

# Dissipation of Certain Pesticides on/in Capsicum in Open Field & Poly House

## Research Article

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### Abstract

Studies were undertaken to establish dissipation of commonly used insecticides at recommended dose both in open field and poly house situations to assess the differences in rate of dissipation, and also to recommend Pre-Harvest Intervals based on MRLs established by CAC and FSSAI by utilizing Carbendazim, Chlorpyrifos, Ethion, Triazophos and Profenophos sprays and capsicum samples collected at regular intervals were analyzed for residues. The dissipation pattern of insecticides in open field conditions showed that initial deposit of 2.47 mg kg<sup>-1</sup> of carbendazim dissipated to 0.04 mg kg<sup>-1</sup> by 15 days whereas Chlorpyrifos 0.468 mg kg<sup>-1</sup> dissipated to BDL by 7th day. Similarly Ethion (0.746 mg kg<sup>-1</sup> dissipated to BDL by 10th day, whereas Triazophos (1.947 mg kg<sup>-1</sup>) dissipated to BDL by 15th day (1.947 mg kg<sup>-1</sup>) to BDL by 10th day. As no MRLs are available for, PHI of 10 days can be recommended as residues dissipated to less than 0.05 mg kg<sup>-1</sup>. As per the Insecticide Act, 1968, profenofos is not recommended for use, but when sprayed at farmer's dose on capsicum, 2.24 mg kg<sup>-1</sup> and 3.71 mg kg<sup>-1</sup> initial deposits were recorded in open field and poly house, respectively. Codex suggests MRL as 10 mg kg<sup>-1</sup>, hence it can be concluded that profenofos application does not leave residues above MRL. Out of five insecticides tested in poly house. Chlorpyrifos and profenofos residues are found in capsicum up to 10 days, while carbendazim, Ethion and Triazophos are detected up to 15 days.

**Keywords:** Open field and poly house dissipation; Pre-Harvest intervals

## Introduction

Capsicum (*Capsicum annum* L.), also called "Bell pepper," is an important commercial crop in India and suffers major quantitative and qualitative loss in production due to capsicum thrips, *Scirtothrips dorsalis* Hood and yellow mite, *Polyphagotarsonemus latus* (Banks) and fruit borers *Helicoverpa armigera* (Hubner). A number of pesticides are being frequently used, to combat these pests. However, some of these insecticides leave residues on fruits and these residues may persist up to harvest. Presence of pesticide residues in the harvested capsicum is posing problem at the time of export and in recent times importing countries have rejected few consignments. Pesticide use has increased rapidly over the last two decades at the rate of 12% per year [1]. Many farm gate capsicum samples showed presence of insecticide residues [2]. As per insecticides Act of 1968 (www.cibrc.nic.in) [3], 37 insecticide formulations are registered

and recommended for use on capsicum targeting various pests. In recent years due to the support of the Government under National Horticulture Mission (NHM), capsicum is widely cultivated under poly house conditions as well as in open field conditions. Since the persistence and dissipation of insecticides, the present study is proposed to monitor the pesticide usage on capsicum, dissipation studies of recommended pesticides, so as to recommend the safe waiting periods based on the Maximum Residue Limits (MRLs) calculated, as it helps in recommending risk mitigation protocols for food safety.

## Materials and Methods

### Residue analysis method

**AOAC Official Method 2007.01 (QuEChERS):** 0 day samples were collected for estimation of deposits of pesticide within 2 hours

of last spray, and the samples of Capsicum were collected at 1, 3, 5, 7, 10, 15, 20 days after last spray or till they reached BDL for residue estimation.

#### Extraction and clean up procedure.

- 2 kg Capsicum Fruits were collected randomly from each plot in polythene bags.
- The samples were homogenized with robot coupe blixer.
- Homogenized  $15 \pm 0.1$  g sample was taken in 50 ml centrifuge tube, and  $30 \pm 0.1$  ml acetonitrile was added.
- The sample was homogenized at 14000-15000 rpm for 2-3 min using heidolph silent crusher.
- The samples were then added with  $3 \pm 0.1$  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  $9 \pm 0.1$  g anhydrous sodium sulphate to remove the moisture content.
- 8 ml of extract was taken in to 15 ml tube, containing  $0.4 \pm 0.01$  gr PSA sorbent (for dispersive solid phase d-SPE cleanup) and  $1.2 \pm 0.01$  g anhydrous magnesium sulphate.
- The sample tube was vortexed for 30 sec then centrifuged for 5 min at 2500-3000 rpm.
- The extract of 1 ml Acetonitrile was transferred into 2 ml vial by filtering through  $0.2 \mu\text{m}$  filter paper for analysis on LC-MS under standard operational conditions (Table 1).

