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Sudhakar S Kelageri
All India Network Project on Pesticide Residues, PJTS Agricultural University, Hyderabad, India

Cherukuri Sreenivasa Rao
All India Network Project on Pesticide Residues, PJTS Agricultural University, Hyderabad, India

Vemuri Shashi Bhushan
All India Network Project on Pesticide Residues, PJTS Agricultural University, Hyderabad, India

Pothula Narayana Reddy
All India Network Project on Pesticide Residues, PJTS Agricultural University, Hyderabad, India

Correspondence
Sudhakar S Kelageri
All India Network Project on Pesticide Residues, PJTS Agricultural University, Hyderabad, India

Dissipation kinetics and decontamination of phosalone residues from tomato under green house and open field conditions

Sudhakar S Kelageri, Cherukuri Sreenivasa Rao, Vemuri Shashi Bhushan and Pothula Narayana Reddy

Abstract
Phosalone an organophosphorus insecticide, recommended in tomato for the control of fruit borer in the form of 35% EC @450 g a.i ha^{-1} by CIBRC. Indian MRL values of phosalone for vegetables are 1 ppm and there are no MRLs for tomato as per CAC. Further there is no specified Pre Harvest Interval [PHI] suggested by CIBRC. In this view a research project was taken to study dissipation pattern of phosalone in both open fields and poly houses, and to address the food safety issue, common household decontamination methods were evaluated for their efficiency in removing phosalone residues from tomato fruits. Phosalone residues were quantified through regular sampling till the residues are below determination level (BDL) of 0.25 mg kg^{-1} following the validated QuEChERS method. Initial deposits of 4.55 mg kg^{-1} were detected in tomato samples collected from the poly house, which dissipated to BDL by 7th day with the half-life of 1.06 days. In open fields, deposits of 2.52 mg kg^{-1} dissipated to BDL by 5th day with the half-life of 1.42 days, based on the study PHI of 3 days and 5 days can recommended in open filed and poly house tomato respectively as the residues were below MRL of 1 ppm by respective days after treatment. Veggy wash found to be very effective in removing flubendiamide residues to an extent of 55.13%, followed by 4% acetic acid solutions (52.34%) and 2% salt solution (47.60%).

Keywords: Tomato, phosalone, residues, dissipation, half-life, MRL

1. Introduction
Tomato *Solanum Lycopersicum* is one of the most important vegetable crops in tropical and subtropical regions of the world. It is consumed in raw form as salad, home-cooked, or processed as juice, paste, or sauce. In India tomato yield is severely affected by various factors among which damage caused by insect pests is important, as the fruit is soft and tender, minor damage will also leads to unacceptability of produce in the market. Among insect pests fruit borer, *Helicoverpa armigera* and tobacco caterpillar *Spodoptera litura* pose the major threat by feeding on leaves and fruits resulting in yield loss ranging from 20 to 60 percent [13, 4]. Phosalone, 6-chloro-3 (diethoxyphosphinothioylsulfanylmethyl)-1,3-benzoxazol-2-one, is an organophosphorus insecticide, recommended in tomato for the control of fruit borers in the form of 35% EC @450 g a.i ha^{-1} by CIBRC. Pesticide residues in fruits and vegetables has affected exports in recent years and should be strictly monitored owing to the high concern about the toxic properties of residues. Studies on farm gate monitoring of vegetables carried out in different parts of India revealed contamination mostly with organophosphorous and synthetic pyrethroids insecticides, indicating clearly the changes in the usage pattern from organochlorine to other groups of pesticides [12]. Further, with the intensive use of pesticides in poly house crops, residues may be accumulated at levels higher than those permitted by the EU or international maximum residue levels. The risk of pesticide residues in foods need to be addressed as per FSSAI (Food Safety and Standards Authority of India) and hence for the protection of consumer health and interests, household risk mitigation methods for removal of pesticide residues in tomato are to be recommended based on the scientific evaluation, as the food habits are changing enormously. Indian MRL values of phosalone for vegetables are 1 ppm and there are no MRLs for tomato as per CAC. Keeping these important issues of concern, the present study was planned to study the dissipation kinetics and risk assessment of phosalone in tomato grown under the poly house and open field conditions and to evaluate decontamination methodologies for removal of phosalone residues.

