International Journal of Research in Agricultural Sciences
Volume 2, Issue 2, ISSN (Online): 2348 – 3997)

Dissipation Dynamics and Risk Assessment of Profenofos, Triazophos and Cypermethrin Residues on Brinjal for Food Safety

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***Abstract*: Dissipation dynamics of profenofos, triazophos, cypermethrin are studied at doses used by farmers for recommending maximum residue limits (MRL) and pre harvest intervals (PHI) for food safety based on chronic hazard exposure assessment. Profenofos @ 500, triazophos @ 500 and cypermethrin @ 50 g ai ha^{-1} sprayed twice and fruit samples analysed following validated QuEChERS method using GC-ECD, GC-TSD, GC-MS/MS (TQD). Fruit samples are detected with deposits of 1.698 mg kg^{-1} profenofos, 1.108 mg kg^{-1} triazophos, and 0.158 mg kg^{-1} cypermethrin dissipated to BDL by 10th, 7th and 5th day, respectively. MRL of 4 mg kg^{-1} for profenofos, 3 mg kg^{-1} for triazophos and 0.4 mg kg^{-1} for cypermethrin is recommended based on chronic hazard exposure assessment parameters (theoretical maximum daily intake), and PHI of 1 day is recommended for food safety taking into consideration of MRLs of Codex and MRLs calculated from present investigation.**

***Keywords:* Brinjal, Insecticides, Food Safety, PHI (Post Harvest Index), Risk Assessment.**

I. Introduction

Brinjal (*Solanum melongena* .) is widely consumed vegetable in India in the form of curry, and also in pickles, home-cooked. During 2012-13 in India, brinjal was cultivated in an area of 879.6 thousand ha with an average annual production of 18226.6 thousand t and productivity of 20.7 t ha^{-1}, which contributed about 9.6% of total vegetable area and 11.2% of total vegetable production.[1] The brinjal yield is affected due to damage caused by leaf hoppers, aphids, caterpillar, flea beetles, leaf miner, spider mites, and fruit and shoot borer [2] and use of synthetic pesticides are very common practice to manage the pest to below threshold levels, but on many occasions, non-judicious use of pesticides lead to imbalance in biotic factors, and also and food safety concerns due to residue contamination in foods. Profenofos, organo thiophosphorous insecticide and acaricide, is the most commonly used against both sap sucking and chewing insects and mites due to its systemic, contact and acaricidal action, is available in India in 50%EC. Triazophos, hetero cyclic organo thio phosphorous insecticide, acaricide and nematicide, registered in India as 40% EC. Cypermethrin, synthetic pyrethroid insecticide and acaricide, commonly used against chewing insects and red spider mites, available in India in 10% EC. As per Insecticide Act, 1968 of India [3] profenofos, triazophos and cypermethrin are not registered for use on brinjal. Further, the reports on national residue monitoring studies and state level monitoring studies conducted by the laboratory revealed that profenofos, triazophos and cypermethrin residues are detected in samples collected from markets, and based on the survey conducted to know the farmer's practice, it is documented that farmers use profenofos 50% EC @ 500 g ai ha^{-1}, triazophos 40% EC @ 500 g ai ha^{-1} and cypermethrin 10% EC @ 50 g ai ha^{-1} for the control of insects and mites, hence profenofos, triazophos and cypermethrin residues were detected in samples collected from markets. Based on the reports of non-registered or non-recommended pesticide residues reported on brinjal, Department of Agriculture and Cooperation, GOI, initiated GAP (Good Agricultural Practices) trials to estimate the dissipation / residues of these pesticides on brinjal for recommending MRLs (Maximum Residue Limits) taking into consideration of risk analysis performed with ADI (Acceptable Daily Intake), food factor and average body weight. The ADI intake for profenofos is of 0-0.03 mg kg^{-1} body weight (bw), and acute reference dose (ARfD) of 1 mg kg^{-1} body weight; ADI for triazophos is 0.001 mg kg^{-1} bw and ARfD of 0.001 mg kg^{-1} bw and ADI and ARfD for cypermethrin is 0.02 mg kg^{-1} bw and ARfD is 0.02 mg kg^{-1} bw as per Codex Alimentarius Commission. To estimate the risk due to pesticide residues present in/on brinjal, food consumption data is very mandatory. As per the National Sample Survey conducted during 2011-12 in India, per capita consumption of brinjal in rural and urban area is 586 and 806 grams per month, respectively.[4] Brinjal contains 200 kcal kg^{-1}, 9 g protein kg^{-1}, and 2 g fat kg^{-1}.[5] Maximum Residue Limits (MRL) for profenofos, triazophos and cypermethrin on brinjal are not set by Government of India.[6] Hence analysis on presence of residues at harvest time following farmers practice is essential to study the risk analysis. A study was conducted during 2012-13 crop seasons to analyze dissipation pattern of profenofos, triazophos and cypermethrin on brinjal in fields so as to recommend MRLs, pre harvest intervals based on the risk analysis.

