Dissipation Pattern of *carbendazim* and *cypermethrin* on Curry Leaf

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Abstract— *Field experiments were conducted during kharif 2014 and 2015 with curry leaf variety Suwasini to study the dissipation pattern of carbendazim 50 EC at 500 g a.i. ha-1 and cypermethrin 10 % EC @ 50 g a i/ha (550 ml/ha) 1 by giving two sprays first at vegetative stage and second 10 days later. The leaf samples collected at 0, 1, 3, 5, 7, 10, 15, 20, 25, 30 and 45th day after second spray and soil samples at 45th day for residue analysis showed initial deposits of carbendazim 16.00 mg kg^{-1} and Cypermetrin 13.09 mg kg^{-1} which ,dissipated to 0.04 mg kg $^{-1}$ and 0. 28 mg kg $^{-1}$ respectively. The residues reached to Below Determination Level (BDL) at 20 and 10 days, respectively. As there were no pesticide recommendations and MRLs fixed for any of the pesticide in curry leaf, the day at which residues reached BDL can be suggested as the safe harvest period for curry leaf.*

Keywords— Dissipation pattern, , curry leaf, BDL, curry leaf.

I. INTRODUCTION

Curry leaf [*Murraya koenigii* (L.) Sprengel] is a leaf spice of the citrus family Rutaceae. Curry leaves form an integral part of spicing up dishes and 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 commercial scale in Tamil Nadu, Karnataka, 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 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. Hence, residues were detected at the farm gate level for export location and led to the rejection of the consignment (Ramakrishnan *et al.*, 2015). According to the report of the Indian delegation at 45th session of the Codex Committee on Pesticide Residues (CCPR) held at Beijing P. R. China (May 6-11, 2013) in agenda no. 11, India is considered for fixation of new and revised MRL's of profenophos, chlorpyriphos, cypermethrin, methyl parathion, triazophos, ethion and quinalphos in curry leaves based on good agricultural practice (GAP) trials and monitoring data and should submit in the prescribed format to Joint FAO/WHO Meeting on Pesticide Residues (JMPR), as a follow-up, for evaluation in 2014 for fixation of MRL on curry leaves. By keeping in view all these most important issues of concern, the present comprehensive study, on dissipation of insecticides viz., carbendazim 50 EC at 500 g a.i. ha^{-1} and cypermetrin 20 EC at 300 g a.i. ha^{-1} was carried out in curry leaf.

II. MATERIAL AND METHOD

A field experiment was conducted during 2014 - 2015 at Department of Agroforestry, College of Agriculture,Professor Jayashankar Telangana State Agricultural University (PJTSAU), Rajendranagar, Hyderabad and the laboratory studies were carried out at the All India Network Project on Pesticide Residues, Hyderabad Centre The insecticidal treatments *viz.*, carbendazim 50 EC at 500 g a.i. ha^{-1} and cypermetrin 20 EC at 300 g a.i. ha^{-1} were sprayed twice, first at vegetative stage and second at 10 days after first spray with hand compression knapsack sprayer and the amount of spray fluid used was 500 L ha^{-1}.

2.1 Sample Collection

The curry leaf samples were collected at 0, 1, 3, 5, 7, 10, 15, 20, 25, 30 and 45th day after second spray for residue analysis. The leaf samples (1 kg) were collected randomly from each treatment in polythene bags and brought to the laboratory immediately for further sample processing.

2.2 Extraction and clean up procedure for leaf samples

The leaf samples were analyzed for both carbendazim and cypermetrin residues following the AOAC official method 2007.01 (QuEChERS) after validation of the method at the laboratory. One kg of leaf samples collected from all treatments was homogenized with robot coupe blixer separately. 7.5 g sample was taken in 50 ml centrifuge tube and added with 30±0.1 ml acetonitrile. The sample was homogenized at 14000-15000 rpm for 2-3 min using Heidolph silent crusher. The samples were then added with 3±0.1 g sodium chloride 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 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±0.01 g GCB (remove chlorophyll). The sample tube was vortexed for 30 sec, followed by centrifugation for 5 min at 2500-3000 rpm. The extract of 1 ml acetonitrile was transferred into 2 ml vial by filtering through 0.22µm filter paper for the analysis on LC-MS under standard operational conditions (Table 1).

