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Persistence and decontamination of insecticide residues in polyhouse grown capsicum (*Capsicum annuum*)

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ABSTRACT

The persistence and dietary intake of deltamethrin, endosulfan and malathion residues from coloured capsicum (*Capsicum annuum* L. var. Orobelle) grown under open ventilated polyhouse, was assessed after following common culinary processes. Each insecticide was applied at two concentrations, i.e. the recommended (single dose) and double the recommended doses. Insecticide residues were extracted using acetone and cleaned up over charcoal and Florisil columns. The determination and quantification of insecticide residues were done by using gas chromatograph equipped with ECD. The application of single dose, i.e. deltamethrin (0.0028%), endosulfan (0.05%) and malathion (0.05%) resulted in initial deposits of 0.667, 3.900 and 2.026 mg/kg; whereas, 0.937, 5.694 and 4.078 mg/kg at double dose, respectively, on capsicum fruits. The RL_{50} values were worked out as 1.90-2.41 days for deltamethrin, 1.75-1.88 days for endosulfan and 1.83 days for both the doses of malathion. Fruits washing under tap water for 1 minute provided relief of 30.05-38.81, 23.58-37.52 and 25.0-27.64% from deltamethrin, endosulfan and malathion residues, respectively, upto 3rd day, irrespective of doses. However, steaming after washing of the fruits provided maximum decontamination from insecticide residues. The endosulfan treated capsicum fruit consumption as salad revealed an appreciable risk to the consumers till 3rd day, whereas, washing+steaming reduced the dietary risk to the day of insecticide application.

Key words: Bell pepper, Culinary processes, Deltamethrin, Endosulfan, Malathion, Persistence, Risk assessment

Capsicum (*Capsicum annuum* L) grown in India is of recent interest due to its high demand for variety of fast food preparations and an important component of diet as salad. The bright coloured vegetables due to naturally occurring compounds act as antioxidants in the human body. The presence of polyphenols, carotenoids and flavonoids carry strong capacity to scavenge free radicals in coloured capsicum (Nadeem *et al.* 2011, Blanco-Ríos *et al.* 2013). To capture international market, polyhouse farming, an alternative new technique for growing capsicum in agriculture has been gaining foothold in rural areas of India. The polyhouse farming in the Himalayan mountainous area has enabled cultivation of regular off and main season crops with high productivity and enhanced shelf life (Rai *et al.* 2004), thus making year round availability of coloured capsicum to rest of country. However, the media attention related to fresh, particularly polyhouse produced vegetables has heightened consumer awareness of produce free from the presence of insecticide residues. A higher presence of pesticides has been recorded in urine of children consuming foods of conventional origin (Curl *et al.* 2003). A number of pests attacking capsicum under polyhouse conditions have been reported in India (Singh *et al.* 2004).To sustain production under polyhouse conditions, pesticides act as an important input for crop health, hygiene. Only *ad hoc* recommendations of deltamethrin, endosulfan and malathion insecticides have been advocated so far for the management of insect-pests on capsicum under protected conditions (Kohli *et al.* 2007). However, these recommendations lack pesticides residue data to ensure the consumers safety. Hence, a systematic study was conducted to investigate the persistence of deltamethrin, endosulfan and malathion on coloured capsicum grown under polyhouse conditions and to assess the consumer health risk of residues following common household practices of treated fruits.

MATERIALS AND METHODS

The Capsicum (*Capsicum annuum* L. var. Orobelle) F_1 hybrid crop was raised in a Quonset type polyhouse with 25% sun light reduction capacity. The seedlings raised in mist chamber were transplanted in 2.5 × 1 m plot size with spacing of 60 × 45 cm in the polyhouse. Insecticides were

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applied thrice at two concentrations (recommended and double the recommended doses). Endosulfan (Thiodan 35 EC) and malathion (Cythion 50 EC) were applied at 0.05 (single dose) and 0.1 (double dose) per cent concentrations, whereas, deltamethrin (Decis 2.8 EC) was applied at 0.0028 (single dose) and 0.0056 (Double dose) per cent concentrations. First spray was given 40 days after transplanting and thereafter, consequent two sprays were given at three weeks interval. The experiment was laid in a randomized block design (RBD), with each treatment replicated three times. The control plots were treated with water only.

