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Dissipation of Certain Pesticide Residues in Curry Leaf

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Abstract: *Dissipation of acephate, Imidacloprid and bifenthrin residues were studied in/on curry leaf (Murraya koenijii; The pesticides were sprayed twice with an interval of 10 days using knapsack sprayer at a rate of 2 ml/ lit of water. Samples were collected from each replicate (one Kg) at time intervals of one hour after application (0 day), 1, 3, 5, 7,10, 15 and 20 days after treatment with acephate, Imidacloprid and bifenthrin or until the residues dissipated to Below Determination Level. Extraction and clean up of the samples were carried out as per AOAC official method 2007.01 Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) after validation of the method at the laboratory, and estimated on LCMSMS. The initial deposits of acephate, Imidacloprid and bifenthrin were found to be 84.33 12.00 and 34.20 mg kg $^{-1}$ respectively. Results showed that acephate, Imidacloprid and bifenthrin rapidly dissipated to Below Determination Level by 25th, 20th and 25th days respectively.*

Keywords: *Dissipation pattern, curry leaf, acephate, Imidacloprid and bifenthrin*

I. Introduction

Curry leaf [*Murraya koenigii* (L.) Sprengel] is a leaf spice of the citrus family Rutaceae. Curry leaves, an inevitable part of spicing up dishes are not a part of mere garnishing. They are rich in medicinal, nutraceutical properties and have even cosmetic uses. In India, of late it is cultivated on the commercial scale in Tamil Nadu, Karnataka, Kerala, Andhra Pradesh and Telangana states and has gained importance as a major spice crop with high export potential (Mohan, 2012). A total of 12 insect pests belonging to 10 families of 5 insect orders were recorded infesting curry leaf plants (Tara and Monika Sharma, 2010). As per Insecticides act, 1968 there is no pesticide recommendation for spray on curry leaf as on today and hence there are no MRL's suggested by Codex Alimentarius Commission. However , farmers are using pesticides indiscriminately that are designed to control the pest even if there are no recommendations for the crop and whether the pest is present or not. Curry leaves constitute a major spice exported from India, and of late become one of the very important condiment spice in global food trade. But recently curry leaves have received red alert message from the European Union the major importers, where the pesticide residue limits were found much beyond the permissible levels. The high dosage of pesticides can even cause serious health troubles like cancer if consumed highly (Ramakrishnan et al, 2015). This created a panic among the mass. Many of the importing countries have imposed checks on imports of curry leaves into their territories especially to monitor pesticide residue levels. Many a times, the MRLs fixed by the importing countries are unilateral since the relatively safe pesticides in producing countries are not monitored for fixing of the residue levels. As there is no Codex MRL for curry leaves, it is suggested that Codex may set MRLs for the commonly used pesticides to avoid trade barriers. This will ensure food safety and consumer health from an overall point of view. By keeping in view all these most important issues of concern, the present study, on dissipation of newer insecticides viz., acephate, Imidacloprid and bifenthrin was carried out in curry leaf.

II. Material And Methods

The field experiment utilizing acephate, Imidacloprid and bifenthrin on curry leaf was carried out at PJTSAU student Farm and dissipation studies at AINP on Pesticide residues, Rajendra nagar, Hyderabad during Rabi 2014. Reference standards of acephate, Imidacloprid and bifenthrin obtained from sigma Aldrich were used as internal standards to correct variability in LCMSMS(Gurley et al. (1995).Standard solutions were prepared with Methanol and suitably diluted to obtain the working standards. The spray application was given twice at an interval 10 days and analysis was carried out on 0 (2 h after application), 1, 3, 5, 7, 10, 15 ,20 and 25 days after the second spray. From each plot, approximately 500 g of curry leaf was harvested, pooled together, packed in plastic bags and transported to the laboratory for processing. Extraction and clean up of the samples were carried out as per AOAC official method 2007.01 (Quechers) after validation of the method at the laboratory using LCMS .The method was validated using chromatographic parameters including recovery, reproducibility and limits of detection (LOD) as proposed by Lee et al, (1995). For recovery experiments, homogenized untreated samples were spiked with three different pesticides at 0.5, 0.25 and 0.05 mg kg^{-1} for each fortification level, three replicates were analyzed by LCMS MS the collected samples were homogenized

with robot coupe blixer, and homogenized 7.5±0.1 g sample was taken in 50 ml centrifuge tube,the sample tube is then added with 30±0.1 ml acetonitrile. the sample is homogenized at 14000-15000 rpm for 2-3 min using heidolph silent crusher. The samples then added with 3±0.1 g sodium chloride (merck) and mixed 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(fisher scientific) to remove the moisture content. 8 ml of extract was taken into 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 sampliq carbon (agilent technologies, usa) .The sample tube was vortexed for 30 sec, followed by centrifugation for 5 min at 2500-3000 rpm. The extract of about 4 ml was transferred into test tubes and evaporated to dryness using turbovap with nitrogen gas and reconstituted with 1 ml methanol in 2 ml vials from borosil by LCMS analysis under standard operational conditions.(Table -1)