II. Materials and Methods

Certified Reference Material (CRM) of profenofos, triazophos and cypermethrin were procured from M/S Sigma Aldrich, Germany, and primary, intermediary and working standards were prepared from the CRMs using acetone and hexane as solvents. Working standards of all the pesticides were prepared in the range of 0.01 ppm to 0.5 ppm in 10 mL calibrated graduated volumetric flask using distilled n-hexane as solvent, and injected in Gas Chromatograph with Electron Capture Detector (ECD) and Thermionic Specific Detector (TSD) with injector split ratio of 1:10 (GC- Agilent 7890B, VF-5ms Capillary Column) and the same was confirmed on Bruker Scion GC-MS/MS (TQD) using MRM method. It was found that the limit of detection for profenofos, triazophos is 0.05 ng in GC-TSD, 0.05 ng for cypermethrin on GC-ECD and the linearity is in the range of 0.05 ng to 5 ng. Prior to field and poly house experiments, QuEChERS method [7] for extraction and clean up was validated as per SANCO/12495/2011 guidelines.[8] Control brinjal fruits (5 kg) were homogenized with high volume homogenizer and 15 g was taken in to 50 mL centrifuge tubes. The required quantity of profenofos, triazophos and cypermethrin intermediary standards are added to each 15 g sample to get fortification levels of 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg^{-1} in three replications each. 30±0.1 mL acetonitrile was added to the tube, and sample was homogenized at 14000-15000 rpm for 2-3 min using Heidolph silent crusher (low volume homogeniser). Then 3±0.1g sodium chloride was added to tube and mixed by shaking gently, and centrifuged 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 to which 9±0.1 g anhydrous sodium sulphate was added to remove the moisture content. 8 mL of extract was taken in to 15 mL tube containing 0.4±0.01g PSA sorbent (for dispersive solid phase d-SPE cleanup) and 1.2±0.01 g anhydrous magnesium sulphate, and the sample tube was vortexed for 30 sec followed by centrifugation for 5 min at 2500-3000 rpm. The extract of (2mL) was transferred into test tubes and evaporated to dryness using turbovap with nitrogen gas and reconstituted with 1mL n-Hexane: Acetone (9:1) for dimethoate analysis. Brinjal samples fortified with profenofos, triazophos and cypermethrin at 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg^{-1} were analyzed and the mean recovery of the residues calculated. Brinjal crop (Popular hybrid Super Sujan) was raised in both poly house and open field laid out in Randomized Block Design at spacing of 60×45 cm with each plot size of 20 m^2 and all Good Agricultural Practices (GAPs) recommended by University were followed. Profenofos 50% EC, Triazophos 40% EC and Cypermethrin 10% EC procured from local market was sprayed @ 500 g a.i. ha^{-1}, 500 g a.i. ha^{-1}, and 50 g a.i. ha^{-1}, respectively twice; first spray at fruit initiation stage followed by second spray at 10 days after first spray, using high volume knapsack sprayer with a spray solution of 500 L ha^{-1}. Brinjal fruit samples were collected at regular intervals i.e. 0, 1, 3, 5, 7, 10, 15, 20 days after last spray for dissipation studies. Half-life and TBDL (Time required for residues to reach below determination level) were calculated as per Hoskins [9] from first-order dissipation kinetics. OECD (Organisation for Economic Co-operation and Development) MRL calculator is used for calculation of MRL and chronic hazard risk analysis was performed using TMDI (Theoretical Maximum Daily Intake) for arriving at MRL for recommendation.