After last spray, 1 kg fruit samples were collected randomly from each replication at periodic intervals of 0, 1, 3, 5, 7 and 10 days. The sample was chopped into small pieces and homogenized in a domestic mixer to get fine homogenate. Sample extraction and cleanup was carried out as per method of Sharma (2007). A sub homogenized sample of 100 g was extracted with 200 ml acetone in a 500 ml conical flask. The extract was filtered through Buchner funnel by fitting a Whatman No. 1 filter paper overlaid with Celite 545 and retained cake was washed with 50 ml acetone to remove traces of insecticide residues. An aliquot of 60 ml (30 g equivalent to sample) was partitioned with 200 ml mixture of hexane and dichloromethane (1:1, v/v). The lower aqueous phase was transferred to another 1 litre seperatory flask and remaining organic phase was retained in the same separatory flask. To the aqueous phase, 10 ml saturated sodium chloride solution was added and again partitioned twice with 100 ml dichloromethane. Pooled organic phase was passed through anhydrous sodium sulphate and evaporated to dryness at 45°C by using vacuum rotary evaporator. Residues were taken up in 3 ml acetone for cleanup.

Cleanup for deltamethrin and endosulfan was done by diluting 3 ml extract with 10 ml of acetone: hexane (1:9) mixture, loaded on 4 g Florisil column and eluted with 50 ml solvent mixture (50 % dichloromethane: 48.5 % hexane: 1.5 % acetonitrile). The eluant was evaporated to dryness and residues were taken up in 3 ml toluene. Finally, 1µl of sample was injected into gas chromatograph for estimation of insecticide residues. For cleanup of malathion, 3 ml sample extract was loaded on to the column and eluted with 200 ml of 2:1 acetone: dichloromethane mixture. The eluant was evaporated to dryness in rotary vacuum evaporator at 45°C and residues were dissolved in 3 ml toluene for estimation by gas chromatography.

Samples collected at 0, 3 and 7 days after last insecticidal application were processed with common household practices like washing, steaming and washing followed by steaming, and then analysed for insecticide residues as per method described above. In the washing treatment, capsicum fruits were washed under a stream of running tap water by hand rubbing for 1 min, dried on a filter paper and then analysed. Steam heating was done in 3 l capacity pressure cooker, steamed till first whistle and then analysed. Washing+steam heating treatment involved first washing under tap water followed by steam heating in pressure cooker.

Insecticide residues were analysed and quantified by using Agilent 6890N gas chromatograph equipped with ultra performance, DB-5, cross linked methyl silicon capillary column (30m × 0.25 mm i.d. with 0.25 µm film thickness) and electron capture detector. Injector and detector temperature was kept at 250°C and 300°C, respectively. Oven temperature programme was ramped as 100°C for 1 minute, 30°C/minute up to 150°C for 2 minutes, 3°C/min up to 205°C and finally it was increased at the rate of 10°C/ minute up to 260°C and kept for 10 minutes. Under these conditions, retention time of deltamethrin, α-endosulfan, β-endosulfan, endosulfan sulfate and malathion was 15.859, 18.951, 22.262, 23.358 and 34.351 minutes, respectively.

Dietary intake of different insecticides through raw consumption of treated coloured capsicum and % ADI was calculated by using the following method (Gupta 1999).

$DI = (FF \times R \times TD)/BW$

$\%\ ADI = (DI \times 100)/ADI$

where, DI - Dietary intake, TD – Total diet (2 kg), FF– Food factor (contaminated capsicum, 250g/TD), R– Residues of respective insecticides, BW– Average body weight (60 kg), ADI- Acceptable daily intake (deltamethrin- 0.01, endosulfan- 0.006 and malathion- 0.03 mg/kg/day).

The insecticides persistence data were analyzed for correlation coefficient, regression equation and residue half life (RL_{50}) as per the method of Hoskins (1961).

