6 min, carbendazim		
Nebulizing gas	Nitrogen		
Nebulizing gas flow	2.0 litres/min		
Pump mode/ flow	Gradient / 0.4 ml/ min		
LC Solvents	A: Ammonium Formate In Water (10Mm) B: Ammonium Formate In Methanol (10Mm)		
LC programme	Time	A Conc	B Conc
	0.01	65	35
	2.00	65	35
	7.00	40	60
	9.00	40	60
	14.00	05	95
	17.00	15	85
	19.00	30	70
21.00	65	35	
24.00	65	35	
Total Time Programme	24 min		

Table 3. Recoveries of Profenofos, carbendazim, Triazophos, Chlorpyrifos, Cypermethrin and Ethion at various fortification levels in curryleaf samples

		Pesticides						
		Profenophos	Carbendazim	Triazophos	Chlorpyrifos	Cypermethrin	Ethion	
Fortification Levels (mg/kg)	0.05	Calculated Level	0.053	0.053	0.043	0.047	0.058	0.05
		Average						
	0.25	% Recovery	106.66	106.66	85.33	94.66	115.33	101.33
		Calculated Level	0.207	0.210	0.210	0.261	0.244	0.205
	Average							
	0.50	% Recovery	83.06	84.00	84.00	104.53	97.60	82.00
Calculated Level		0.488	0.578	0.488	0.578	0.574	0.506	
Average								
	% Recovery	98.06	115.60	97.60	115.60	114.86	101.13	

Results of fortification and recovery studies in curryleaf:

The control curryleaf samples were fortified at 0.50 mg/kg, 0.25 and 0.05 mg/kg levels adding required quantity of Profenofos, Triazophos, Ethion, Chlorpyrifos, Carbendazim and Cypermethrin standards and replicated thrice. The following are the recoveries of Profenofos, Triazophos, Ethion, Chlorpyrifos, carbendazim and Cypermethrin at three different fortification levels (Table-3).

3. Results and Discussion

The residues of, Triazophos, Chlorpyrifos, Ethion, Carbendazim, Cypermethrin and Profenofos in curryleaf samples have got substantial reduction by different house

hold processing methods. The reduction percentage and residue levels are presented in Table 4 and depicted in Fig 1.

Triazophos

Triazophos is a non-systemic, broad spectrum, organophosphorus insecticide with contact and stomach action, acaricide with some nematicidal properties. Frying curry leaf for 10 min was found to be most effective with 27.53 per cent decontamination when compared to other treatments. The percentage removal of triazophos residues due to various decontamination treatments in descending order are tamarind water (27.20%) > biowash (25.43%) > butter milk (24.44%) > formula 1 (24.40%) > tap water (18.43%) > acetic acid (17.74%) > drying (17.33%) > lemon water (14.06%) > salt solution (13.84%) > sodium

bicarbonate (12.66%) > cooking (9.07%). Based on the percentage removal of residues, it is statistically proved that there is significant difference in the efficiency of decontaminating methods in removing triazophos residues. Percentage removal of triazophos residues from various decontamination methods is depicted in Fig 2.

Chlorpyrifos

Various decontamination methods were evaluated in order to know their efficiency in removing chlorpyrifos residues from curry leaf. Results revealed that all the treatments significantly differed among each other in their efficiency in removing chlorpyrifos residues. Dipping in formula 1 for 10 min was found to be most effective with 58.76 per cent decontamination when compared to other treatments. Next promising treatment was dipping in Butter milk (46.55%). Other treatments like tamarind water (44.28%), tap water (43.37%), cooking (40.27%), biowash (39.13%), sodium bicarbonate (35.28%), frying (32.09%), lemon water (27.81%), acetic acid (22.26%), salt solution (6.97%) and

drying (2.23%) were also found to remove significant amount of residues from curry leaf. Percentage removal of chlorpyrifos residues from various decontamination methods is depicted in Fig 3.

Ethion

Curry leaf sprayed with recommended dose of ethion were collected two hours after spraying and subjected to various decontamination methods. Among the different treatments employed, dipping leaves in Formula 1 solution for 10 min was found to be more effective with 19.44 per cent decontamination than other treatments. Biowash and sodium bicarbonate were found to be next promising treatments with 17.54 per cent decontamination followed by salt solution (15.15%), tamarind water (14.39%), cooking (12.99%), butter milk (12.03%), acetic acid (10.45%), drying (6.67%), tap water (4.14%), frying (2.84%) and lemon water (2.19%). Percentage removal of ethion residues from various decontamination methods is depicted in Fig 4.

