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Risk analysis of profenofos on tomato in poly house and open fields and risk mitigation methods for removal of profenofos residues from tomato for food safety.

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ABSTRACT

Profenofos is a broad spectrum foliar insecticide and acaricide with contact and systemic action, widely used on tomato in India for the management of sap sucking insects and mites. Profenofos is not registered for use in India on tomato, and hence Maximum Residue Limits are not available as per Food Safety and Standards Authority of India. However, use of profenofos in poly house and open fields is very common, and hence profenofos residues are found in market samples. A research project was taken to study dissipation pattern of profenophos 50% EC in both open fields and poly houses, when applied twice @ 500 g a.i. ha^{-1}, first spray at fruit initiation followed by second spray at 10 days interval as per the farmers practice. Profenofos residues were quantified through regular sampling till the residues are below determination level (BDL) of 0.05 mg kg^{-1} following the validated QuEChERS method. The qualitative and quantitative analysis of profenofos was performed on GC-FPD and GC-MSMS (TQD). Initial deposits of 3.25 mg kg^{-1} were detected in tomato samples collected from poly house, which dissipated to BDL by 15th day with half-life of 2.43 days. In open fields, deposits of 1.51 mg kg^{-1} dissipated to BDL by 7th day with half-life of 1.55 days, indicates that dissipation is slow in poly house compared to open fields due to various factors, and in both situations initial deposits are lower than the MRL (10 mg kg^{-1}) of Codex Alimentarius Commission hence a pre-harvest interval of 1 day is recommended. MRL of 7 mg kg^{-1} in poly house tomato and 3 mg kg^{-1} in open field tomato is recommended based on the OECD (Organization for Economic Co-operation and Development) calculator and chronic hazard exposure assessment taking into consideration of average body weight, national per capita tomato consumption and acceptable daily intake (ADI) of profenofos. Among various decontamination methods tested, veggy wash found to be very effective in removing profenofos residues to an extent of 75.84% which can be recommended as risk mitigation method for food safety, followed by 4% acetic acid solution (71.22%) and tap water wash was least effective (37.60%) in removing profenofos residues from tomato.

Highlights

- Maximum residue limits of 7 mg kg-1 profenofos are recommended on tomato based on risk analysis, and veggy wash removes profenofos residues on tomato up to 76%.

Keywords: profenofos, tomato, poly house, open fields, dissipation, risk analysis, decontamination methods, food safety.

Tomato (Lycopersicon esculentum Mill.) is widely consumed vegetable in India usually in the form of curry, and also in raw form as salad, home-cooked, or processed as juice, paste, or sauce. Tomato contains 200 kcal kg^{-1}, 9 g protein kg^{-1}, and 2 g fat kg^{-1} (Gopalan *et al.*, 1991). During 2012-13 in India, tomato 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 (Indian horticulture database 2014). In recent years, due to better cost benefit ratio, farmers are growing in controlled atmosphere conditions, majorly in poly house, besides regular open fields during crop seasons. The tomato yield in India is considerably lower because of several factors of which the damage caused by leaf hoppers, aphids, caterpillar, flea beetles, leaf miner, spider mites, and fruit borer (Singh *et al.*, 1989) is economical. The 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 (O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioate), an organo phosphate 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. Profenofos is available in India in 50% EC formulation. As per Insecticide Act, 1968 of India (cibrc.nic.in), profenofos is not registered for use on tomato and hence maximum residue limits (MRLs) are not fixed by Food Safety and Standards Regulation, 2011 of India. Further, the reports on national residue monitoring studies and state level monitoring studies conducted by the laboratory revealed that profenofos residues are detected in samples collected from markets. 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} for the control of insects and mites in both open field and poly house conditions, hence profenofos residues were detected in market samples. The acceptable daily intake for profenofos is of 0-0.03 mg kg^{-1} body weight, and acute reference dose of 1 mg kg^{-1} body weight (Codex Alimentarius Commission). As per the National Sample Survey conducted during 2011-12 in India, per capita consumption of tomato in rural and urban area is 586 and 806 grams per month, respectively (NSSO, 2014). Analysis on presence of residues at harvest time following farmers practice is essential to study the risk analysis so as to recommend Maximum Residue Limits (MRLs) and Pre-Harvest Intervals (PHIs). Hence, a comprehensive research was conducted during 2012-13 crop seasons to assess dissipation pattern of profenofos on tomato in both open field and poly house situations so as to recommend pre harvest intervals based on the risk analysis taking in to consideration of acceptable daily intake, per capita tomato consumption and average body weight. Extended studies were also conducted to establish the recommendations for removal of profenofos residues from tomato with simple house hold techniques to contain risk of profenofos residues from tomato.