Certified Reference Materials (CRMs) of Carbendazim, Chlorpyrifos, Ethion, Triazophos and Profenophos from Dr. Erhenstorfer, Germany were utilized for preparing working standards which were injected into were injected to Liquid Chromatograph with Photo Diode Array (PDA) Detector for estimating the lowest quantity of these pesticides under standard operating parameters Table 1. One micro litre of each working standard was injected for the study. The retention times of Carbendazim, Chlorpyrifos, Ethion, Triazophos and Profenophos were 9.95, 11.87, 6.99 and 5.27 min, respectively. Under standard operating parameters Table 1. Working standards of Carbendazim, Chlorpyrifos, Ethion, Triazophos and Profenophos (0.05 ppm, 0.075 ppm, 0.10 ppm, 0.25 ppm and 0.50 ppm) were injected 6 times and the linearity lines were drawn the LOD (limit of detection) for Carbendazim, Chlorpyrifos, Ethion, Triazophos and Profenophos 0.05 ng, and the linearity is in the range of 0.05 ng to 0.10 ng.

#### Method validation and fortification recoveries

Prior to pesticide application and field sample analysis, the residue analysis method was validated following the SANCO document (12495/2011). The capsicum fruits (5 kg) collected from untreated control plots were brought to the laboratory, homogenized using Robot Coupe Blixer (High volume homogenizer) and 15 g sample was taken in to 50 ml centrifuge tubes. The required quantity of Carbendazim, Chlorpyrifos, Ethion, Triazophos and Profenophos intermediary standard prepared from CRMs were added to each

sample to get fortification levels of 0.05 ppm, 0.25 ppm and 0.5 ppm, in three replications each. These fortification levels are selected to know the suitability of the method to detect and quantify pesticides in capsicum below Maximum Residue Limits (MRLs) of Codex Alimentarius Commission (CAC).

The AOAC official method 2007.01 (Pesticide Residues of Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate) was slightly modified to suit to the facilities available at the laboratory and the same was validated for estimation of LOQ (Limit of Quantification) of above mentioned pesticides in Capsicum matrix. The Quechers method was followed as presented in the flow chart. The final extract of the sample i.e. 2 ml equal to 1 g of the sample was evaporated using turbovap and made up to 1 ml (equal to 1 g sample) using suitable solvent for analysis on GC, while for LC analysis, filtered 1 ml final extract (equal to 0.5 g sample) was directly injected in LC and the residues of pesticides recovered from fortified samples were calculated using the following formula.

Sample weight (15 g) X aliquot taken

Wt of the Sample analysed = \_\_\_\_\_

Volume of acetonitrile (30 ml)

#### Fortification and recovery studies

The untreated/control Capsicum samples were fortified at 0.05, 0.25, and 0.50 mg/kg levels adding required quantity of Carbendazim, chlorpyrifos, ethion, profenophos and triazophos standards. All the fortified levels were replicated thrice. Recoveries of all pesticides at three fortification levels are presented in Table 2.

The recovery of carbendazim is 101.13 % from the Capsicum

**Table 1:** LC-MS-MS instrument parameters.

LC-MS/MS	SHIMADZU LC-MS/MS 8040
Detector	Mass Spectrophotometer
Column	KINETEX, 100X3, 2 $\mu$ m
Column Oven Temperature	40 °C
Retention time (RT)	2.02 min
Nebulizing gas	Nitrogen
Nebulizing gas flow	2.0 lit/min
Pump Model/flow	Gradient/0.4ml/min
LC Programme	A: Ammonium formate in water - 65%
	B: Ammonium formate in methanol - 35%
Total Programme	4 min
Precursor ion	192
Quantifier ion	159.95
Qualifier ion	139.95