III. Results and Discussion

Method validation

Fortification and recovery test results were presented in Table 1, 2 and 3 and the method followed for qualitative and quantitative estimation of profenofos, triazophos and cypermethrin is suitable up to 0.05 mg kg^{-1} levels as the recoveries obtained are 92.67%, 93.33% and 88%, respectively at 0.05 mg kg^{-1} fortification level.

Dissipation and risk analysis for profenofos

Dissipation studies conducted for profenofos on brinjal indicate that initial residues of 1.709 mg kg^{-1} dissipate to 0.879 mg kg^{-1} in 24 hours, 0.401 mg kg^{-1} by 3rd day, 0.184 mg kg^{-1} by 5th day, 0.068 mg kg^{-1} by 7th day, and BDL (0.05 mg kg^{-1}) by 10th day. The calculated half-life is 1.57 days, and TBDL is 12.08 days. Gupta et al [10] reported that residues of profenofos dissipated with half-life of 2.2-5.4 days, and Sahoo et al [11] reported that profenophos spray on brinjal @ 500 g a.i. ha^{-1} first at 50% flowering stage and subsequently at 15 days intervals, resulted in to initial deposit of 1.37 mg kg^{-1} dissipating to BDL in 15 days, and similar results also reported by Ahmad et al [12] on brinjal. Experimental results of Radwan et al [13] shows that at application of very high dose @ 1280 g a.i. ha^{-1} on three crops *viz.*, green pepper, hot pepper and brinjal results in very high initial deposit of 10-11 mg kg^{-1} on pepper, and 4.50 mg kg^{-1} on brinjal, which dissipated to BDL in 2 weeks. However, the studies conducted by various workers [10, 14-15] on dissipation on profenophos on different crops clearly indicate that when applied at recommended dose, the initial deposits are less than 3 mg kg^{-1} and dissipate to BDL in 7-10 days depending on the crop, except on cardamom. Based on the OECD calculator, proposed MRL is 4 mg kg^{-1}, and hazard exposure assessment conducted taking into consideration of national averages of body weights, per capita brinjal consumption, ADI, risk analysis indicator i.e. TMDI calculated in 0.00196 which is less than ADI values, and hence MRL of 4 mg kg^{-1} is recommended based on farmers practice. Pre-harvest interval of 1 day is proposed taking in to consideration of MRLs of CAC and present experiment results. Various workers suggested safe waiting periods varying from 1 to 14 days, but as per CCPR guidelines based on CAC MRLs (10 mg kg^{-1}) it is not necessary to recommend waiting periods till the residues reach BDL, and taking into consideration of MRLs set by CAC and results on dissipation dynamics on brinjal in present study, it can be scientifically concluded and recommended that PHI of 1 day as the initial deposits are below 10 mg kg^{-1}.