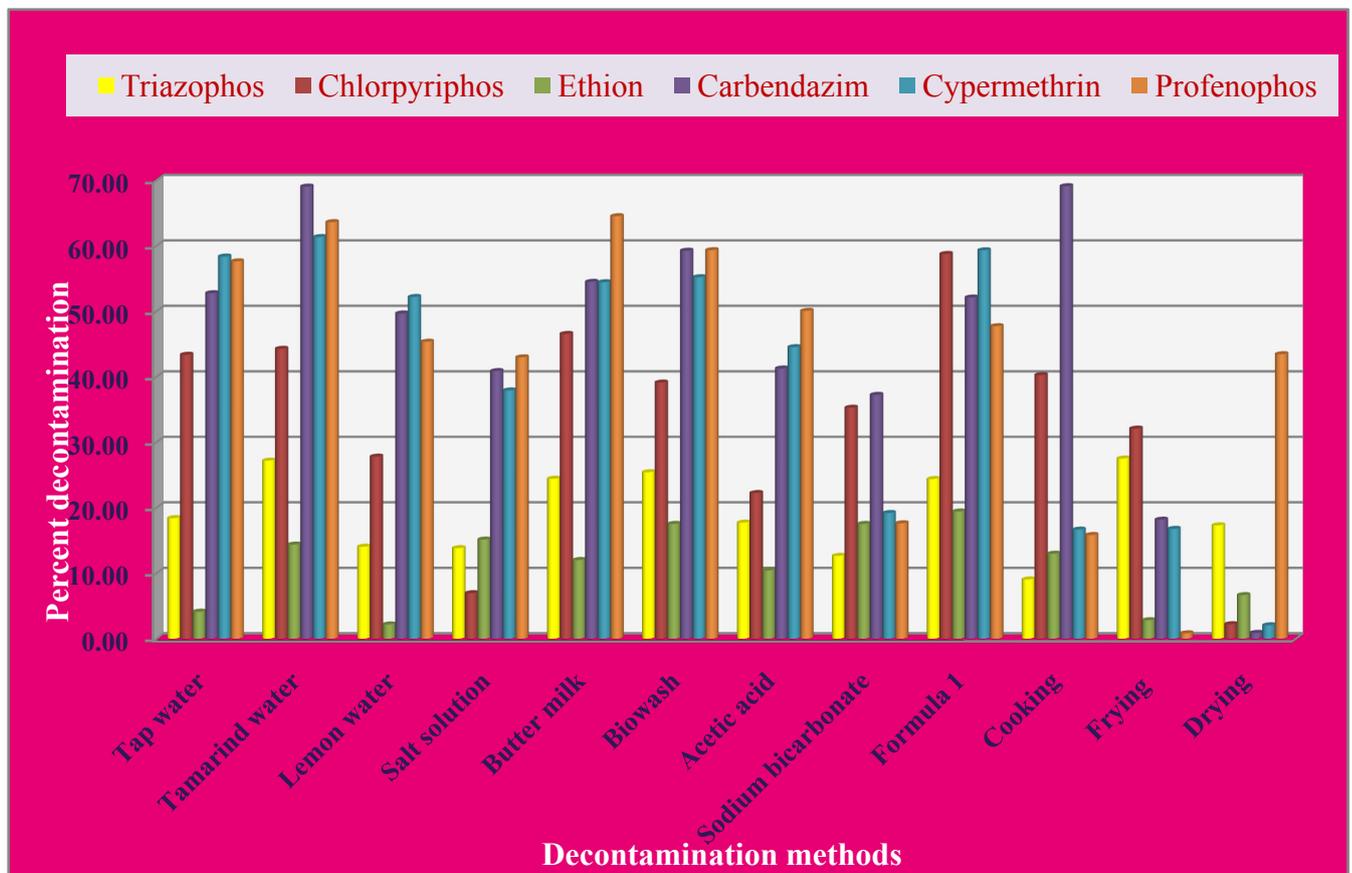


Figure 1. Per cent removal of pesticide residues from curry leaf by various decontamination methods