**Table 2:** Recovery of different pesticides at various fortification levels in/on capsicum samples.

Pesticides	0.05 mg/kg		0.25 mg/kg		0.5 mg/kg	
	Mean Calculated Level (mg/kg)	Mean % Recovery	Mean Calculated Level (mg/kg)	Mean % Recovery	Mean Calculated Level (mg/kg)	Mean % Recovery
Carbendazim	0.050	101.13	0.238	95.45	0.484	96.95
Chlorpyrifos	0.047	95.73	0.264	105.83	0.519	103.97
Ethion	0.049	98.74	0.261	104.58	0.589	117.88
Triazophos	0.045	90.39	0.239	95.96	0.485	97.04
Profenophos	0.044	89.20	0.244	97.80	0.468	93.60

samples fortified at 0.05 mg/kg, and 95.45% recovery at 0.25 mg/kg fortified level while the samples fortified with 0.50 mg/kg have shown the recovery of 96.95%. The recovery of Chlorpyrifos is 95.73% from the Capsicum samples fortified at 0.05 mg/kg, and 105.83% recovery at 0.25 mg/kg fortified level while the samples fortified with 0.50 mg/kg have shown the recovery of 103.97%. The recovery of Ethion is 98.74% from the Capsicum samples fortified at 0.05 mg/kg, and 104.58% recovery at 0.25 mg/kg fortified level while the samples fortified with 0.50 mg/kg have shown the recovery of 117.88%. The recovery of Triazophos is 90.39 % from the Capsicum samples fortified at 0.05 mg/kg, and 95.96% recovery at 0.25 mg/kg fortified level while the samples fortified with 0.50 mg/kg have shown the recovery of 97.04%. The recovery of Profenophos is 89.2% from the capsicum samples fortified at 0.05 mg/kg, and 97.8% recovery was seen at 0.25 mg/kg fortified level while the samples fortified with 0.50 mg/kg have shown the recovery of 93.6%.

## Results and Discussion

The dissipation pattern of all the pesticides on capsicum in open field conditions and playhouse are presented hereunder in Tables 3 and 4, Figures 1 and 2.

### Dissipation of carbendazim

The initial deposit and subsequent residues of carbendazim on capsicum in open field and poly house situation at an interval of 0, 1, 3, 5, 7, 10, 15 and 20 days after second spray are presented in Tables 3 and 4. In open field situation, the initial deposit of 2.47 mg kg<sup>-1</sup> gradually dissipated to 1.98, 1.31, 1.02, mg kg<sup>-1</sup> at 1, 3 and 5 days, respectively. The per cent dissipation was 19.83, 46.96 and 58.70, respectively. The residues fell Below Determination Level (BDL) of 0.05 mg kg<sup>-1</sup> by 15<sup>th</sup> day. There are no maximum residue limits (MRL) for carbendazim on capsicum as per Codex Alimentarius Commission (CAC), while Food Safety and Standards Authority of India [4] suggests 2 mg kg<sup>-1</sup> for fruits and vegetables, and hence safe waiting period of 1 day can be suggested, as the initial deposits are less than the MRL. In poly house, initial deposits of 3.80 mg kg<sup>-1</sup> were detected, which dissipated to 0.08 mg kg<sup>-1</sup> by 15<sup>th</sup> day after the second spray. The residues of 2.85, 2.57, 1.46, 1.08 and 0.51 mg kg<sup>-1</sup> were recorded at 1, 3, 5 and 7 and 10 days, respectively and dissipated to 25, 32.36, 61.57, 71.57 and 86.57 per cent on 1, 3, 5 and 7 and 10<sup>th</sup> day, respectively. The results showed that the residues of carbendazim

reached Below Determination Level (BDL) of 0.05 mg kg<sup>-1</sup> in 20 days. There are no maximum residue limit for carbendazim in capsicum as per Codex Alimentarius Commission (CAC) while Food Safety and Standards Authority of India (FSSAI) suggests 2 mg kg<sup>-1</sup> for vegetables, and hence based on the present study, PHI of 15 days can be recommended as the residues degraded to BDL by 15<sup>th</sup> day. It is evident that there is clear difference in dissipation pattern of carbendazim in poly house and open fields. Initial deposit of 2.47 mg kg<sup>-1</sup> was recorded in open fields, where as it was 3.80 mg kg<sup>-1</sup> in poly house.

