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REDUCTION OF ENDOSULFAN RESIDUES IN BRINJAL FRUITS DURING PROCESSING

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ABSTRACT

Laboratory experiments were carried out to find out the dissipation and to assess the effect of household processing like washing, boiling and washing plus boiling on reduction of endosulfan residues on brinjal (*Solanum melongena* L.) at room temperature and refrigerated conditions. Half life periods were 3.30 and 3.41 days at room temperature and 3.81 and 3.72 days at refrigerated conditions for single and double dose, respectively, showing faster dissipation at room temperature. The processing results indicated that washing plus boiling led to more than 72 per cent loss of endosulfan residues. Washing, boiling as well as washing plus boiling were found comparatively more effective in samples kept at room temperature than under refrigerated conditions.

Key words: Endosulfan, Brinjal, Processing, Half-life period, Storage, Residues, Reduction.

INTRODUCTION

Vegetables are essential components of our diet due to their nutritional value. Fruits, nuts, and vegetables play a significant role in human nutrition, especially as sources of vitamins (C, A, B_6, thiamine, niacin, E), minerals, and dietary fiber[1-3]. In near future, there is a need of around 5-6 million tones of vegetables to feed over 1.3 billion Indian population expected by the year 2020[4]. The total area under vegetables crops is 71, 31, 000 hectares with total annual production of 11, 01, 06000 tonnes[5]. However, several factors limit their productivity, mainly insect pests and diseases, due to increased pest menace there is an average loss of 40% in different crops[6].

Among vegetables, brinjal (*Solanum melongena* L.) is an important vegetable crop grown extensively in India. This crop assumes a special significance among vegetables as it gives better returns over investment to the farmers. But it suffers heavily at fruiting stage due to attack of shoot and fruit borer causing 70% damage to the crop thus making it totally unfit for human consumption[7,8]. In order to combat the insect pest problem, lot of pesticides is used by the vegetable growers for better yield and quality. Insecticides are repeatedly applied during the entire period of growth and sometimes even at the fruiting stage. It accounts for 13-14% of total pesticide consumption as against 2.6% of cropped area[9]. Indiscriminate use of pesticides particularly at fruiting stage and non adoption of safe waiting period leads to accumulation of pesticides residues in consumable vegetables. Contamination of vegetables with pesticide residues has been reported by many

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researchers[10-12]. Scientists and food processors have long been interested in the effect of processing on pesticide residues in food commodities. The extent to which pesticide residues are removed by processing depends on a variety of factors, such as chemical properties of the pesticides, the nature of food commodity, the processing step and the length of time the compound has been in contact with the food[13,14]. The presence of pesticide residues is a major bottleneck in the international trade of food commodities. Because of the invariable presence of pesticides, it becomes imperative to find some alternatives for decontamination of foods. Endosulfan, 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepine-3-oxide ($C_9H_6C_{16}O_3S$) is a broad spectrum organochlorine insecticide and acaricide. Technical endosulfan contains a mixture of alfa (α) and beta (β) isomers in the approximate ratio 70:30. In soil and plant surfaces, endosulfan sulfate is the primary degradation product of endosulfan. It is a highly toxic pesticide and suspected endocrine disrupter[15-17]. This insecticide is very effective for the control of a large variety of insects and mites of brinjal and other crops. The objective of this study is to investigate the persistence, dissipation behaviour of endosulfan and to assess the effect of some processes like washing, boiling and washing plus boiling on reduction of its residues on brinjal fruits.

EXPERIMENTAL

Materials and methods

Marketable size of brinjal was procured from vegetable market, out of which 0.5 kg was kept as control. Brinjal fruits were sprayed in the laboratory with aqueous solution of commercial formulation (Thiodan 35% EC). Aqueous solution of endosulfan was uniformly sprayed to drenching level on 5 kg brinjal for each treatment to get the intended deposits of 4.5 mg kg^{-1} (Single Dose, T_1) and 9.0 mg kg^{-1} (Double Dose, $T_{2)}$. Samples were left at room temperature for 2 h for attachment and penetration of the pesticide in the brinjal fruits. The treated samples were divided in to two lots. Lot one was kept at room temperature (41-42.5°C) and the second lot was kept in refrigerator (5°C). Samples from treated lots were processed in triplicates on 0 (2 h after spray) 3, 5 and 7days after treatment for knowing persistence of endosulfan and the effects of washing, boiling/cooking and washing plus boiling on reduction of residues. During processing, washing was done for 30 sec under running tap water and for boiling; water was added to brinjal fruits and boiled till softness. After processing, extraction and clean-up was performed in a similar way as unprocessed samples.