Table 4. Effectiveness of various decontamination methods

S. No.		Mean per cent removal of pesticide residues (%) ± SD											
		Pesticides											
		Triazophos 40 EC		Chlorpyrifos 20 EC		Ethion 50 EC		Carbendazim 50 WP		Cypermethrin 10 EC		Profenophos 50 EC	
Decontamin-ation methods		Initial deposit (mg kg ⁻¹)	18.19	14.73		21.04		16.00		13.09		19.83	
		Residues after treatment (mg kg ⁻¹)	Percent removal	Residues after treatment (mg kg ⁻¹)	Percent removal	Residues after treatment (mg kg ⁻¹)	Percent removal	Residues after treatment (mg kg ⁻¹)	Percent removal	Residues after treatment (mg kg ⁻¹)	Percent removal	Residues after treatment (mg kg ⁻¹)	Percent removal
1	Tap water	14.84±0.36	18.43±1.11	8.34±0.34	43.37±1.20	20.17±0.37	4.14±1.10	7.56±0.40	52.77±1.23	5.45±0.31	58.36±1.30	8.40±0.36	57.64±1.32
2	Tamarind water	13.24±0.32	27.20±0.29	8.21±0.36	44.28±1.11	18.01±0.43	14.39±0.50	4.95±0.40	69.05±0.42	5.06±0.48	61.35±0.37	7.21±0.31	63.62±0.61
3	Lemon water	15.63±0.36	14.06±0.23	10.63±0.33	27.81±0.67	20.58±0.43	2.19±0.40	8.05±0.28	49.68±0.35	6.26±0.51	52.19±0.38	10.84±0.33	45.36±0.43
4	Salt solution	15.67±0.55	13.84±0.39	13.70±1.44	6.97±0.46	17.85±0.68	15.15±0.36	9.46±0.04	40.88±2.81	8.12±2.14	37.93±2.50	11.31±0.05	42.98±2.19
5	Butter milk	13.74±1.15	24.44±0.55	7.87±0.81	46.55±0.95	18.51±0.81	12.03±0.95	7.28±1.85	54.51±0.74	5.96±1.04	54.45±0.72	7.03±1.57	64.53±0.72
6	Biowash	13.56±0.95	25.43±0.23	8.97±0.75	39.13±0.42	17.35±1.37	17.54±0.36	6.52±1.11	59.26±0.34	5.86±1.20	55.22±0.37	8.06±1.10	59.34±0.40
7	Acetic acid	14.96±0.40	17.74±0.50	11.45±0.43	22.26±1.11	18.84±0.36	10.45±0.29	9.39±0.32	41.27±1.32	7.26±0.36	44.53±1.30	9.90±0.31	50.08±1.23
8	Sodium bicarbonate	15.89±0.28	12.66±0.35	9.53±0.51	35.28±0.38	17.35±0.33	17.54±0.43	10.04±0.55	37.25±0.39	10.58±1.44	19.21±0.46	16.33±0.68	17.63±0.36
9	Formula 1	13.75±0.95	24.40±0.81	6.07±0.95	58.76±0.81	16.95±0.55	19.44±1.15	7.66±2.19	52.11±0.05	5.32±2.50	59.33±2.14	10.36±2.81	47.74±0.04
10	Cooking	16.54±1.85	9.07±0.74	8.80±1.04	40.27±0.72	18.31±1.57	12.99±0.72	4.94±0.40	69.11±1.10	10.91±0.37	16.67±1.20	16.69±0.34	15.85±1.11
11	Frying	13.18±0.49	27.53±0.86	10.00±0.56	32.09±0.32	20.44±0.29	2.84±0.28	13.09±0.75	18.19±1.85	10.89±2.19	16.79±1.10	19.66±0.40	0.84±0.95
12	Drying	15.04±0.17	17.33±0.95	14.40±0.45	2.23±0.34	19.64±0.23	6.67±0.40	15.86±0.95	0.90±0.32	12.82±0.61	2.07±0.37	11.21±0.38	43.46±1.85