Materials and Methods

Chemicals and Reagents: Certified Reference Materials (CRM) of profenofos (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 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. Profenofos 50% EC was procured from local market.

Analytical Instruments and Limits of Detection

Working standards were injected in Gas Chromatograph (Agilent 7890 B) with Electron

Capture Detector (ECD) and Thermionic Specific Detector (TSD) with injector split ratio of 1:10 using VF-5ms Capillary Column) and confirmatory analysis was done on Bruker Scion 436 GC-MS/MS Triple Quadrupole Detector (EI) using Multiple Reaction Monitoring (MRM) method (Qualifier ions: 339>188, 339>251, 339>269, 139>97; Quantifier Ions: 139>97). It was found that the limit of detection for profenofos is 0.05 ng in GC-TSD with linearity range of 0.05 ng to 5 ng.

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 profenofos 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 sample was homogenized 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 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 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 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

Table 1 : Recovery of profenofos residues from tomato

Replication	Fortification 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	0.049	98.59	0.238	95.14	0.480	95.95
R2	0.047	94.49	0.236	94.21	0.479	95.77
R3	0.047	94.49	0.240	96.13	0.470	94.07
Mean		95.85		95.16		95.27
SD		2.367		0.960		1.025
RSD		2.469		1.008		1.076

Table 2. Decontamination Methods for removal of profenofos residues from tomato

S.No	Treatment	Details of treatment
T1	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.
T2	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.
T3	Dipping in 0.1% baking soda) (NaHCo3)	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.
T4	Soaking in 4% acetic acid solution	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.
T5	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.

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 for applying recovery factor while calculating the residues in samples. Fortification and recovery test results were presented in Table 1 and the method followed for qualitative and quantitative estimation of profenofos is suitable up to 0.05 mg kg^{-1} levels as the recoveries obtained are 95.85%, 95.16% and 94.07, respectively at 0.05, 0.25 and 0.50 mg kg^{-1} fortification level. The residues detected below 0.05 mg kg^{-1} were mentioned as levels Below Determination Level (BDL) in all cases.

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. Profenofos 50% EC procured from local market was sprayed @ 500 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 profenofos.

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 pesticide residues from tomato were presented in Table 2. After decontamination treatments, the samples were shade dried for 10 min placing on clean blotting papers and analysed for residues remaining on tomato.

Table 3. Dissipation of dimethoate residues in open fields and poly house situations

Days after treatment	Residues in Poly House (mg kg-1)						Residues in Open Field (mg kg-1)					
	R1	R2	R3	R4	Mean+SD	% dissipation	R1	R2	R3	R4	Mean+SD	% dissipation
0 (2 h)	3.26	3.33	3.22	3.18	3.25	0	1.51	1.57	1.46	1.50	1.51	0
1	2.77	2.62	2.65	2.64	2.67	17.84	0.86	0.82	0.83	0.82	0.83	45.03
3	1.70	1.76	1.62	1.58	1.66	48.92	0.45	0.44	0.46	0.37	0.43	71.52
5	0.71	0.71	0.70	0.70	0.70	78.46	0.25	0.19	0.27	0.26	0.24	84.10
7	0.41	0.43	0.37	0.41	0.41	87.38	BDL	BDL	BDL	BDL	BDL	100.00
10	0.18	0.20	0.19	0.15	0.18	94.46	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 = 3.523+ (-0.124) X (log*1000 residue)						Y = 3.184 + (-0.194) X (log*1000 residue)					
R2	0.995						0.961					
Half-life (days)	2.427						1.552					
TBDL (days)	17.22						11.94					

BDL Below Determination Level of 0.05 mg kg^{-1}

Calculation Methods

Half-life and TBDL (Time required for residues to reach below determination level) were calculated as per Hoskins (1966) from first-order dissipation kinetics. OECD (Organization 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 taking in to consideration of national per capita tomato consumption, average body weight and ADI of profenofos. In case of decontamination studies, per cent removal of profenofos was calculated.