2.3 Extraction and clean up procedure for soil samples

The soil samples were analyzed for both carbendazim and cypermetrhin residues following the QuEChERS method after validation of the method in the laboratory. Two kg of soil was collected from each plot in Polythene bags. The soil samples were pooled treatment wise, mixed well and about 200 g of the representative sample was drawn by quartering method. The soil samples were dried at room temperature under shade, ground, passed through 2 mm sieve and a representative 10 g sample was taken into 50 ml centrifuge tube. The sample tube is then added with 20±0.1 ml acetonitrile. The sample is then added with 1±0.1 g sodium chloride and 4±0.1 g Magnesium sulphate mixed by shaking gently followed by centrifugation for 3 min at 3300 rpm to separate the organic layer. The top organic layer of about 10 ml was taken into the 15 ml centrifuge tube containing 1.5±0.1 g magnesium sulphate and 0.25 g PSA and sonicated for 1 min to remove air bubbles and centrifuged for 10 min at 3000rpm. The extract of about 1 ml (0.5 g sample) was transferred into vials for LCMS/MS analysis under standard operational conditions (Table 1).

TABLE 1
LCMS OPERATING PARAMETERS FOR ANALYSIS

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^oC
Retention Time (RT)	Carbendazim - 16.64 min
	Cypermethrin - 17.39 min
Nebulizing gas	Nitrogen
Nebulizing flow gas	2.0 litres/min
Pump Mode/ flow	Gradient/ 0.4 ml/min
LC Programme	A : Ammonium formate in water (10 Mm) - 65%
	B : Ammonium formate in methanol (10 Mm) - 35%
Total Time of Programme	24 min

2.4 Fortification and Recovery studies

The untreated control leaf samples were fortified with required quantity of carbendazim and cypermetrhrin to obtain 0.5, 0.25 and 0.05 mg kg^{-1} fortification levels and the samples were extracted and cleaned up as per QuEChERS method to validate the suitability of method. The recovery of carbendazim is 85.49, 115.7 and 88.62 per cent from the curry leaf fortified at 0.5, 0.25 and 0.05 mg kg^{-1} (Table-2), while cypermetrhin fortified at 0.5, 0.25 and 0.05 mg kg^{-1} has shown the recovery of 91.68, 115.07 and 90.37 per cent(Table-3). Hence, the limit of quantification (LOQ) is 0.05 mg kg^{-1} in curry leaf for both carbendazim and cypermetrhrin.

TABLE 2
RECOVERY OF CARBENDAZIM RESIDUES IN CURRY LEAF

1	Recoveries of carbendazim from fortified curry leaf samples					
	Fortified level (mg kg^{-1})					
	0.05		0.25		0.50	
	Residues recovered (mgkg^{-1})	**Recovery (%)**	**Residues recovered (mgkg^{1})**	**Recovery (%)**	**Residues recovered (mgkg^{1})**	**Recovery (%)**
R_1	0.052	96.67	0.218	114.61	0.528	94.74
R_2	0.053	95.19	0.213	117.61	0.541	92.39
R_3	0.05	100.62	0.204	122.70	0.533	93.77
Mean		97.49		118.30		93.64
SD		2.81		4.09		1.18
RSD		2.88		3.46		1.26

TABLE 3
RECOVERY OF CYPERMETHRIN RESIDUES IN CURRY LEAF

Details	Recoveries of cypermethrin from fortified curry leaf samples					
	Fortified level (mg kg^{-1})					
	0.05		0.25		0.50	
	Residues recovered (mg kg^{-1})	Recovery (%)	Residues recovered (mg kg^{-1})	Recovery (%)	Residues recovered (mg kg^{-1})	Recovery (%)
R_1	0.049	102.54	0.217	115.18	0.576	86.86
R_2	0.048	104.76	0.211	118.40	0.571	87.59
R_3	0.05	100.87	0.203	123.24	0.546	91.63
Mean		102.72		118.94		88.69
SD		1.95		4.05		2.57
RSD		1.90		3.41		2.90

III. RESULTS AND DISCUSSION

The initial deposits of carbendazim and cypermetrhin were 16.00 and 13.09 mg kg^{-1}, respectively which dissipated to below determination levels (< 0.05 mg kg^{-1}) at 20 and 10 days, respectively. The soil samples at 45 das did not show any residues of both carbendazim and cypermetrhin . The half-life values were 1.81 and 1.31 days, respectively. As there were no MRLs fixed, the day at which residues reached to BDL was suggested as safe harvest i.e., 20 and 10days, respectively. Soil samples collected at harvest (45th day) were free from the residues of both pesticides (Table 4 and 5 and figs 1and 2).The variation of results pertaining to the initial deposits (16.00 and 13.09 mg kg^{-1}) may be due to variation in dosages of application, change in matrix and climatic conditions. Results are in agreement with the work done by Swarupa *et al.* (2015), who reported the initial deposit of 15.40 mg kg^{-1} of in curry leaf which reached BDL by 15th day with half-life of 1.5 days and safe waiting period of 14.08 days.