Extraction

All reagents and solvents were of analytical grade and distilled before use. Test samples (200 g) from each treatment in triplicate were macerated in mixer grinder from which a representative sample (25 g) was extracted with hexane: acetone (4 : 1 v/v) by shaking on mechanical shaker for one hour. The extract was filtered through 2-3 cm bed of anhydrous sodium sulphate and rinsed twice with 10 mL hexane: acetone (4 : 1 v/v). The extract was concentrated to about 10 mL and then subjected to cleanup.

Clean- up

Concentrated extract was cleaned as per method of Kathpal and Dewan[18]. To the extract, 3 mg activated charcoal was added, shaken for one minute and allowed the contents to settle down. Supernatant was filtered through Whatman filter paper No.1. with a layer of anhydrous sodium sulphate and rinsed thrice with 15 mL mixture of hexane: acetone (4 : 1 v/v). Cleaned extract was concentrated just to dryness using rotary evaporator and coloured complex was prepared as per method of Maitlen et al.[19].

Estimation

Residues were estimated by measuring the absorbance of coloured complex at 520 nm wavelength. Results of both treatments and conditions in triplicate were subjected to statistical analysis using two factorial ANOVA.

RESULTS AND DISCUSSION

Prior to taking up analysis of brinjal for quantification of their residue contents, recovery tests were performed for validation of analytical methodology to be adopted. Average recoveries of endosulfan for three replicates spiked at the levels of 0.25 and 0.50 mg kg^{-1} were in the range of 88.77- 91.66 per cent. The experimental data shown in Table 1 indicate that the application of endosulfan (Thiodan 35EC) at single dose (4.5 mg kg^{-1}) and double dose (9.0 mg kg^{-1}) up to drenching level on brinjal fruits in the laboratory resulted in an initial deposits on brinjal fruits to the extent of 3.95 and 8.25 mg kg^{-1}at single and double dose, respectively. The insecticide dissipated very fast just after its application in both the dose and fell abruptly to the level of 1.55 and 3.00 mg kg^{-1} on 3rd day. From 3rd day onwards, there was a gradual degradation of endosulfan residues till 7th day. It is evident from the table that on initial day, the dissipation rate was slightly higher in case of double dose as compared to the single dose. Further, it was observed that in single dose residues reached below maximum residue limit (MRL) of 2 mg kg^{-1} on 3rd day whereas in double dose, residues reached below MRL valve on 7th day under room temperature.

Under refrigerated conditions (Table 1), trend of dissipation was similar as of room temperature except the extent of per cent dissipation. In this case, after 3 days, per cent dissipation was 56.96 and 61.81 percent at single and double dose, respectively whereas the per cent dissipation on corresponding days and doses at room temperature were 60.7 and 63.6 percent which clearly indicate slightly slow degradation under refrigerated conditions. The half -life periods were 3.30 and 3.41 days at single and double dose, respectively under room temperature. The present findings are almost similar to earlier results[20] who reported half –life period of 2.5 and 2.92 days at doses of 0.05 and 0.10 % under field conditions. Singh and Kavadia[21] have reported half-life period of 5 days in brinjal. Under refrigerated conditions, the half -life periods were 3.81 and 3.72 days at single and double dose, respectively. Slow degradation of insecticides under freezing conditions has also been reported by earlier researchers[22,23]. Statistically analyzed data for samples stored at room temperature showed that the interaction between days and treatment was found to be significant and critical difference ($p = 0.05$) for treatments was found to be 0.0466 while for days, it was 0.0659. At refrigerated conditions also, interaction between days and treatment was found to be significant and critical difference ($p = 0.05$) for treatments was found to be 0.0497 while for days, it was 0.0703. During present investigations, the loss of endosulfan at all the corresponding time intervals was slightly more at room temperature as compared to the refrigerated conditions.

Table 1: Persistence of endosulfan residues in brinjal at room temperature

Days after treatment	Residue (mg Kg^{-1})* at room temperature			
	Single dose (4.5 mg Kg^{-1})		Double dose (9.0 mg Kg^{-1})	
	Initial deposit ± SD	% Dissipation	Initial deposit ± SD	% Dissipation
0	3.95 ± 0.002	-	8.25 ± 0.014	-
3	1.55 ± 0.006	60.7	3.00 ± 0.011	63.6
5	1.19 ± 0.010	69.8	2.35 ± 0.005	71.5
7	0.90 ± 0.003	77.2	1.92 ± 0.002	76.7
	Correlation coefficient r = -0.9746 **Regression equation y = 3.5453-0.09099x** **$t_{1/2}$ = 3.30 d**		**Correlation coefficient r = -0.9511** **Regression equation y = 3.8465-0.08820x** **$t_{1/2}$ = 3.41 d**	