Results and Discussion

Tomato fruits collected at regular intervals from profenofos sprayed research plots of open field and poly house were analysed and the data is presented in Table 3. In poly house experiments, initial deposits of 3.25 mg kg^{-1} were detected after 2 hrs of last spray, which dissipated to 2.67 mg kg^{-1} by 1st day, 1.66 mg kg^{-1} by 3rd day, 0.70 mg kg^{-1} by 5th day, 0.41 mg kg^{-1} by 7th day, 0.18 mg kg^{-1} by 10th day and BDL of 0.05 mg kg^{-1} by 15th day. The calculated half-life is 2.427 days and TBDL of 17.22 days. In open field situations, initial deposits of 1.51 mg kg^{-1} were detected after 2 hrs of last spray, which dissipated to 0.83 mg kg^{-1} by 1st day, 0.43 mg kg^{-1} by 3rd day, 0.24 mg kg^{-1} by 5th day and BDL of 0.05 mg kg^{-1} by 7th day. The calculated half-life is 1.551 days and TBDL of 11.94 days. The results are in full agreement with the findings of Sahoo *et al.*, (2004) who reported that profenophos spray on tomato @ 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 Romeh et al (2009) on tomato. Experimental results of Radwan *et al.*, (2004a) 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 (Gupta *et al.*, 2011, Renuka *et al.*, 2006, Katroju *et al.*, 2014) on dissipation on profenophos on different crops clearly indicate

Table 4. Chronic hazard exposure assessment for recommending profenofos MRLs on tomato

OECD MRL calculator Date sets	Poly House	Open Field
Total number of data (n)	7	5
Percentage of censored data (%)	14	20
Number of non-censored data	6	4
Lowest residue	0.040	0.050
Highest residue	3.250	1.510
Median residue	0.700	0.430
Mean	1.273	0.612
Standard deviation (SD)	1.278	0.579
Correction factor for censoring (CF)	0.905	0.867
Proposed MRL estimate	Poly House	Open Field
Highest residue	3.250	1.510
Mean + 4 SD	6.384	2.928
CF X 3 Mean	3.455	1.591
Unrounded MRL	6.384	2.928
Rounded MRL	7.000	3.000
Risk Analysis	Poly House	Open Field
Average human body weight (kg)	55	
National per capita intake of tomato	806 g $month^{-1}$	
Daily intake of crop (C) = kg $person^{-1}$	0.027	
Consumption of crop C(F_C) = kg kg bw^{-1}	0.00049	
ADI for profenofos (mg kg bw^{-1})	0.03	
TMDI = F_c X MRL (from OECD calculator)	0.00344	0.00147
TMDI v/s ADI	TMDI < ADI	TMDI < ADI
Proposed MRL (mg kg^{-1})	7.00	3.00
Codex MRL (mg kg^{-1})	10	
FSSAI (India) MRL (mg kg^{-1})	Not Available	
EU MRL (mg kg^{-1})	10	

that when applied at recommended dose, the initial deposits are less than 3 mg kg^{-1} and dissipates to BDL in 7-10 days depending on the crop, except on cardamom.