Table-1 LCMS/MS Instrument parameters

LC-MS/MS	SHIMADZU LC-MS/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		
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 2. Recovery of acephatate, imidacloprid and bifentrhin at various fortification levels in Curry leaf

pesticides	0.05 mg/kg		0.25 mg/kg		0.5 mg/kg	
	Calculated Level (mg kg -1) Average	% Recovery	Calculated Level (mg kg-1) Average	% Recovery	Calculated Level (mg kg-1) Average	% Recovery
Acephate	0.053	106.66	0.207	83.06	0.488	98.06
Imidacloprid	0.043	85.33	0.210	84.00	0.488	97.60
Bifentrhin	0.047	94.66	0.261	104.53	0.578	115.60

III. Results And Discussion

The results showed that the analyses by LCMS-MS gave good recoveries (Table-2) of all pesticides at different concentrations with low RSDs (reproducibility) indicating good performance of extraction, clean up, and chromatographic parameters for residue determination in curry leaf samples. The LOD was 5 ppb for all the three pesticides. Dissipation pattern and linearity of all the pesticides are given in figs 1-6 . Dissipation of all the three pesticides was studied over a period of 25 days (Table 3). It is observed that acephate recorded initial deposits of 84.33 mg kg ^{1} which dissipated to BDL by 25 days with quick dissipation from 7th day .The initial deposit of 12.00 mg kg^{-1}of imidacloprid dissipated quickly and reached BDL by 20th day .The initial deposit of bifenthrin at 34.20 mg kg ^{1} dissipated to BDL by 25th day .The difference in dissipation of these pesticides around 20-25 days which is much longer than other crops could be attributed to different formulations, crop foliage and agro climatic conditions. Residues of acephate declined more than 50% by 5th day after spraying. The residues of bifenthrin dissipated by 50% in 3 days, whereas imidacloprid fell to 50% by 10 days. Since the maximum residue limit (MRL) of acephate, imidacloprid and bifentrhin on curry leaf are not fixed by either PFA (prevention of food adulteration, India) or codex (codex alimentarius commission, FAO/WHO) based on the persistence pattern the safe pre harvest interval (PHI) or waiting period of acephate, imidacloprid and bifentrhin on curry leaf have can be fixed as 20 days. Intensive cultivation technologies produce high infestation of crops by some pests and diseases, trigger off major losses of quality crops and initiate the use of more pesticides. The increase in frequency and magnitude of residues in the curry leaf samples 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 study 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: 3 Residues and dissipation of pesticides in curry leaf.

Days after Treatment	Acephate		imidacloprid		Bifenthrin	
	Mean (mg kg^{-1})	Sd +	Mean (mg kg^{-1})	Sd +	Mean mg kg^{-1})	Sd +
0	84.33	1.00	12.00	0.18	34.29	1.16
1	81.39	5.63	11.16	0.28	27.60	1.23
3	72.01	3.70	8.18	0.14	17.35	1.02
5	42.98	2.26	7.77	0.27	13.95	0.35
7	19.84	0.26	6.60	0.37	11.89	0.31
10	10.78	0.54	4.59	0.34	8.82	0.50
15	3.05	0.12	1.41	0.18	3.49	0.24
20	0.59	0.11	0.47	0.01	0.62	0.02
25	BDL	BDL	BDL	BDL	BDL	BDL

BDL= Below determination level (< 0.05 mg/kg)