### Dissipation of Chlorpyrifos

The dissipation pattern of Chlorpyrifos on capsicum in open field and poly house situation at an interval of 0, 1, 3, 5, 7, 10, 15 and 20 days after second spray are presented in Tables 3 and 4. The initial deposit of 0.468 mg kg<sup>-1</sup> on capsicum in open field situations dissipated to 0.269, 0.082 and 0.043 mg kg<sup>-1</sup> at 1, 3 and 5 days with per cent dissipation of 42.52, 82.48 and 90.81, respectively. The residues of Chlorpyrifos dissipated to below determination limit of 0.05 mg kg<sup>-1</sup> by 7<sup>th</sup> day. There are no MRLs suggested for Chlorpyrifos in capsicum by either Codex Alimentarius Commission (CAC) or by Food Safety and Standards Authority of India (FSSAI), since no MRLs are available, PHI of 5 days can be recommend as the residues dissipated to 0.043 mg kg<sup>-1</sup>. In poly house, initial deposits of 0.882 mg kg<sup>-1</sup> Chlorpyrifos detected at 2 hours after last spray, dissipated to Below Determination Level (BDL) of 0.05 mg kg<sup>-1</sup> by 10<sup>th</sup> day after last spraying on capsicum and the initial deposits dissipated to 0.657, 0.488, 0.128, 0.094 and 0.055 mg kg<sup>-1</sup> by 1, 3, 5 7 and 10 days after last spray, respectively. The dissipation pattern showed decrease of residues from first day to 7<sup>th</sup> day and residues dissipated by 67.56, 70.27, 81.08 and 86.48% at 1, 3, 5 and 7 days, respectively. The regression equation was  $Y = 0.256 + (-0.035) X$  with R<sup>2</sup> of 0.607.

### Dissipation of Ethion

The Capsicum samples collected at regular intervals were analyzed for Ethion residues and the residue data is presented in Tables 3 and 4. The initial deposits of 0.746 mg/kg of Ethion were detected in Capsicum samples in open fields dissipated to below detectable level by 7<sup>th</sup> day. In the poly house the initial deposits of 2.813 dissipated to 2.186 by 1<sup>st</sup> day and 0.013 by 15<sup>th</sup> day.

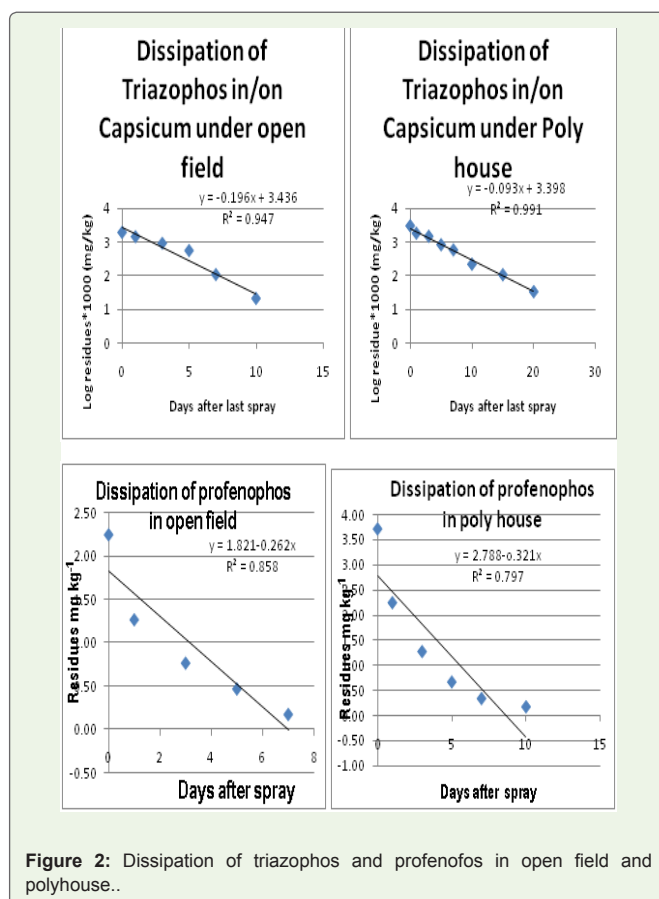
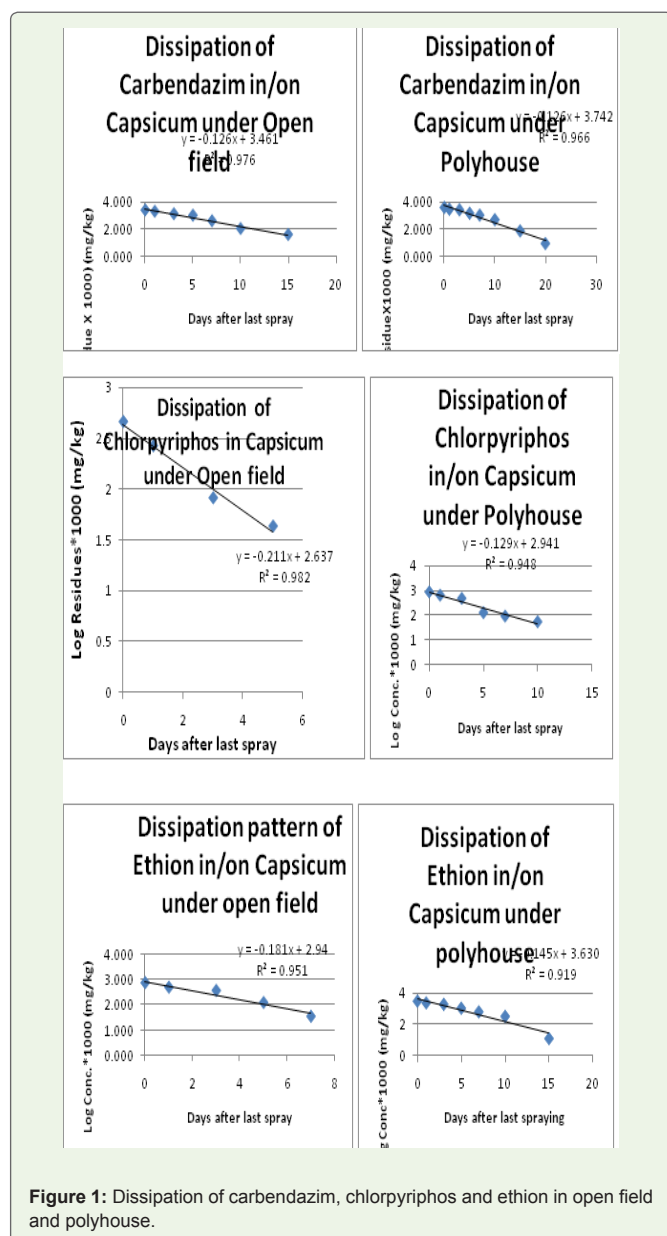
**Table 3:** Dissipation of different insecticides in capsicum in open fields.