2. Materials and Methods

2.1 Chemicals and Reagents

Certified Reference Materials (CRM) of phosalone (96.9% purity) were procured from M/S Sigma Aldrich, Germany, and primary, intermediary and working standards were prepared from the CRMs using GC PR grade acetone and hexane as solvents. Working standards of phosalone 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. Primary Secondary Amine (Agilent), magnesium sulfate anhydrous (Emsure grade of Merck), sodium sulfate anhydrous (Emparta ACS grade of Merck), acetonitrile (HPLC gradient grade of Merck), acetic acid glacial (HPLC grade of Merck), acetone (Emplure grade of Merck), n-hexane (HPLC grade of Merck) were used during the study for sample preparation. Phosalone 35% EC was procured from local market.

2.2 Analytical Instruments and Limits of Detection

The working standards of phosalone were injected in Gas Chromatograph with Electron Capture Detector (ECD) and Thermionic Specific Detector (TSD) for estimating the lowest quantity of these pesticides which can be detected with injector split ratio of 1:10 under standard operating parameters (Table 1). It was found that the LOD (limit of detection) for phosalone is 0.01 ng, and the linearity is in the range of 0.01 ng to 0.10 ng.

2.3 Method validation

Prior to field experiments, QuEChERS (Quick Easy Cheap Effective Rugged Safe) method for extraction and clean-up was validated as per SANCO/12571/2013 guidelines. Tomato fruits (5 kg) collected from control plots were homogenized with high volume homogenizer (Robot Coupe Blixer 7L) and 15 g was taken in to 50 mL centrifuge tubes. The required quantity of phosalone intermediary standards is 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 the sample was homogenized for 2-3 min using Heidolph silent crusher (low volume homogeniser). Then 3±0.1g sodium chloride was added to the tube and mixed by shaking gently, and centrifuged for 3 min at 2500-3000 xg with Remi R-238 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 clean up) 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 xg. The extract of (2mL) was transferred into test tubes and evaporated to dryness using concentration work
station (Turbovap LV of Caliper life sciences) with nitrogen gas and reconstituted with 1mL n-Hexane: Acetone (9:1) for dimethoate analysis. Tomato samples fortified with phosalone 0.25 mg kg^{-1} and 0.5 mg kg^{-1} were analysed and the mean recovery of the residues using the method was 95.16% and 97.43% respectively and the results show that the method is suitable for the analysis of phosalone residues up to 0.25 mg kg^{-1}, and the limit of quantitation (LOQ) is 0.25 mg kg^{-1}. Fortification and recovery test results were presented in Table 2. The residues detected below 0.25 mg kg^{-1} were mentioned as levels Below Determination Level (BDL) in all cases.

2.4 Field experiments and sample collections

Tomato crop (Popular hybrid Nirupama) 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. Phosalone 35% EC procured from local market was sprayed @ 450 g a.i. ha^{-1} 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}. Pest damage free and crack free tomato fruits of 5 kg were collected from each plot in separate polythene bags and brought to laboratory. Samples were collected at regular intervals i.e. 0, 1, 3, 5, 7, 10, 15, 20 days after last spray for dissipation studies. For evaluation of risk mitigation/ decontamination methods, zero day samples were collected separately in large quantities and made into 6 sets, each in 4 replications. One set of sample is analyzed for initial deposits of flubendiamide. The remaining sets of samples were subjected to various decontamination methods separately and the residues were calculated to know the efficiency of the various decontamination methods in removal of pesticide residues from the tomato samples. The decontamination / risk mitigation methods selected for evaluation of efficiency in removal of phosalone residues from tomato are presented in Table 3. After decontamination treatments, the samples were shade dried for 10 min placing on clean blotting papers and analyzed for residues remaining on tomato.

2.5 Calculation Methods

In order to calculate the rate of degradation and half-life of phosalone on tomato fruits, Hoskin's (1961) [3] linear regression equation was followed.

$Y = a + b X$

Where,

Y - Log of tolerance limit

a - Log of initial deposit

b - Slope of the regression line

In case of decontamination studies, per cent removal of flubendiamide was calculated.

3. Results and Discussion

Tomato fruits collected at regular intervals from phosalone sprayed research plots of open field and poly house was analyzed and the data is presented in Table 4. In poly house experiments initial deposit of 4.55 mg kg^{-1} was recorded at 2 hours after the last spray, which dissipated to Below Determination Level (BDL) of 0.25 mg kg^{-1} by 7th day after last spraying on tomato. The initial deposits were dissipated to 3.62, 2.09 and 0.86 mg kg^{-1} by 1, 3 and 5 days after last spray, respectively. The dissipation pattern showed a decrease of residues from the first day to 5th day. The residues dissipated by 20.43, 54.06, 81.09% at 1, 3 and 5 days, respectively. The half-life of phosalone on tomato was 1.06 days. Where as in case of open field studies initial deposit of 2.52 mg kg^{-1} was recorded at 2 hours after last spray, dissipated to 1.55 and 0.60 mg kg^{-1} by 1 and 3 days, respectively, and BDL of 0.25 mg kg^{-1} by the 5th day. The dissipation pattern showed fast decrease / dissipation of residues from the first day to 3rd day where the residues dissipated by 38.49% and 76.19% at 1 and 3 days, respectively, with half-life of 1.42 days.

