Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(6): 1966-1970
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Received: 21-09-2017
Accepted: 26-10-2017

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Evaluation of decontamination methods for removal of pesticide residues from tomato *Solanum lycopersicum*

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Abstract
The study has been designed to determine the extent of pesticide residues removal from tomato fruits through common house hold practices. Experiment was conducted during 2013-14, kharif season. Tomato crop was planted in student's farm, College of Agriculture, Rajendranagar, Hyderabad. The most commonly used pesticides such as dimethoate, lambda cyhalothrin, phosalone, flubendiamide, and profenophos were sprayed twice at recommended doses first at fruit formation stage and 10 days after first spray. Samples were collected at 2 hours after second spray to quantify the deposits further samples were subjected to various household treatments, each in three replications, and analysed for residues using validated QuEChERS method and using GC-ECD, (GAS Chromatography Electron capture detector), Thermionic Specific Detector (TSD) and Liquid Chromatograph with Photo Diode Array (PDA) Detector so as to estimate the per cent removal and their effectiveness. Out of all treatments, veggy wash a formulation prepared by AINP on Pesticide Residues proved to be the most efficient by removing pesticides residues in the range of 55-76%. The next promising treatment was 4% acetic acid solution (52-71%), flowed by 2% salt solution (39-58%), 0.1% baking soda solution (39-52%) and tap water (17-39%).

Keywords: Tomato, residues, food safety, decontamination

1. Introduction

Tomato *Solanum lycopersicum*, is one of the important vegetable crop grown in tropical and subtropical regions of the world. It is consumed as fresh or in multiple of processed forms [14]. Due to the presence of antioxidants including carotenes, (Lycopene, β-carotene), ascorbic acid, and phenolic compounds consumption of tomato is recommended to avoid several diseases [9]. In India productivity of tomato is hampered mainly by the insect-pests viz, fruit borer *Helicoverpa armigera*, tobacco caterpillar *Spodoptera litura*, whitefly *Bemisia tabaci*, serpentine leaf miner *Liriomyza trifolii* and recent invasive pest south american tomato pinworm, *Tuta absoluta* are of importance [7, 13, 15]. Despite of IPM recommendations Indian farmers tend to rely heavily on pesticides for plant protection purposes, indiscriminate use of pesticides without following GAPs (Good Agricultural Practices) has led to the contamination of soil and crop produce by pesticide residues [16]. Exposure to pesticides results in acute and chronic health problems. Increasing incidence of cancer, chronic kidney diseases, suppression of the immune system, sterility among males and females, endocrine disorders, neurological and behavioural disorders, especially among children, have been attributed to chronic pesticide poisoning [17]. Safe food idea is not new, but after the formation of World Trade Organization (WTO) and agreement on Sanitary and Phytosanitary (SPS) measures in international trade, the food exports and imports standards have been implemented by all countries [3]. The legal standard for pesticide residues worldwide is Maximum Residues Limit (MRL), which is set by regulatory authorities at national (FSSAI-India) and international level (Codex Alimentarius Commission) [3]. Like other vegetables tomato is majorly consumed as a salad, hence risk of pesticide residues getting in to food chain and bio magnification always exists. The risk of pesticide residues in foods need to be addressed as per FSSAI 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 [6].

Decontaminating the fresh fruits and vegetables by various household methods, have found effective in reducing pesticide residues [2]. Among various methods available soaking of fruits and vegetables in various organic solutions followed by washing them with tap water found effective and easy way of reducing pesticide load from fresh products [2, 11].With this background the present study was conducted to evaluate simple house hold decontamination methodologies for their efficiency in removing pesticide residues from tomato fruits.

2. Materials and Methods

2.1 Field Experiment: The present experiment was conducted during 2013-14, kharif season. Tomato crop was planted in student's farm, College of Agriculture, Rajendranagar, Hyderabad.

2.1.1 Layout of the field experiment: The present experiment was
laid out in a Randomized Block Design (RBD) with six treatments including untreated control replicated four times. A popular tomato hybrid Nirupama (TO-1988) was chosen for the study.