Cont…

Residue (mg Kg^{-1})* at refrigerated conditions				
0	3.95 ± 0.002	-	8.25 ± 0.014	-
3	1.70 ± 0.007	56.9	3.15 ± 0.007	61.8
5	1.35 ± 0.011	65.8	2.47 ± 0.005	70.0
7	1.10 ± 0.008	72.1	2.27 ± 0.012	72.4
	Correlation coefficient r = -0.9672		**Correlation coefficient r = -0.9372**	
	Regression equation y = 3.5457-0.07898x		**Regression equation y = 3.8439-0.0808x**	
	$t_{1/2}$ = 3.81d		**$t_{1/2}$ = 3.72d**	

Average of three replicates

CD (p = 0.05) for treatments = 0.0466; for Days = 0.0659; for Days × Treatment = Significant (at Room Temperature)

CD (p = 0.05) for treatments = 0.0497; for Days = 0.0703; for Days × Treatment = Significant (at Refrigerated Conditions)

Effect of washing

Data presented in Table 2 reveal that on washed brinjal fruits the initial average residues on 0 day were 2.13 mg kg^{-1} indicating 46.1 per cent loss of endosulfan in single dose. The residues after three day of treatment declined to the level of 0.86 mg kg^{-1} resulting in 44.5 per cent reduction. After 5, and 7days of treatment, endosulfan reduced to the level of 0.68 and 0.55 mg kg^{-1} resulting in 42.8 and 38.9 per cent loss, respectively due to washing. In case of double dose, dissipation due to washing varied from 46.9 to 40.1 per cent between 3 to 7 days after treatment. It was observed that due to washing, the residues reached well below MRL value of 2 mg kg^{-1} on 3rd day in both the doses. Under refrigerated conditions, residues reached to the level of 1.02 mg kg^{-1} showing 40.00 percent reduction on 3rd day. After 5, and 7 days of treatment, endosulfan reduced to the level of 0.85 and 0.79 mg kg^{-1} resulting in 37.0 and 28.2 per cent loss, on respective days due to washing in single dose. In double dose, residues reached to the levels of 1.90, 1.56 and 1.50 mg kg^{-1} on 3rd, 5th and 7th day after treatment with per cent reduction of 39.7, 36.8 and 33.9 on respective days. It was observed that due to washing under this condition also, the residues reached well below MRL value of 2 mg kg^{-1} on 3rd day in both the doses. As per earlier reports[24], although by washing, residues were reduced to some extent but not completely as pesticide residues after spraying rapidly spread in to wax and cuticulas. Thus, washing the vegetable would be insufficient in removing the pesticides. This hypothesis was confirmed in literature and in the present study[24-26]. Effects of the washing steps were not associated with water solubility of this pesticide because water solubility of endosulfan at 20^{o}C is 0.32 g /L[17].

Effect of boiling/cooking

Initial deposit of 3.95 mg kg^{-1} at single dose reduced to 1.50 mg kg^{-1} on 0 day due to boiling/cooking of brinjal fruits resulting in 62.0 per cent reduction. Further, the insecticide after boiling/cooking remained to the level of 0.65, 0.55 and 0.44 mg kg^{-1} indicating 58.0, 53.8 and 51.1 per cent reduction after 3, 5 and 7 days after treatment, respectively. In case of double dose, the residue reached to the levels of 1.42, 1.10 and 0.95 mg kg^{-1} after 3, 5 and 7 days after treatment and obtained 56.6, 53.2 and 50.5 per cent loss of endosulfan at corresponding time intervals. It is evident from the data that the insecticide in boiled/cooked brinjal fruits was below MRL on 0 day in single dose and on 3rd day in double dose. In 3 day samples under refrigerated conditions, the residues reduced to a level of 0.85 mg kg^{-1} showing 55.9 per cent reduction in single dose. Residues further dissipated to the levels of 0.63 and 0.57 mg kg^{-1} after 5th and 7th day of treatment showing thereby reduction to the tune of 53.3 and 48.2 per cent on the respective days. In case of double dose, the residues recorded on 3rd, 5th and 7th days were 1.40, 1.17 and 1.15 mg kg^{-1} showing 55.5, 52.6 and 49.3 per cent reduction on corresponding days, respectively. It is revealed from the data that the residues in boiled/cooked brinjal fruits were below MRL of 2 ppm on 0 day in single dose and on 3rd day in double dose under refrigerated conditions.