Dissipation and risk analysis for triazophos

Initial deposits of 1.108 mg kg^{-1} triazophos were detected in brinjal samples collected from plots sprayed with triazophos 40%EC @ 500 g ai ha^{-1}, which dissipated to 0.666 mg kg^{-1} by 1st day. The triazophos residues were below detectable level of 0.05 mg kg^{-1} in 7 days, with a calculated half-life of 1.14 days, and 9.49 days to come to

BDL. Statistical formulae applied as per OECD procedure, and the suggested MRL is 3 mg kg^{-1}, and based on the proposed MRL, TMDI is more than ADI, and hence PHI of 1 day is recommended to avoid the risk of triazophos residues for food safety. MRLs are not set legally both by CAC and FSSAI, and hence based on the present studies conducted taking into consideration of farmers practice, MRL of 3 mg kg^{-1} can be recommended.

Dissipation and risk analysis for cypermethrin

Spray of cypermethrin 10%EC twice @ 50 g ai ha^{-1} as per the farmers practice, resulted an initial deposits of 0.158 mg kg^{-1} which dissipated to BDL of 0.05 mg kg^{-1} by 5th day after spray. The half-life is 2.41 days, and calculated TBDL is 15.79 days. Gupta et al [10] reported that residues of cypermethrin on fruits dissipated with half-life of 2-3.6 days. The studies conducted by Nilufar Nahar et al [16] recorded 0.55 mg kg^{-1} initial residues of cypermethrin on brinjal when sprayed at recommended dose. Based on the dissipation dynamics of cypermethrin on brinjal, MRL of 0.4 mg kg^{-1} is calculated as per OECD MRL calculator. The ADI of cypermethrin is 0.02 mg kg^{-1} as per CAC, and the taking into consideration of national data, the calculated TMDI is less than ADI, and hence cypermethrin spray on brinjal can be allowed provided the data requirements for registration is satisfied. Based on the farmers practice, and the MRL of CAC and MRL calculated from present studies, PHI of 1 day can be recommended for food safety. In India, MRLs for cypermethrin on brinjal are not fixed as per FSSAI, since the pesticide is not registered for use on brinjal as per Insecticide Act.

IV. Conclusion

As per the Insecticide Act, profenofos, triazophos and cypermethrin are not registered for use on brinjal, and also MRLs are not fixed as per FSSAI. MRLs for profenofos and cypermethrin are fixed by CAC. The present study conducted based on the results of national monitoring data base and also farmers practice (profenofos 50% EC application @ 500 g ai ha^{-1}, triazophos 40% EC @ 500 g ai ha^{-1} and cypermethrin 10% EC @ 50 g ai ha^{-1} twice; first at fruit initiation followed by 10 days interval), to assess the dissipation pattern for recommending MRLs and PHIs through risk analysis. MRL of 4 mg kg^{-1} for profenofos, 3 mg kg^{-1} for triazophos and 0.4 mg kg^{-1} for cypermethrin is recommended based on OECD MRL calculator and chronic hazard exposure assessment parameters, and PHI of 1 day is recommended taking into consideration of MRLs of CAC and MRLs calculated from present investigation.

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International Journal of Research in Agricultural Sciences
Volume 2, Issue 2, ISSN (Online): 2348 – 3997)

Table1: Fortification and recovery results.

PROFENOFOS						
Replication	Fortified Levels					
	0.05 mg kg^{-1}		0.1 mg kg^{-1}		0.5 mg kg^{-1}	
	Calculated Level (mg kg^{-1})	% Recovery	Calculated Level (mg kg^{-1})	% Recovery	Calculated Level (mg kg^{-1})	% Recovery
R1	0.046	92	0.096	96	0.457	91.4
R2	0.047	94	0.097	97	0.466	93.2
R3	0.046	92	0.097	97	0.459	91.8
Average		92.67		96.67		92.13
TRIAZOPHOS						
Replication	Fortified Level					
	0.05 mg kg^{-1}		0.1 mg kg^{-1}		0.5 mg kg^{-1}	
	Calculated Level (mg kg^{-1})	% Recovery	Calculated Level(mg kg^{-1})	% Recovery	Calculated Level (mg kg^{-1})	% Recovery
R1	0.046	92	0.094	94	0.52	104
R2	0.049	98	0.092	92	0.49	98
R3	0.045	90	0.094	94	0.50	100
Average		93.33		93.33		100.67
CYPERMETHRIN						
Replication	Fortified Level					
	0.05 mg kg^{-1}		0.1 mg kg^{-1}		0.5 mg kg^{-1}	
	Calculated Level (mg kg^{-1})	% Recovery	Calculated Level (mg kg^{-1})	% Recovery	Calculated Level (mg kg^{-1})	% Recovery
R1	0.046	92	0.092	92	0.475	95.0
R2	0.044	88	0.095	95	0.487	97.4
R3	0.042	84	0.095	95	0.482	96.4
Average		88		94		92.27