Days after last spray	Residues of different insecticides(mg kg <sup>-1</sup> )					Dissipation%				
	Carbendazim	Chlorpyrifos	Ethion	Triazophos	Profenophos	Carbendazim	Chlorpyrifos	Ethion	Triazophos	Profenophos
0	2.47	0.468	0.746	1.947	2.24	0	0.00	0.0	0.00	0
1	1.98	0.269	0.500	1.444	1.26	19.83	42.52	32.98	25.83	43.75
3	1.31	0.082	0.371	0.923	0.76	46.96	82.48	50.27	52.59	66.07
5	1.02	0.043	0.125	0.558	0.47	58.70	90.81	83.24	71.34	79.01
7	0.40	BDL	0.036	0.108	0.18	83.80	BDL	95.17	94.45	91.96
10	0.11		BDL	0.021	BDL	95.54		BDL	98.92	100
15	0.04			BDL		98.38			-	
20	2.47					0				

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

**Table 4:** Dissipation of different insecticides in capsicum in poly house.

Days after last spray	Residues of different insecticides(mg kg <sup>-1</sup> )					Dissipation%				
	Carbendazim	Chlorpyrifos	Ethion	Triazophos	Profenophos	Carbendazim	Chlorpyrifos	Ethion	Triazophos	Profenophos
0	3.80	0.882	2.813	2.911	3.71	0	0.0	0.00	0.00	0
1	2.85	0.657	2.186	1.772	2.24	25.00	25.51	22.29	39.13	39.62
3	2.57	0.488	1.794	1.446	1.27	32.36	44.67	36.22	50.33	65.76
5	1.46	0.128	1.032	0.816	0.66	61.57	85.49	63.31	71.97	82.21
7	1.08	0.094	0.604	0.578	0.33	71.57	89.34	78.53	80.14	91.10
10	0.51	0.055	0.315	0.222	0.17	86.57	93.76	88.80	92.37	95.41
15	0.08	BDL	0.013	0.109	BDL	97.89	BDL	99.54	96.26	100
20	0.01			0.035		99.73			98.80	



### Dissipation of Triazophos

The Capsicum samples collected at regular intervals were analyzed for Triazophos residues and the residue data is presented in Tables 3 and 4. The initial deposits of 1.947 mg/kg of Triazophos detected in Capsicum samples in open field conditions dissipated to below detectable level by 10<sup>th</sup> day whereas in poly house situation the initial deposit of 2.911 dissipated to BDL by 20 days.