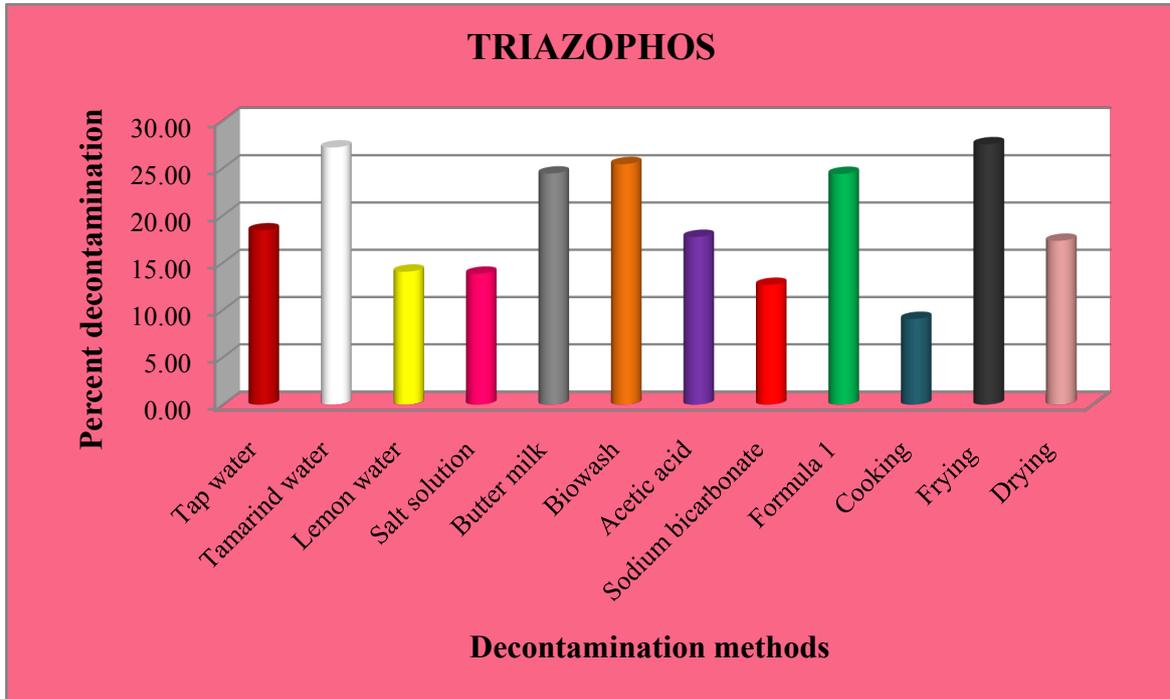


Figure 2. Per cent removal of triazophos residues from curry leaf by various decontamination methods

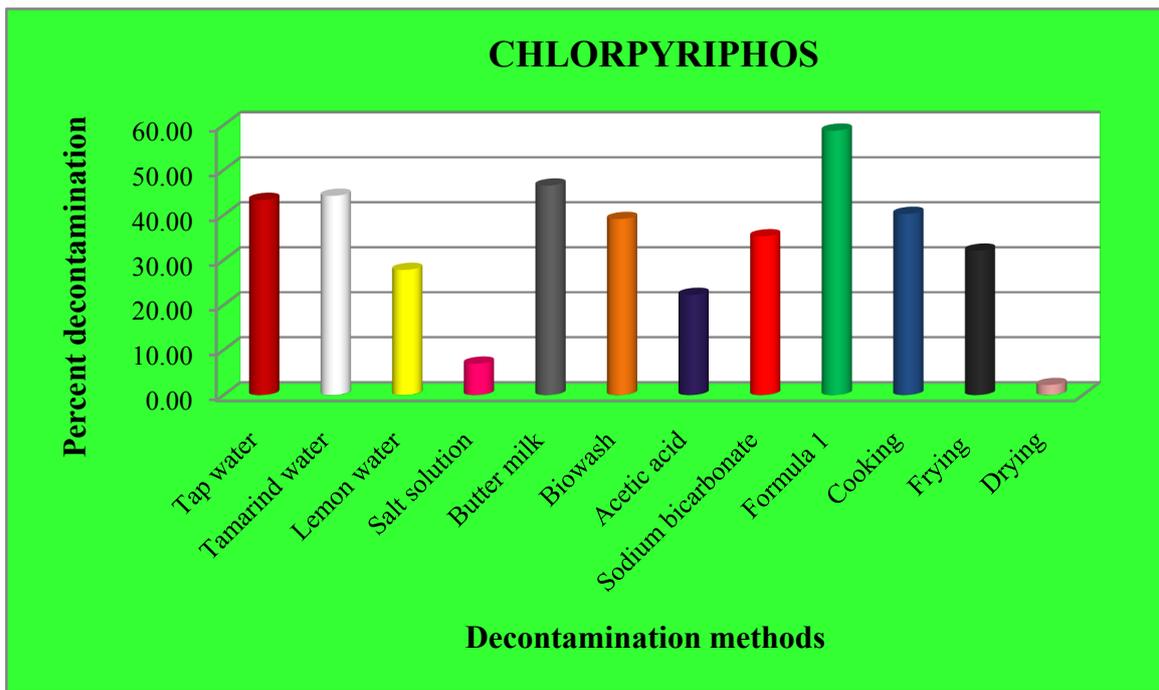


Figure 3. Per cent removal of chlorpyrifos residues from curry leaf by various decontamination methods