Table 2: Dissipation dynamics of profenofos, triazophos and cypermethrin on brinjal

Days after spray	Residues (mg kg^{-1}) in Profenofos sprayed plots				Residues (mg kg^{-1}) in triazophos sprayed plots				Residues (mg kg^{-1}) in cypermethrin sprayed plots			
	R1	R2	R3	Mean±SD	R1	R2	R3	Mean±SD	R1	R2	R3	Mean±SD
0	1.673	1.713	1.709	1.698±0.022	1.102	1.111	1.112	1.108±0.006	0.149	0.167	0.158	0.158±0.009
1	0.858	0.898	0.880	0.879±0.020	0.698	0.701	0.599	0.666±0.058	0.104	0.123	0.144	0.124±0.020
3	0.411	0.398	0.393	0.401±0.009	0.139	0.211	0.129	0.160±0.045	0.064	0.068	0.068	0.067±0.002
5	0.191	0.182	0.180	0.184±0.006	0.061	0.050	0.059	0.057±0.006	BDL	BDL	BDL	BDL
7	0.070	0.071	0.063	0.068±0.004	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
15	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Soil	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Regression equation (log*1000 residues)	Y=3.188+(-0.191)X				Y=3.051+(-0.264)X				Y=2.206+(-0.125)X			
R^2	0.995				0.995				0.996			
Half-life (days)	1.57				1.14				2.41			
TBDL (Days)	12.08				9.49				15.79			

BDL=Below Determination Level (<0.05 mg kg^{-1} for profenofos, triazophos and cypermethrin)

Table 3: Chronic Hazard Exposure Assessment for recommending MRL and PHI for profenofos, triazophos and cypermethrin on brinjal.

OECD MRL calculator Date sets	**Profenofos**	**Triazophos**	**Cypermethrin**
Total number of data (n)	6	4	3
Percentage of censored data (%)	17	0	0
Number of non-censored data	5	4	3
Lowest residue	0.040	0.057	0.067
Highest residue	1.698	1.108	0.158

Median residue	0.293	0.413	0.124
Mean	0.545	0.498	0.116
Standard deviation (SD)	0.644	0.486	0.046
Correction factor for censoring (CF)	0.889	1.000	1.000
Proposed MRL estimate			
Highest residue	1.698	1.108	0.158
Mean X 4 SD	3.122	2.442	0.300
CF X 3 Mean	1.453	1.493	0.349
Unrounded MRL	3.122	2.442	0.349
Rounded MRL	4.00	3.00	0.4
Risk Analysis			
Average human body weight (kg)	55		
National per capita intake of brinjal	806 g / person		
Daily intake of crop (C) = kg person $^{-1}$	0.027		
Consumption of crop C(F_C) = kg kg bw^{-1}	0.00049		
ADI (mg kg bw^{-1})	0.03	0.001	0.02
TMDI = F_c X MRL (from OECD calculator)	0.00196	0.00147	0.0002
TMDI v/s ADI	TMDI < ADI	TMDI > ADI	TMDI < ADI
Proposed MRL (mg kg^{-1})	**4**	**3**	**0.4**
Codex MRL (mg kg^{-1})	10	NA	0.2
FSSAI MRL (mg kg^{-1})	NA	NA	NA
Proposed PHI (days)	1	1	1