2.1.2 Selection of pesticides: In India CIBRC (Central Insecticides Board and Registration Committee) has recommended 28 insecticides for use on tomato (CIBRC), out of these 28 insecticides we have selected 4 most commonly used insecticides viz., dimethoate, λ- cyhalothrin, phosalone, flubendiamide and profenophos although it is not recommended by CIBRC, it was included in the study by considering usage by farmers and detection of profenophos residues in farm gate tomato samples.

2.1.3 Application of insecticidal treatments: All the selected insecticide treatments and dosages applied as foliar sprays are presented in Table 1. The first spray was given after fruit initiation and second spray was given at 10 days after first spray. A total of two sprays were given during the experiment.

2.2 Laboratory Experiment

2.2.1 Preparation of working standards: Certified Reference Materials (CRMs) of Dimethoate, λ-cyhalothrin, Phosalone, Flubendiamide and Profenophos were purchased from Dr. Erhenstorfer, Germany. Primary standards, 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. All the standards were stored in deep freezer maintained at -20 ^{0}C.

2.2.2 Limit of Detection and Linearity: The working standards of dimethoate, λ-cyhalothrin, phosalone and profenophos were injected in Gas Chromatograph with Electron Capture Detector (ECD) and Thermionic Specific Detector (TSD) and flubendiamide standards were injected in Liquid Chromatograph with Photo Diode Array (PDA) Detector for estimating the lowest quantity of these pesticides which can be detected under standard operating parameters as given below in Table 2 and 3. It was found that the LOD (limit of detection) for all pesticides is 0.01 ng, and the linearity is in the range of 0.01 ng to 0.10 ng except for flubendiamide where LOD is 0.05 ng, and the linearity is in the range of 0.05 ng to 0.10 ng.

2.2.3 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 Dimethoate, λ-cyhalothrin, Phosalone, Flubendiamide and Profenophos intermediary standard prepared from CRM is added to each 15 g sample to get fortification levels of 0.05 ppm, 0.25 ppm and 0.5 ppm 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 homogenizer). 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 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 all pesticides except flubendiamide. For flubendiamide filtered 1 ml final extract was directly injected in HPLC and the residues of pesticides recovered from fortified samples were calculated using the following formula.

$$\text{Residues (mg kg}^{-1}) = \frac{\text{Sample peak area X conc of std (ppm) X µl std. injected X Final volume of the sample}}{\text{Standard Peak area X weight of sample analyzed X µl of sample injected}}$$

$$\text{Wt of the Sample analyzed} = \frac{\text{Sample weight (15 g) X aliquot taken}}{\text{Volume of acetonitrile (30 ml)}}$$

Tomato samples fortified with test insecticides 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 using the method is presented in table 4. Results show that the method is suitable for the analysis of dimethoate, lambda cyhalothrin, flubendiamide and profenophos residues up to 0.05 mg kg^{-1}, and the limit of quantitation (LOQ) is 0.05 mg kg^{-1}.Where as in case of phosalone method is suitable for the analysis up to 0.25 mg kg^{-1}, and the limit of quantitation (LOQ) is 0.25 mg kg^{-1}.

2.2.4 Evaluation of Decontamination methods: 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 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 methods selected for evaluation are presented in Table 5. After decontamination treatments, the samples were shade dried for 10 min placing on clean blotting papers and analyzed for residues remaining on tomato.

2.3 Statistical analysis: Fortification and recovery were calculated as percentage of insecticide residues recovered. Initial deposits of pesticide residues after two hours of spray were calculated by taking the average of four replications. Standard Deviation (SD) and Relative Standard Deviation (RSD) were calculated to know the reliability of the data. Removal of insecticide residues by decontamination methods were measured in per cent and expressed as mean per cent removal. Critical Difference @ 5% was calculated.