3.1 Dissipation of carbendazim

The dissipation dynamics of carbendazim when sprayed @ 500 ml ha^{-1} was studied in field situation by collecting leaf samples at 0, 1, 3, 5, 7, 10, 15, 20, 25, 30 and 45 days and soil samples at 45 days after the second spray and results are presented in table 4 and figure 1.Initial deposits of 16.00 mg kg^{-1} of carbendazim was detected at 2 hours after last spray, which dissipated to 14.28, 10.71, 7.03, 3.19, 1.07 and 0.04 mg kg^{-1} by 1, 3, 5, 7, 10 and 15 days, respectively. The residues reached below detectable level (BDL) at 20th day after last spray. The dissipation pattern showed decrease of residues from first day to 20th day and residues dissipated by 10.75, 33.06, 56.06, 80.06, 93.31, 99.75 and 100.00 per cent at 1, 3, 5, 7, 10, 15 and 20 days, respectively. There were no residues in soil samples collected at 45 days after last spray. The regression equation was Y = 4.461 + (-0.166) X with R^2 of 0.93. Half- life was worked out by using linear semi-logarithmic regression analysis (Hoskins, 1961). There are no maximum residue limit (MRL) for carbendazim on curry leaf as per Codex Alimentarius Commission (CAC) and Food Safety and Standards Authority of India (FSSAI). Hence, safe waiting period of 20 days can be suggested. The half life of carbendazim on curry leaf was 1.81 days. Mohapatra *et al.* (1998) reported that carbendazim sprayed @ of 250 and 500 g a.i. ha^{-1}on grape berries showed half-life values of 5 days and safe waiting periods of 2 and 5 days, respectively. In mango, pre harvest spray of carbendazim @ 0.05 and 0.1 % a.i. ha^{-1} showed initial deposits of 2.48 and 5.28 mg kg^{-1} in whole fruit and 1.23 and 2.51 mg kg^{-1} in pulp at both the doses, respectively. For whole fruit, the safe waiting period of 2.5 and 7 days were recommended at both the doses, respectively (Bhattacherjee and Pandey, 2010). Carbendazim 12 per cent in combined formulation SAAF-75 WP sprayed on mango @ 90 and 180 g a.i. ha^{-1} showed initial deposits of 1.12 and 1.95 mg kg^{-1}, reached BDL by 7th day and half-life values of 1-5 days (Ahila *et al.*, 2015). The difference in dissipation dynamics may be due to difference in matrix and also the dosages applied.