Table 2: Effect of processing on reduction of residues

Days after treatment	At room temperature							
	Single dose	% Reduction			Double dose	% Reduction		
	Initial* deposit (mg Kg^{-1})	W	B	W+B	Initial deposit (mg Kg^{-1})	W	B	W+B
0	3.95	2.13	1.50	1.10	8.25	4.38	3.20	2.20
		(46.1)	(62.0)	(72.1)		(46.9)	(61.2)	(73.3)
		0.53	**0.37**	**0.27**		**0.53**	**0.38**	**0.26**
3	1.55	0.86	0.65	0.50	3.00	1.68	1.42	0.97
		(44.5)	(58.0)	(67.7)		(44.0)	(56.6)	(67.6)
		0.55	**0.42**	**0.32**		**0.56**	**0.47**	**0.32**
5	1.19	0.68	0.55	0.45	2.35	1.34	1.10	0.89
		(42.8)	(53.8)	(62.2)		(42.9)	(53.2)	(62.1)
		0.57	**0.46**	**0.37**		**0.57**	**0.46**	**0.37**
7	0.90	0.55	0.44	0.37	1.92	1.15	0.95	0.80
		(38.9)	(51.1)	(58.9)		(40.1)	(50.5)	(58.3)
		0.61	**0.48**	**0.41**		**0.59**	**0.49**	**0.041**

Days after treatment	Under refrigerated conditions							
	Single dose	% Reduction			Double dose	% Reduction		
	Initial deposit (mg Kg^{-1})	W	B	W+B	Initial deposit (mg Kg^{-1})	W	B	W+B
0	3.95	2.13	1.50	1.10	8.25	4.38	3.20	2.20
		(46.1)	(62.0)	(72.1)		(46.9)	(61.2)	(73.3)
		0.53	**0.37**	**0.27**		**0.53**	**0.38**	**0.26**
3	1.70	1.02	0.85	0.60	3.15	1.90	1.40	1.18
		(40.0)	(55.9)	(64.7)		(39.7)	(55.5)	(62.5)
		0.60	**0.50**	**0.35**		**0.60**	**0.44**	**0.37**
5	1.35	0.85	0.63	0.55	2.47	1.56	1.17	1.03
		(37.0)	(53.3)	(59.2)		(36.8)	(52.6)	(58.3)
		0.62	**0.46**	**0.40**		**0.63**	**0.47**	**0.41**
7	1.10	0.79	0.57	0.49	2.27	1.50	1.15	1.00
		(28.2)	(48.2)	(55.4)		(33.9)	(49.3)	(55.9)
		0.71	**0.51**	**0.44**		**0.66**	**0.50**	**0.44**

Average of three replicates; W: Washing; B: boiling; W+B: Washing followed by Boiling Figures in parenthesis is % reduction of residues

Bold and underlined figures represents processing factor

Effect of Washing + boiling/cooking

It has been observed from data (Table 2) that washing followed by boiling was found to be more effective in reducing the residues in both doses and conditions. In this process, residues reduced by 72.10

per cent in single dose and 73.30 per cent in double dose on 0 day at room temperature. Thereafter, reduction of residues was 67.70, 62.20 and 58.90 percent on 3rd, 5th and 7th days after application in single dose whereas at double dose percent reduction on respective days were 67.60, 62.10 and 58.30. At refrigerated conditions trend of reduction was almost similar to room temperature. Here, per cent reduction of residues in single dose was recorded from 72.10 to 55.40 and from 73.30 to 55.90 per cent in double dose from 0 to 7 days studies. Raha et al.[27] reported 51.00 per cent reduction of endosulfan residues in brinjal by washing which are in agreement with present results. Randhawa et al.[28] observed 15-30 per cent reduction of endosulfan residues on brinjal by washing which is in full conformity with the present findings. Present results are in agreement with earlier results of Raha et al.[27] who reported 74 per cent reduction of endosulfan from brinjal fruits on 0 day on which endosulfan was applied @ 0.05 %. Singh[23] applied endosulfan @ 0.05, 0.10 and 0.20% on cauliflower and observed the reduction from 51.24 to 97.90 per cent on 0 day. Washing removed 30.62 per cent residues of endosulfan from tomato by washing by Pala and Bilisli[29]. Thus present findings are in confirmation with the earlier reports.

Food processing studies are designed to measure changes in residue levels when raw commodity is converted to processed commodity. Changes are simply expressed as processing factors, which is formulated as residue level in processed commodity over residue level in raw commodity[30]. In the present study, processing factors (Residues in processed fruit/ Residues in unprocessed fruits e.g. 0.86/1.55 = 0.55) for endosulfan were also calculated (Table 2). These could be used to model exposure to these pesticide found in diets.

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