In general, the studies conducted by various groups of scientists [10, 5]
on dissipation of phosalone on tomato, suggests that an initial deposit of 7.40-7.80 mg kg^{-1} were recorded, which dissipated

to BDL by 7-10 days depending up on the stage of application at a dose of 700 g ai ha^{-1}, and in the present study, an initial deposit of 2.52 mg kg^{-1} in open field, and 4.55 mg kg^{-1} in poly house from 450 g ai ha^{-1} applied plots suggests that the results are in full agreement with the general trend. Further, based on the reports suggests that when applied at 0.05% level on okra and black gram, respectively, initial deposits of phosalone around 10-11 mg kg^{-1} was recorded [7, 1].

The findings of present investigation are in agreement with research reports that [6] initial deposits of 4.74 mg kg^{-1} phosalone dissipated and persisted up to 7th day with half-life values of 2.96 days when applied @ 310 g a.i. ha^{-1} on round gourd. Further reports reveal that phosalone when applied @ 700 g a.i. ha^{-1} on brinjal reached Below Determination Level (BDL) by 5th day with half-life value of 1.56 [9]. Based on the available literature on dissipation of phosalone on various vegetable crops, it is clear that in most cases, the initial deposits are in the range of 7 to 10 mg kg^{-1} at recommended doses which dissipated to BDL in 7-15 days, depending up on the season and crop. In the present study on tomato, phosalone dissipation pattern is in agreement with all available literature clearly indicating that phosalone dissipates to BDL in 5-7 days depending up on the crop management practices. The efficiency of various risk mitigation methods for removal of phosalone residues from tomato is presented in Table 5. The percentage removal of phosalone residues from tomato when subjected to different decontamination solutions at 2 hours after spraying showed that dipping fruits in veggy wash solution for 10 min followed by tap water wash for 30 sec were found to be more effective (55.13%) than other treatments. Acetic acid solution of 4% (52.34%) was found to be next promising treatment, followed by 2% salt solution (47.60%), 0.1% baking soda solution (44.40%) and tap water wash (39.06%).

The present findings are in agreement with research reports that [11] maximum percent removal (51.30%) of phosalone residues from brinjal fruits achieved by bio wash treatment which contains similar ingredients as veggy wash used in our studies, followed by 0.1% baking soda solution (33.60%) and acetic acid solution of 4% (22.40%). Furthermore reports reveal that highest percent removal of phosalone residues from tomato fruits was achieved by 2% salt solution (52.00%) followed by cooking (55.1%), veggie wash(50.00%), 0.1% baking soda solution (32.90%) and acetic acid solution of 4% (26.20%) [2]. Maximum percent removal of phosalone from chilli fruits by veggie wash treatment (74.77%), followed by acetic acid solution of 4% (67.87%), 2% salt solution (65.05%), 0.1% baking soda solution (55.75%) and tap water (29.80%) were observed [8] which are in agreement with the findings of present investigation.

Table 1: Standard operating parameters of GC

Gas Chromatograph	Gas Chromatography- AGILENT- 7890B
Column	VF-5ms Capillary Column 30 m length, 0.25 mm Internal Diameter, 0.25 □m film thickness; 1% methyl siloxane
Column Oven (0C)	Initial 50^0C for 1 min - increase @ 20^0C/min upto 325^0C – hold for 14 min
Detectors	Electron Capture Detector (ECD) Thermionic Specific Detector (TSD)
Detector Temperature (0C)	300
Injector Temperature (0C)	280
Injector Status	Split Ratio: 1:10
Carrier Gas	Nitrogen, Iolar II, Purity 99.999%
Carrier Gas Flow (ml min^{-1})	1 ml min^{-1}
Make-up Flow (ml min^{-1})	35 ml min^{-1}
Retention time (min)	Phosalone 6.99 min
Total run time (min)	28.75 min