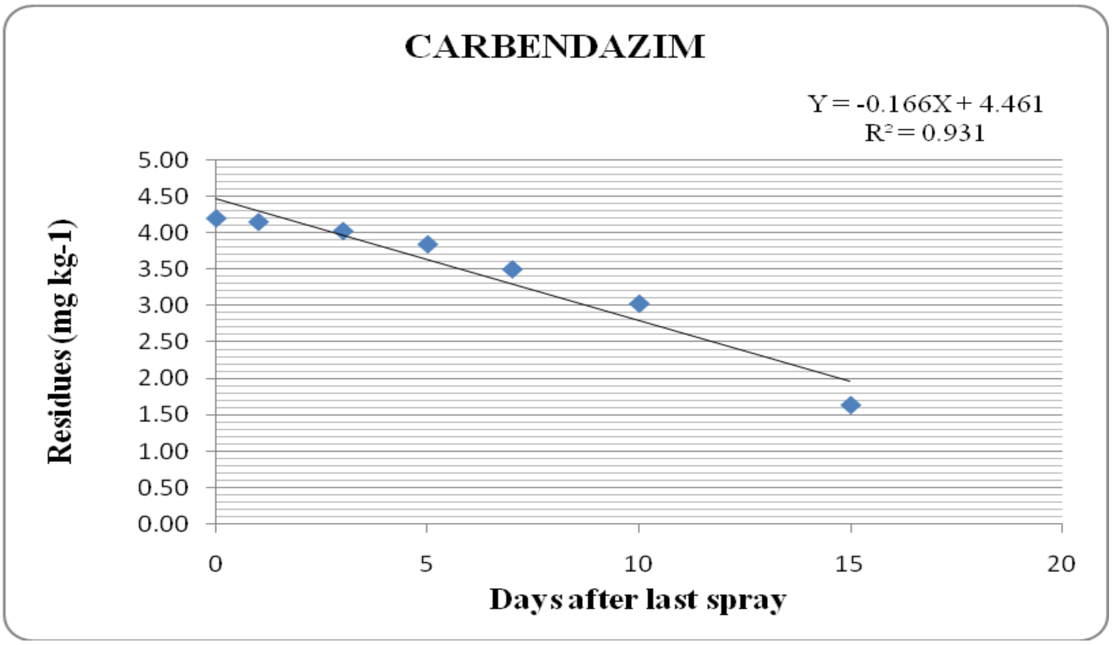

FIGURE.1. DISSIPATION OF CARBENDAZIM IN CURRY LEAF

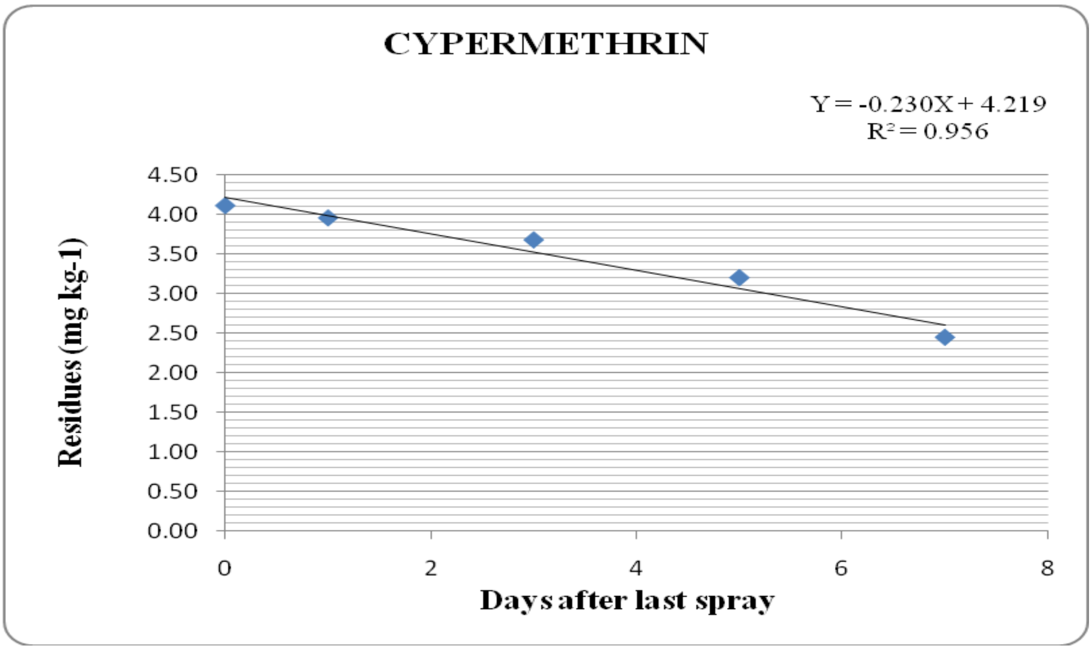

FIGURE 2 DISSIPATION OF CYPERMETHRIN IN CURRY LEAF

TABLE- 4
DISSIPATION OF CARBENDAZIM IN CURRY LEAF

Days after last spray	Residues of carbendazim (mg kg^{-1})				Dissipation (%)
	R_1	R_2	R_3	Average	
0	16.38	15.59	16.04	16.00	0.00
1	14.09	13.87	14.89	14.28	10.75
3	10.55	11.09	10.49	10.71	33.06
5	7.69	6.99	6.41	7.03	56.06
7	3.09	3.48	2.99	3.19	80.06
10	1.02	1.29	0.91	1.07	93.31
15	0.01	0.04	0.08	0.04	99.75
20	BDL	BDL	BDL	BDL	100.00
Soil (45th day)	BDL	BDL	BDL	BDL	-
Regression equation	Y = 4.461 + (-0.166) X				
R^2	0.93				
Half-life (Days)	1.81				
BDL : Below Detectable Level (< 0.05 mg kg^{-1})					