Carbendazim

Carbendazim is a systemic fungicide with protective and curative action. Cooking of curry leaf for 10 min in pressure cooker was found to be most effective treatment with 69.11 per cent reduction in residues of carbendazim than other treatments. The percentage removal of carbendazim residues due to various decontamination treatments in descending order are tamarind water (69.05%) > biowash (59.26%) > butter milk (54.51%) > tap water (52.77%) > formula 1

(52.11%) > lemon water (49.68%) > acetic acid (41.27%) > salt solution (40.88%) > sodium bicarbonate (37.25%) > frying (18.19%) > drying (0.90%). Based on the percentage removal of residues, it was statistically proved that there is significant difference in the efficiency of decontaminating solutions in removing carbendazim residues. Percentage removal of carbendazim residues from various decontamination methods is depicted in Fig 5.

Cypermethrin

Cypermethrin residues in curry leaf were removed significantly when subjected to different decontamination solutions after two hours of spraying. Results revealed that dipping in tamarind water for 10 min was found to be most effective than other treatments. In this treatment residues were reduced up to 61.35 per cent. Next promising treatment

was formula 1 (59.33%) followed by tap water (58.36%), bio wash (55.22%), butter milk (54.45%), lemon water (52.19%), acetic acid (44.53%), salt solution (37.93%), sodium bicarbonate (19.21%), frying (16.79%), cooking (16.67%) and drying (2.07%). Percentage removal of cypermethrin residues from various decontamination methods is depicted in Fig 6.

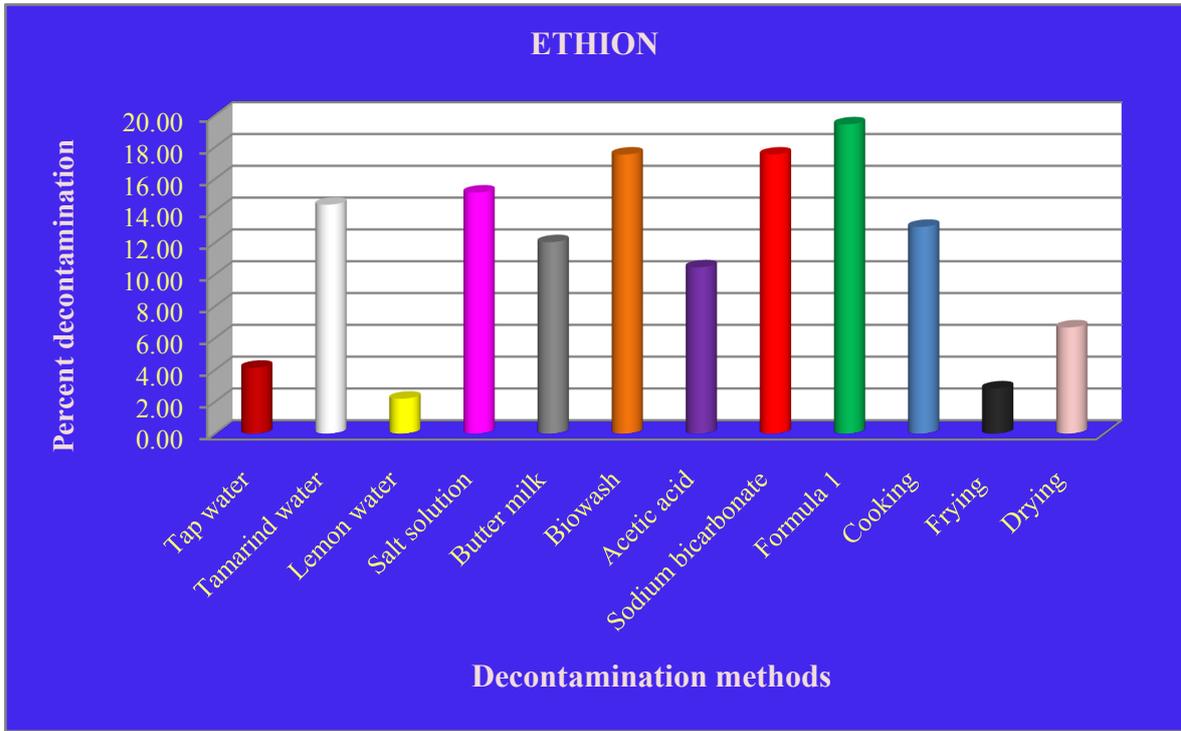


Figure 4. Per cent removal of ethion residues from curry leaf by various decontamination methods