Various groups suggested safe waiting periods varying from 1 to 14 days, but based on CAC MRLs (10 mg kg-1) it is not necessary to recommend pre- harvest intervals till the residues reach BDL, and taking into consideration of MRLs set by CAC and results on dissipation dynamics on tomato in present study, it can be scientifically concluded and recommended that PHI of 1 day is exceedingly well as the initial deposits as well below 10 mg kg-1. In India, as per Food Safety and Standards Authority of India (FSSAI) MRLs are not fixed for profenofos on tomato. Hence, risk analysis is necessary for setting MRLs based on supervised field trials. Based on the present studies in poly house, as per OECD calculator, MRL of 7 mg kg-1 can be suggested since the TMDI calculated based on OECD MRL is not more than the ADI of 0.03 mg kg body weight. Hence, MRL of 7 mg kg-1 is suggested based on the risk analysis. Similarly, in open field studies, OECD calculator suggest MRL of 3 mg kg-1 for profenofos on tomato, and further this MRL can be taken as proposed MRL as the risk analysis studies indicates that TMDI is lower than the ADI (Table 4)

The efficiency of various risk mitigation methods for removal of profenofos residues from tomato is presented in Table 5. The percentage removal of profenophos residues from tomato when subjected to different decontamination solutions at 2 hours after spraying showed that dipping in veggy wash solution for 10 min was found to be most effective removing 75.84% residues, than other treatments. The next promising treatment was 4% Acetic acid solution (71.22 %), followed by 2% salt solution (55.31 %), 0.1% Baking soda solution (47.60 %) and tap water (37.60 %). Based on the percentage removal of residues, it was statistically proved that there is significant difference in the efficiency of decontaminating solutions in removing residues of above mentioned pesticides. In the present study, veggy wash, a formulation prepared by AINP on Pesticide Residues proved to be the most efficient in removing various pesticides. Many workers (Radwan *et al.*, 2004b; Jayakrishnan *et al.*, 2005; Zhang *et al.*, 2006; Klinhom, 2008; Liang *et al.*, 2012) suggested that washing with 1% acetic acid solution, 0.1% NaHCO3 solution removes various pesticide residues in different vegetables, and the extent of removal varies from type of pesticide and vegetable. Research conducted by Abou-Arab, 1999 showed that washing of tomato fruits with 10% salt solution removed 90.80 and 82.40% of dimethoate and profenophos residues and tap water wash was the least effective treatment, washing of tomato fruits with water removed dimethoate and profenophos residues up to 18.80 and 22.17% respectively. Studies conducted by Cherukuri et al (2014) and Shashi et al (2014) reveals that washing of brinjal and tomato with 2% salt solution is effective in removing various pesticides. Based on the test reports, it can be concluded that profenophos can be removed from tomato for food safety with simple house processing methods, and out of all methods, washing with AINP formulation i.e. veggy wash proved to be the best, and also economical. So, this result can be propagated and popularized among home makers

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

Treatments	Mean of profenofos detected (mg kg-1)*	Amount removed (mg kg-1) **	Percent removed
Tap water wash	0.94 + 0.072	0.57 + 0.042	37.60 + 0.95
2% salt solution	0.68 + 0.039	0.87 + 0.054	55.31 + 0.23
0.1% baking soda solution	0.79 + 0.046	0.76 + 0.055	47.60 + 0.75
4% acetic acid solution	0.43 + 0.061	1.12 + 0.091	71.22 + 0.42
Veggy wash	0.37 + 0.019	1.18 + 0.101	75.84 + 1.37

C. D. at 5% = 1.57; Initial deposit = 1.51mg kg^{-1}; * Mean of three replications; ** Amount removed = Initial deposit-Mean of replicates of each treatments.

for removal of pesticides from tomato when used as fresh vegetable salad, and also create confidence that they eat safe food without pesticide residues.

Conclusions

Dissipation pattern of profenophos varies from open field situation to poly house conditions when sprayed as per farmers practice. The risk analysis conducted based on MRLs calculated using OECD calculator, ADI of CAC, per capita tomato consumption in India and average body weight concludes that MRLs of 7.0 mg kg^{-1} and 3.0 mg kg^{-1} can be suggested in poly house and open field situations, respectively, as the TMDI do not exceeds the ADI. Profenofos application @ 500 g ai ha^{-1} twice can be included in GAPs of Tomato in India with MRL of 3 mg kg^{-1} in open field cultivation. Further, as house hold risk mitigation method, washing of market tomatoes with veggy wash or 2% salt solution can be followed for removal of profenofos residues as food safety method.

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