3. Results And Discussion

The tomato samples were collected from plots treated with recommended doses of dimethoate, lambda-cyhalothrin, phosalone, flubendiamide and profenophos to estimate the initial deposits, and efficiency of different decontamination methods was evaluated through quantification of their residues after subjecting to risk mitigation methods. Initial deposits of test insecticides 2hrs after spray and mean per cent removal of insecticides by various decontamination methods are presented in table 6 and 7 respectively. In the present study, veggy wash, a formulation prepared by AINP on Pesticide Residues proved to be the most efficient in removing various pesticides. The next promising treatment was dipping in 4% acetic acid solution for 10 min followed by tap water wash for 10 sec, these findings are in agreement with the reports of Dikshit *et al.,* (1984) [4] who revealed that washing of cowpea with 1% acetic acid solution was capable of removing 85.70 and 88.60% of metasystox and carbaryl residues. Research findings of Radwan *et al.,*(2004) [10] revealed that washing of hot pepper, sweet pepper and brinjal with 2% acetic acid removed pirimophos-methyl residues by 76.61, 95.74 and 94.58%. 2% salt solution was found to be third best treatment except in case of profenophos where 0.1% baking soda solution was found as third best treatment. These results are in agreement with the findings of Reddy and Rao (2004) [12] wherein 72.80, 67.50, 51.80 and 58.20% removal of acephate, chlorpyriphos, quinalphos and bifenthrin residues from grapes was achieved by dipping them in 2% salt solution for 10 min, followed by water wash. Dipping in 0.1% baking soda (NaHCo3) solution for 10 min followed by tap water wash was the 4th best treatment in removing residues from tomatoes. These findings are in line with the findings of Liang *et al.,*(2012) [8] where in washing of cucumber with 2% NaHCO3 was efficient enough to remove the trichlorfon, dimethoate, dichlorovos, fenitrothian and chlorpyrifos residues by 73.20, 58.70, 96.40, 51.10 and 77.80%. Tap water wash was the least effective treatment and the findings of present investigations are in agreement with the reports of Abou-Arab (1999) [1] that washing of tomato fruits with water removed dimethoate and profenophos residues up to 18.80 and 22.17 % respectively. Further reports of Jayakrishnan *et al.,* (2005) [5] reveals washing of tomato fruits with water removed lambda cyhalothrin residues by 29-30%.

Table 1: Details of Insecticides treatments

Treatment	Common Name of Insecticide	Dosage (g a.i ha^{-1})	Trade Name & Formulation
T_1	Dimethoate	300	Rogor 30% EC
T_2	λ-cyhalothrin	15	Karate 5 % EC
T_3	Phosalone	450	Zolone 35% EC
T_4	Flubendiamide	48	Takumi 20% WG
T_5	Profenophos	500	Curacron 50% EC
T_6	Control		

Table 2: 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 mm film thickness; 1% methyl siloxane
Column Oven (0C)	Initial 50 ^{0}C for 1 min - increase @ 20 ^{0}C/min upto 325 ^{0}C – 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)	Dimethoate 9.95 min λ-cyhalothrin 11.87 min Phosalone 6.99 min Profenophos 5.27 min
Total run time (min)	28.75 min

Table 3: Standard operating parameters of HPLC

HPLC	SHIMADZU LC-20		
Detector	HPLC Photo Diode Array Detector (PDA)		
Column	HPLC Column Kinetex 5U –C18 column, 100 X 4.6 mm ID		
Wave Length	254 nm		
Solvents in Pump A Solvents in Pump B	Water Acetonitrile		
Solvents Gradient Program	Water: Acetonitrile (40:60) mixture run for 10 min		
Solvents Gradient rate	0.8 ml min-1		
Quantity of sample injected	20 µl		
Run time	10 min		
Retention time	Flubendiamide – 4.12 min		
LC Program	Time	Acetonitrile	Water
	0.00 10.0	60 80	40 20

Table 4: Recovery of insecticide residues in tomato

Insecticides	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 %
Dimethoate	0.049	99.23	0.236	94.68	0.331	88.27
Lambda Cyhalothrin	0.045	91.44	0.262	105.08	0.567	113.46
Phosalone	-	-	0.237	95.16	0.487	97.43
Profenophos	0.047	95.85	0.238	95.16	0.426	95.27
Flubendiamide	0.059	117.79	0.259	104.00	0.489	97.93
Residues recovered (mg kg^{-1}) and Recovery % presented in table is the average of three replications.						