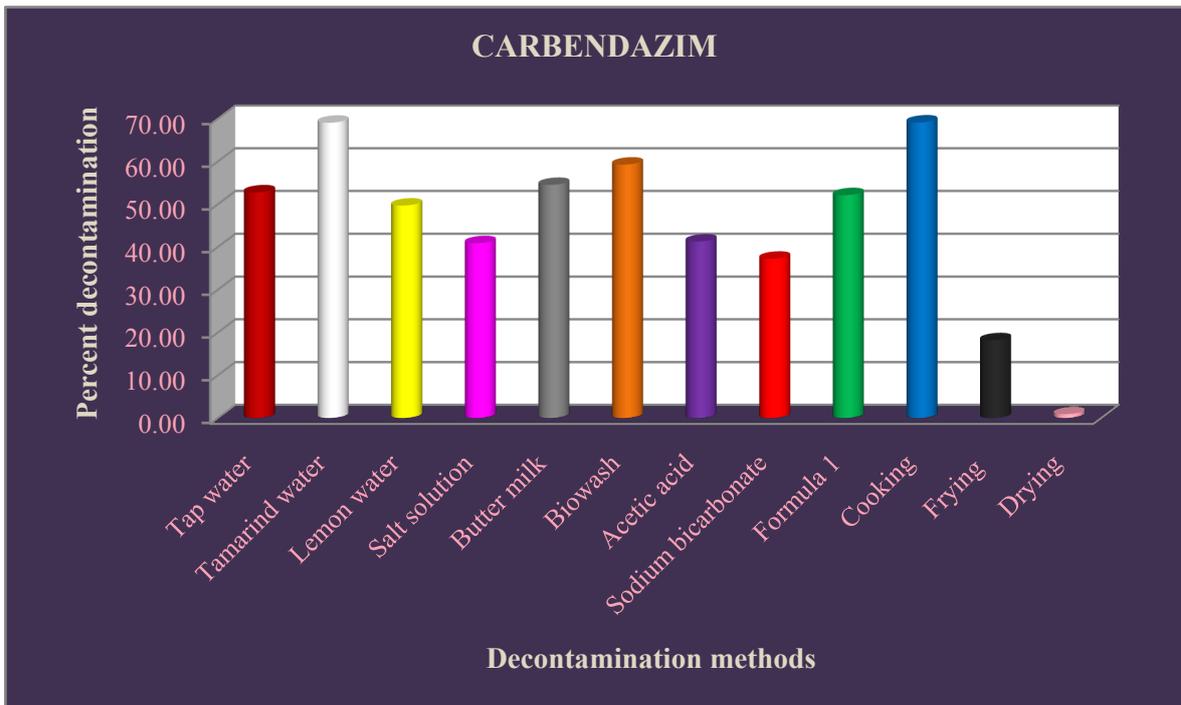


Figure 5. Per cent removal of carbendazim residues from curry leaf by various decontamination methods