### Dissipation of Profenophos

The residue data of profenophos from the studies conducted in



open field and poly house situation is presented in Tables 3 and 4. Profenophos is not registered for use on capsicum as per Insecticide Act, 1968, but based on the survey reports, it is very much evident that profenophos is most popular and commonly used pesticide and hence, field trial was conducted based on the farmer's practice dose @ 500 g a.i ha<sup>-1</sup> to study the dissipation pattern. The study revealed that the initial deposits of 2.24 mg kg<sup>-1</sup> (at 2 hours after last spray) dissipated to Below Determination Level (BDL) of 0.05 mg kg<sup>-1</sup> by 10<sup>th</sup> day after last spraying on capsicum. The initial deposits dissipated to 1.26, 0.76, 0.47 and 0.18 mg kg<sup>-1</sup> by 1, 3, 5 and 7 days after last spray, respectively. The residues dissipated by 43.75, 66.07, 79.01 and 91.96% at 1, 3, 5 and 7 days, respectively. The regression equation was  $Y = 1.821 + (-0.262) X$  with R<sup>2</sup> of 0.858. As the Indian Government does not recommend profenophos on capsicum, MRLs are also not set. However, maximum residue limit for profenophos in capsicum are set by Codex Alimentarius Commission (CAC) and European Union (EU) as 10 mg kg<sup>-1</sup>. Based on the present investigations, it can be concluded and recommended that a PHI of 1 day is good for food safety when profenophos is applied. In poly house experiment, initial deposits of 3.71 mg kg<sup>-1</sup> of profenophos were detected at 2 hours after last spray, which dissipated to Below Determination Level (BDL) of 0.05 mg kg<sup>-1</sup> by 15<sup>th</sup> day after last spraying on capsicum, indicating that profenophos dissipation is slow in poly house compared to open fields. The initial deposits dissipated to 2.24, 1.27, 0.66, 0.33 and 0.17 mg kg<sup>-1</sup> by 1, 3, 5, 7 and 10 days after last spray, respectively. The residues dissipated by 39.62, 65.76, 82.21, 91.10 and 95.41% at 1, 3, 5, 7 and 10<sup>th</sup> days, respectively. The regression equation was  $Y = 2.788 + (-0.321) X$  with R<sup>2</sup> of 0.797. As the Indian Government does not recommend profenophos on capsicum, MRLs are also not set. However, maximum residue limit for profenophos in capsicum are set by Codex Alimentarius Commission (CAC) and European Union (EU) as 10 mg kg<sup>-1</sup>. Based on the present investigation, it can be concluded and recommended that a PHI of 1 day is good for food safety when profenophos applied on capsicum in poly house. From Tables 3 and 4, it is evident that there is a clear difference in dissipation pattern of profenophos in poly house and open fields. Initial deposit of 3.71 mg kg<sup>-1</sup> was recorded in poly house, whereas in open fields it was 2.24 mg kg<sup>-1</sup>. Profenophos reached Below Determination Level (BDL) of 0.05 mg kg<sup>-1</sup> by 15<sup>th</sup> day in poly house whereas, in open field trial by 10<sup>th</sup> day. The results are in full agreement with the findings of Ahmad et al. [5] who reported that profenophos spray on tomato @ 500 g a.i. ha<sup>-1</sup>, resulted in initial deposit of 2.58 mg kg<sup>-1</sup> dissipating to BDL in

15 days and similar results were also reported by Sahoo et al. [6] on tomato. Various workers suggested safe waiting periods varying from 1 to 14 days, but as per CCPR guidelines based on CAC MRLs (10 mg kg<sup>-1</sup>) 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 capsicum in present study, it can be scientifically concluded and recommended that PHI of 1 day is exceedingly good as the initial deposits are well below 10 mg kg<sup>-1</sup>. Experimental results of Radwan et al. [7] show that at application of very high dose @ 1280 g a.i. ha<sup>-1</sup> on three crops *viz.*, green pepper, hot pepper and brinjal resulted in very high initial deposit of 10-11 mg kg<sup>-1</sup> on pepper and 4.50 mg kg<sup>-1</sup> on brinjal, which dissipated to BDL in 2 weeks. However, the studies conducted by various workers [8-10] on dissipation on profenophos on different crops clearly indicates that when applied at recommended dose, the initial deposits are less than 3 mg kg<sup>-1</sup> and dissipate to BDL in 7-10 days depending on the crop, except on cardamom.

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