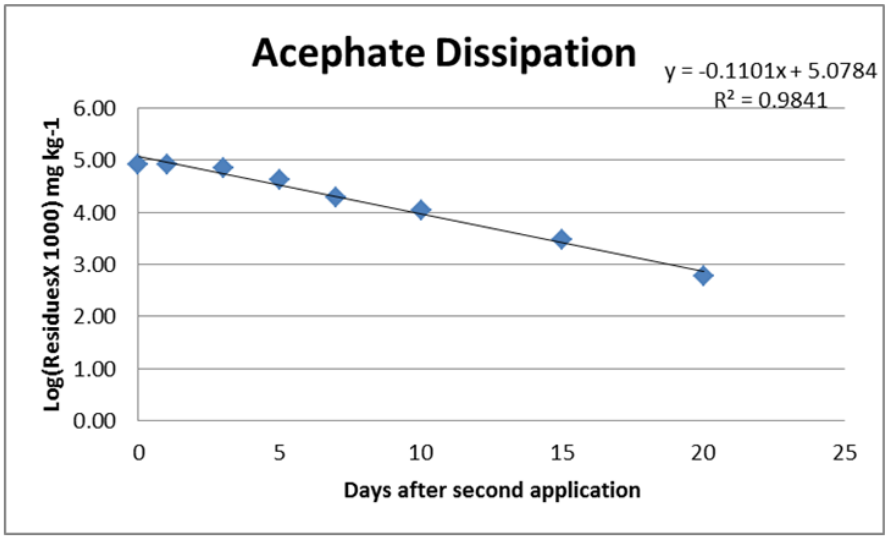

Fig.1 Semi logarithmic graph showing dissipation kinetics of Acephate in Curry Leaf

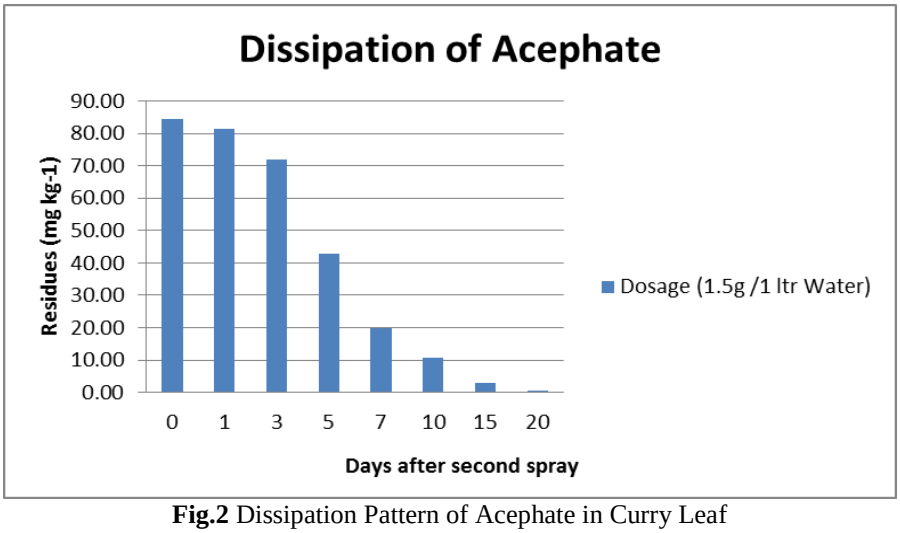

Fig.2 Dissipation Pattern of Acephate in Curry Leaf

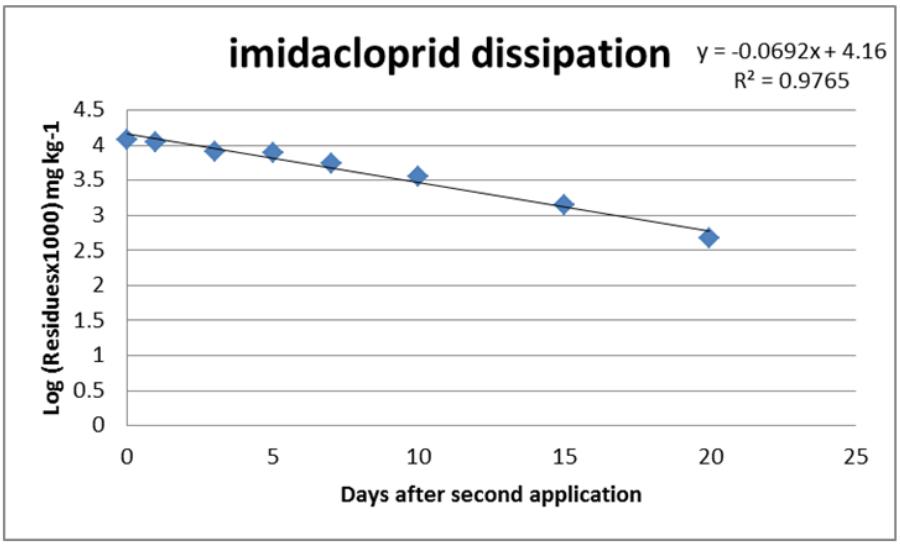

Fig.3 Semi logarithmic graph showing dissipation kinetics of imidacloprid in Curry Leaf