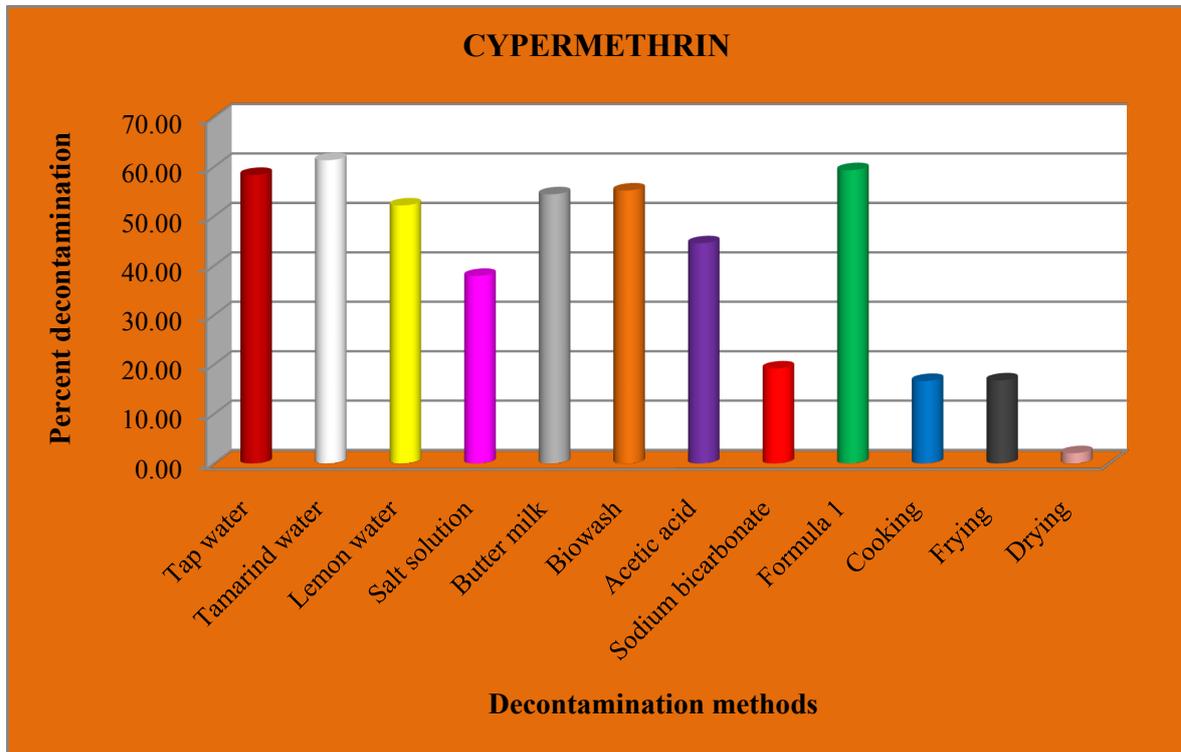


Figure 6. Per cent removal of cypermethrin residues from curry leaf by various decontamination methods

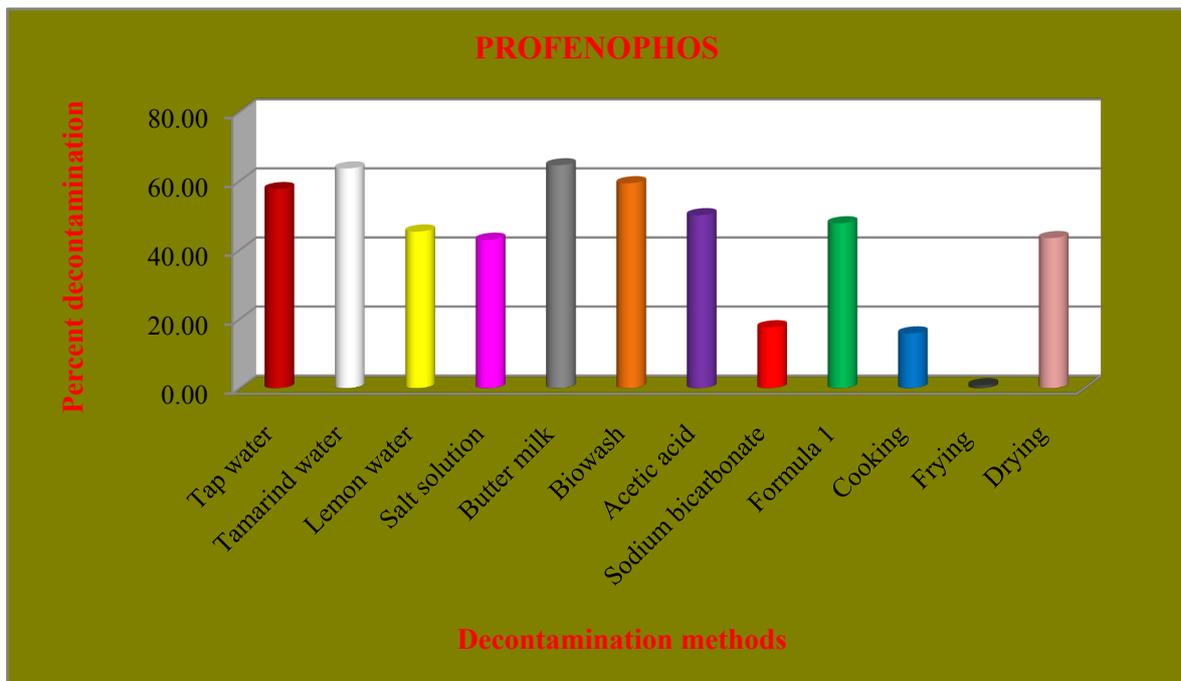


Figure 7. Per cent removal of profenophos residues from curry leaf by various decontamination methods