Table 2: Recovery of phosalone residues from tomato

Replication	Fortified level (mg kg^{-1})					
	0.05 mg kg^{-1}		0.25 mg kg^{-1}		0.50 mg kg^{-1}	
	Residues recovered (mg kg^{-1})	Recovery%	Residues recovered (mg kg^{-1})	Recovery%	Residues recovered (mg kg^{-1})	Recovery%
R1	-	-	0.224	89.67	0.499	99.73
R2	-	-	0.242	96.94	0.498	99.50
R3	-	-	0.247	98.87	0.465	93.07
Mean	-	-		95.16		97.43
SD				4.851		3.781
RSD				5.097		3.881

Table 3: Decontamination Methods for removal of phosalone residues from tomato

S. No	Treatment	Details of treatment
T_1	Tap water wash	4 L of tap water was taken into the plastic tub of 7 L capacity and 2 Kg of tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_2	Soaking in 2% salt solution	4 L of 2% salt solution was prepared by mixing 80 g of table salt in 4 L of water in plastic tub of 7 L capacity and 2 Kg tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_3	Dipping in 0.1% baking soda	4 L of 0.1% baking soda solution was prepared by mixing 4 g of baking soda in 4 L of water in plastic tub of 7 L capacity and 2 Kg tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_4	Soaking in 4% acetic acid	4 L of 4% acetic acid solution was prepared by mixing 160 ml of acetic acid glacial 100% in 4 L of water in plastic tub of 7 L capacity, mixture was kept for 1 min and 2 Kg of tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_5	Veggy wash	4 L of veggy wash was prepared by mixing 160 ml of acetic acid glacial 100%, 4 g of baking soda and lemon juice of 4 lemons in 4 L of water in plastic tub of 7 L capacity, mixture was kept for 1 min and 2 Kg tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.

Table 4: Dissipation of phosalone residues in poly house and open field situations

Replication	Residues in Poly House (mg kg^{-1})						Residues in Open field(mg kg^{-1})					
	R1	R2	R3	R4	Mean	% dissipation	R1	R2	R3	R4	Mean	% dissipation
0	4.51	4.73	4.72	4.24	4.55	0	2.34	2.58	2.72	2.44	2.52	0
1	3.65	3.59	3.63	3.62	3.62	20.43	1.68	1.50	1.49	1.53	1.55	38.49
3	2.00	2.00	2.16	2.19	2.09	54.06	0.63	0.70	0.55	0.54	0.60	76.19
5	0.95	0.78	0.77	0.94	0.86	81.09	BDL	BDL	BDL	BDL	BDL	100.00
7	BDL	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	BDL	100.00
10	BDL	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	BDL	100.00
15	BDL	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	BDL	100.00
20	BDL	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	BDL	100.00
Regression equation	Y = 4.313 + (-0.652) X						Y = 2.259 + (-0.485) X					
R^2	0.983						0.949					
Half-life	1.06 days						1.42 days					
(BDL) Below Determination Level : < 0.25 mg kg^{-1}												

Table 5: Removal of phosalone residues from tomato fruits with different decontamination methods

Treatments	Mean of phosalone detected (mg kg-1)*	Amount removed (mg kg-1) **	Percent removed
Tap water wash	1.54 ± 0.045	0.98 ± 0.090	39.06 ± 0.72
2% salt solution	1.32 ± 0.049	1.20 ± 0.077	47.60 ± 1.57
0.1% Baking soda solution	1.40 ± 0.057	1.11 ± 0.091	44.40 ± 0.72
4% Acetic acid solution	1.20 ± 0.031	1.31 ± 0.058	52.34 ± 1.04
Veggy wash	1.13 ± 0.065	1.39 ± 0.074	55.13 ± 0.74
C. D. at 5% = 2.10; Initial deposit = 2.52 mg kg^{-1}; * Mean of three replications; ** Amount removed = Initial deposit-Mean of replicates of each treatments.			

4. Conclusions

Dissipation pattern of phosalone varies from open field to poly house conditions when sprayed as per farmers practice. As per the Insecticide Act of 1968, Pre Harvest Intervals are not recommended for phosalone on tomato, as Codex MRLs are not fixed. Although FSSAI given MRL of 1 ppm on vegetables. Based on the present studies a safe waiting period (PHI) of 3 days can be recommended in open fields and 5 days in poly house conditions as residues were below MRL of 1 ppm by respective days after treatment. Among decontamination methodologies evaluated, veggie wash, 2% salt solution, and 4% acetic acid/vinegar solution, proved effective in removal of phosalone residues from tomato fruits.

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