TABLE 5
DISSIPATION OF CYPERMETHRIN IN CURRY LEAF

Days after last spray	Residues of cypermethrin (mg kg^{-1})				Dissipation (%)
	R_1	R_2	R_3	Average	
0	13.03	12.92	13.32	13.09	0.00
1	9.39	8.98	9.04	9.14	30.18
3	5.05	4.58	4.79	4.81	63.25
5	1.49	1.52	1.77	1.59	87.85
7	0.46	0.06	0.32	0.28	97.86
10	BDL	BDL	BDL	BDL	100.00
Soil (45th day)	BDL	BDL	BDL	BDL	-
Regression equation	Y = 4.219 + (-0.230) X				
R^2	0.96				
Half-life (Days)	1.31				
BDL : Below Detectable Level (< 0.05 mg kg^{-1})					

3.2 Dissipation of cypermethrin:

The dissipation dynamics of cypermethrin when sprayed @ 550 ml ha^{-1} was studied in open field situation by collecting leaf samples at 0, 1, 3, 5, 7, 10, 15, 20, 25, 30 and 45 days and soil at 45 days after the second spray and results are presented in Table 4 and figure 2.The results showed that, initial deposits of 13.09 mg $kg^{-1}$of cypermethrin were detected at 2 hours after last spray, which dissipated to 9.14, 4.81, 1.59 and 0.28 mg kg^{-1} by 1, 3, 5 and 7 days, respectively. The residues reached below detectable level (BDL) at 10th day after last spray. The dissipation pattern showed decrease of residues from first day to 10th day and residues dissipated by 30.18, 63.25, 87.85, 97.86 and 100.00 per cent at 1, 3, 5, 7 and 10 days, respectively. There were no residues in soil samples collected at 45 days after last spray. The regression equation was Y = 4.219 + (-0.230) X with R^2 of 0.96. Half- life was worked out by using linear semi-logarithmic regression analysis (Hoskins, 1961). There are no maximum residue limit (MRL) for cypermethrin on curry leaf as per Codex Alimentarius Commission (CAC) and Food Safety and Standards Authority of India (FSSAI). Hence, safe waiting period of 10 days can be suggested. The half life of cypermethrin on curry leaf was 1.31 days. Kumar *et al.* (2000) observed safe waiting period of 1.8 days when cypermethrin @ 300 g a.i. ha^{-1} was sprayed on chilli. Gupta *et al.* (2011) reported that residues of cypermethrin on fruits dissipated with half-life of 2-3.6 days. The studies conducted by Nilufar Nahar *et al.*(2012) recorded initial residues of 0.55 mg kg^{-1} of cypermethrin on tomato. Gagan jyot *et al.*(2013) found that combined formulation Nurelle-D 505, cypermethrin 5 % on chilli showed initial deposit of 0.32 and 0.44 mg kg^{-1}@ 1 & 2 l ha^{-1}, respectively with half-life and safe waiting period of (2.51, 2.64) days and (1,1) day, respectively. At both doses residues reached BDL by 7^{th} day. The work of Subhash *et al.* (2014) on cypermethrin sprayed on okra @ 100, 200 and 300 g a.i. ha^{-1} showed initial deposits of 0.378, 0.685 and 0.862 mg kg^{-1}, which fell to BDL by 15, 17 and 19 days, respectively. Cherukuri *et al.* (2015) reported that cypermethrin 10 EC @ 50 g a.i. ha^{-1} sprayed on tomato showed initial deposit of 0.158 mg kg^{-1} which reached BDL by 5^{th} day. Safe waiting period and half-life values were 1 and 2.41 days, respectively. Singh *et al.* (2015b) reported initial deposits of 1.46 and 3.11 mg kg^{-1}, safe waiting period of 1 day and half-life value of 4.43 and 4.7 days, respectively when super fighter 25 EC was sprayed @ 50 and 100 g a.i. ha^{-1} on chilli. At both dosages residues reached to BDL by 25^{th} day. This difference in dissipation pattern may be due to difference in matrix, agroclimatic conditions, dosages and formulations of insecticide.

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