Profenophos

The percentage removal of profenophos residues in curry leaf when subjected to different decontamination solutions after two hours of spraying showed that dipping in butter milk for ten min was found to be most effective removing 64.53 per cent residues, than other treatments. The next promising treatment was washing with tamarind water (63.62%), followed by bio wash (59.34%), tap water

(57.64%), acetic acid (50.08%), formula 1 (47.74%), lemon water (45.36%), drying (43.46%), salt solution (42.98%), sodium bicarbonate (17.63%), cooking (15.85%) and frying (0.84%). Fig -7. Based on the percentage removal of residues, it was statistically proved that there is significant difference in the efficiency of decontaminating methods in removing residues of above mentioned pesticides. Per cent removal of pesticide residues from curry leaf by various

decontamination methods is depicted in fig 1.

Scientists and food processors have long been interested in the effect of processing on pesticide residues in food commodities. The extent to which pesticide residues are removed by processing depends on a variety of factors, such as chemical properties of the pesticides, the nature of food commodity, the processing step and the length of time the compound has been in contact with the food (Farris et al., 1992., Holland et al., 1994 and Kumar et al., 2010). In a developing country like India, dissipation techniques at the household level can serve as an effective tool in reducing risk related to dietary exposure to residues and henceforth controlling pesticide related adversities. Washing is the most common form of processing which is a preliminary step in both household and commercial preparation. Loosely held residues of several pesticides are removed with reasonable efficiency by varied types of washing processes (Street, 1969). Several studies have examined the effects of washing solutions on removing pesticide residues from various food commodities as follows. To minimize dietary exposure to pesticides, it is pertinent to explore strategies that effectively help in reducing the residue content at individual level. Twelve simple, labour-less and cost effective unit operations were imparted to curry leaf samples for reducing dietary consumption of pesticide residues which can be even followed in poor populace. Out of all treatments imparted each pesticide has its own treatment of reduction. There is no common treatment observed which can remove all pesticides effectively. Triazophos was effectively removed by frying (27.53%). In case of other pesticides scenario is like chlorpyrifos (formula 1 - 58.76%), ethion (formula 1 - 19.44%), carbendazim (cooking - 69.11%), cypermethrin (tamarind water - 61.35%) and profenophos (butter milk - 64.53%). The results obtained in the present studies are in line with the work done by Pallavi *et al.* (2014) who observed a loss of 62.4, 54.4, 56.2, 68.2 and 45.6 per cent of malathion, chlorpyrifos, quinalphos, profenophos and cypermethrin residues in curry leaf by treating with 2 per cent common salt for 15 min and reduction of 41.8, 47.3, 51.48, 52.77 and 50.25 by treating with 2 per cent vinegar for 15 min. she also observed reduction upto 66.7, 11.4, 41.9, 48.3 and 22.7 per cent of malathion, chlorpyrifos, quinalphos, profenophos and cypermethrin by treating with 2 per cent buttermilk for 15 min. whereas by by treating with 2 per cent vinegar for 15 min the loss was 41.8, 47.3, 51.48, 52.77 and 50.25 per cent respectively. Randhawa *et al.* (2008) also reported that washing reduced cypermethrin residues by 33.42-35 per cent in brinjal. Chlorpyrifos residues were reduced by 33 per cent in spinach by washing (Randhawa *et al.* 2007). Wasim *et al.* (2010) reported that washing with 4 per cent acetic acid solution, biowash and formula 1 play a role in the reduction of pesticide residues in cabbage. Direct cooking process has shown less effect in the removal of residues when compared with 2 per cent salt water washing because of all the processed pesticides are having the property of high water solubility. Similar results were observed by Shashi *et al.* (2014) in tomato. The results of

earlier workers like Raghu *et al.* (2015) were similar to our findings as they found that profenophos residues reduced to 47.64 per cent, 78.48 per cent, 65.48 per cent and 28.95 per cent by 2 per cent by salt solution, bio wash, 4 per cent acetic acid solution and 0.1 per cent baking soda solution in chilli. Geetha (2015) got reduction of chlorpyrifos residues by boiling (54.43%) in spinach which is in line with present work. Shashi *et al.* (2015) observed similar results of reducing the residues of profenophos in brinjal by 47.07 per cent by running tap water washing which proved that washing is an effective and easy tool to dislodge surface residues to greater extent in a simple way.

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