Table 5: Decontamination methods for removal of pesticide residues from tomato fruits

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 6: Pesticide Residues (mg/kg) in tomato samples collected at 2 hours after spray

Pesticide	Residues (mg kg^{-1})					SDEV	% RSD	MRL (mg kg^{-1})		
	R1	R2	R3	R4	Average			FSSAI	CAC	EU
Dimethoate	1.31	1.33	1.31	1.31	1.31	0.01	0.85	2.00	NA	0.02
Lambda Cyhalothrin	0.13	0.13	0.13	0.13	0.13	0.001	0.52	NA	NA	0.1
Phosalone	2.34	2.58	2.72	2.44	2.52	0.16	6.49	1.00	NA	0.01
Profenophos	1.51	1.57	1.46	1.50	1.51	0.04	3.17	NA	10.00	10.00
Flubendiamide	0.93	0.87	0.92	0.87	0.90	0.03	3.46	NA	2.00	0.2
SDEV: Standard Deviation, RSD: Relative Standard Deviation, FSSAI: Food Safety and Standards Authority of India CAC: Codex Alimentarius Commission, EU: European Union										

Table 7: Percent removal of pesticide residues over control

Insecticides	Mean per cent removal of insecticides (%) ± SD					
	Decontamination methods					CD (5%)
	Tap water	2% salt solution	0.1% Baking soda solution	4% Acetic acid solution	Veggy wash	
Dimethoate	23.29 ± 1.44 (1.01)	58.69 ± 0.46 (0.54)	52.30 ± 0.68 (0.63)	65.49 ± 0.36 (0.45)	76.77 ± 0.04 (0.31)	1.55
Lambda cyhalothrin	29.43 ± 2.81 (0.09)	48.02 ± 2.14 (0.07)	39.59 ± 2.50 (0.08)	59.31 ± 0.05 (0.05)	68.87 ± 2.19 (0.04)	3.89
Phosalone	39.06 ± 0.72 (1.54)	47.60 ± 1.57 (1.32)	44.40 ± 0.72 (1.40)	52.34 ± 1.04 (1.20)	55.13 ± 0.74 (1.13)	2.10
Flubendiamide	17.71 ± 1.85 (0.74)	39.75 ± 0.95 (0.54)	45.30 ± 0.81 (0.49)	61.63 ± 0.55 (0.34)	65.39 ± 1.15 (0.31)	1.55
Profenophos	37.60 ± 0.95 (0.94)	55.31 ± 0.23 (0.68)	47.60 ± 0.75 (0.79)	71.22 ± 0.42 (0.43)	75.84 ± 1.37 (0.37)	1.57
Figures in the parenthesis are concentration of insecticide residues mg kg^{-1}						

4. Conclusion
Based on the study results, it can be concluded that pesticides such as dimethoate, lambda-cyahlothrin, phosalone, flubendiamide and 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. Apart from this 2% salt solution and 4% vinegar solution was also found effective, which can be easily prepared at house hold level. Therefore this result can be propagated and popularized among home makers for removal of pesticides from tomato when used as fresh vegetable salad, and also create confidence that they eat safe food without pesticide residues.

5. Acknowledgement
This research was supported by ICAR-All India Network Project on Pesticide Residues and PJTSAU, Hyderabad. I am thankful Dr. Cherukuri Sreenivasa Rao who provided insight, expertise and guidance that greatly assisted the research. I would also like to show my gratitude to ICAR, New Delhi for providing Junior Research Fellowship, during research period.

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