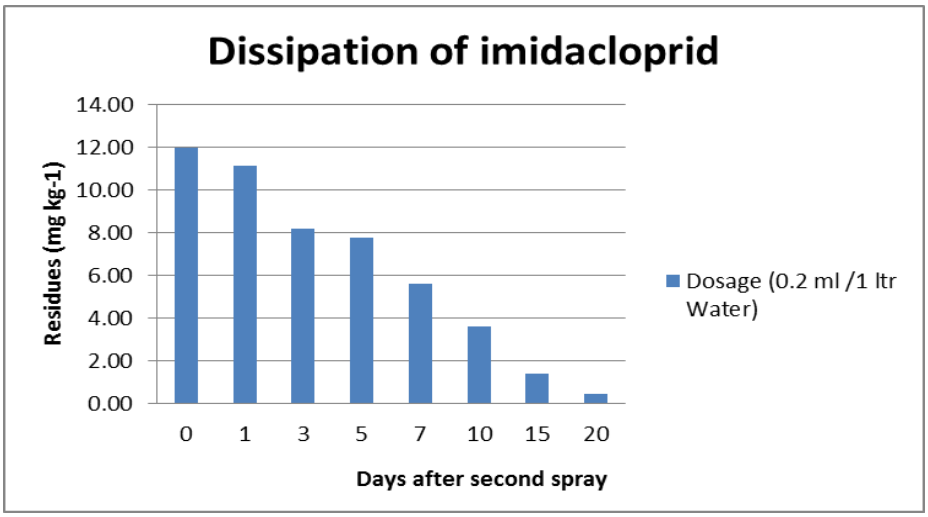

Fig.4 Dissipation Pattern of imidacloprid in Curry Leaf

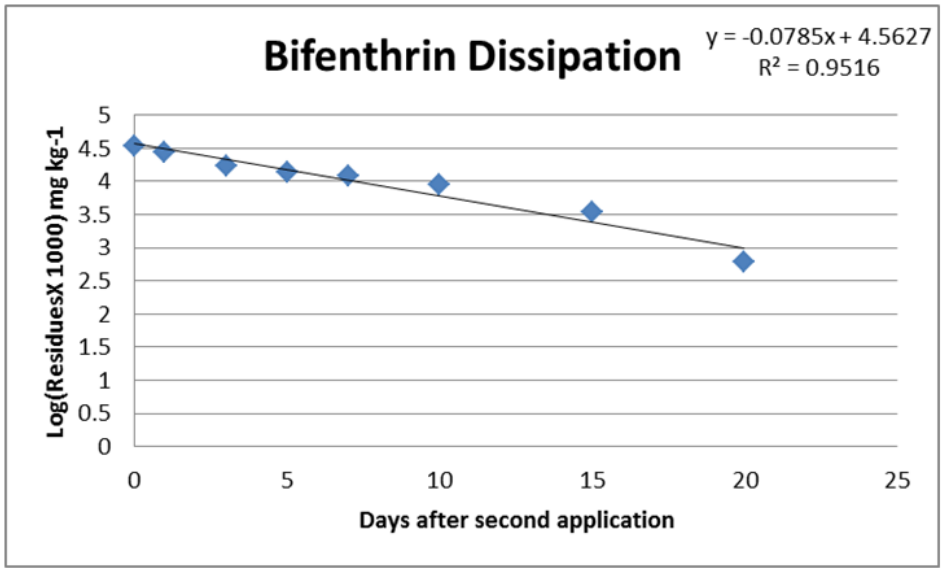

Fig.5 Semi logarithmic graph showing dissipation kinetics of bifenthrin in Curry Leaf

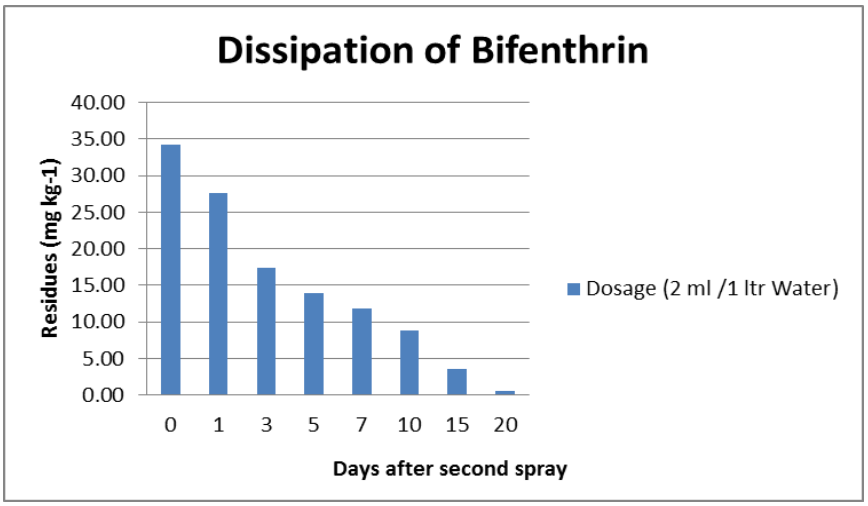

Fig.6 Dissipation Pattern of bifenthrin in Curry Leaf

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