RESULTS AND DISCUSSION

The analytical method was validated by fortification at different concentrations following recovery studies. Capsicum samples were fortified with each deltamethrin and malathion at 0.05, 0.75, 1.00 and 2.0 mg/kg; and at 0.01, 0.05, 0.75, 1.00 and 2.0 mg/kg with each α-endosulfan, β-endosulfan and endosulfan sulphate with triplicate determinations. Based on these recovery studies, the limit of determination (LOD) was 0.05 mg/kg for deltamethrin and malathion; and 0.01 mg/kg for α-endosulfan, β-endosulfan and endosulfan sulfate. The recoveries obtained were 86.60-92.31% from deltamethrin, 88.80-91.39% from malathion, 85.00-89.56% from α-endosulfan, 87.00-91.70% from β-endosulfan and 86.00-91.33% from endosulfan sulfate. These recoveries were considered satisfactory, thus supported the method, when they were between 70 and

Table 1 Recovery of deltamethrin and malathion from fortified capsicum fruits

Amount of insecticide added (mg/kg)	Deltamethrin		Malathion	
	Amount recovered (mg/kg)	Recovery (%)	Amount recovered (mg/kg)	Recovery (%)
0.05	0.0433	86.60	0.0444	88.80
0.75	0.6726	89.69	0.6759	90.12
1.00	0.9168	91.68	0.9086	90.86
2.00	1.8462	92.31	1.8278	91.39

Table 2 Recovery of endosulfan from fortified capsicum fruits

Amount of insecticide added (mg/kg)	α-endosulfan		β-endosulfan		Endosulfan sulfate	
	Amount recovered (mg/kg)	Recovery (%)	Amount recovered (mg/kg)	Recovery (%)	Amount recovered (mg/kg)	Recovery (%)
0.01	0.0085	85.00	0.0087	87.00	0.0086	86.00
0.05	0.0432	86.40	0.0443	88.60	0.0439	87.80
0.75	0.6590	87.86	0.6718	89.58	0.6630	88.40
1.00	0.8863	88.63	0.9091	90.91	0.9074	90.74
2.00	1.7912	89.56	1.8341	91.70	1.8266	91.33

120% (Trevisan *et al.* 2005) (Table 1 and 2).

The endosulfan residues were expressed in the text as total of α-endosulfan, β-endosulfan and endosulfan sulfate.

Persistence studies

The data on the persistence presented in Table 3 revealed that initial deposits of deltamethrin on capsicum fruits were 0.667 and 0.937 mg/kg at single and double doses, which reduced to half in 2.41 and 1.90 days, respectively. Half life values of endosulfan initial deposits on capsicum fruits were 1.75 and 1.88 days at single and double doses, respectively. Initial deposits of malathion on capsicum fruits at single and double doses were 2.026 and 4.078 mg/kg, respectively, which degraded to its half in 1.83 days at both the applied doses.

The residues of deltamethrin and malathion persisted for 7 days and endosulfan for 10 days on capsicum fruits.

Effect of decontamination processes

The household processes, such as washing, peeling, and cooking often lead to significant decline in the amount of pesticide residues (Schattenberg *et al.* 1996). The deltamethrin sprayed capsicum fruits washed with water revealed 30.05-36.84% residue relief on 0 day, while it was found to increase up to 38.81% on 3rd day. Washing of 0 and 3 day old endosulfan treated capsicum fruits resulted in reduction of 23.58-33.60% residues. In present investigation, 25.00-27.64% relief of malathion residues was obtained after washing of fruits with running tap water.

Steaming of capsicum fruits was observed to provide a relief of 55.12-76.49% from deltamethrin, 43.13-63.90% from endosulfan and 53.03-100% from malathion residues at different day intervals.

Tap water washing of capsicum followed by steaming enhanced relief up to 89.44, 70.02 and 85.86% from deltamethrin, endosulfan and malathion residues, on 0 day, respectively. The relief on 3rd day was 100, 80.19 and 92.97% for the respective insecticides, whereas on 7th day it was increased up to 95.60-100% even in endosulfan and malathion treated fruits.

Dietary risk assessment

The residue risk was worked out by taking into account the ADI of the insecticide, as there were no MRLs fixed for deltamethrin, endosulfan and malathion by Codex and FSSAI on coloured capsicum. The dietary intake of deltamethrin and malathion through washed capsicum was observed in the range of 0.0005- 0.0025 and 0.0022-0.0126 mg/kg body weight/day, respectively, till 3rd day of insecticides treatment. This intake was less than that of 0.01 and 0.03 mg/kg body weight/day, the ADIs established for the respective insecticides. However, water washed capsicum fruits taken as salad from endosulfan treatment on 0 and 3rd day exhibited a health risk to the consumers. The daily intake of endosulfan

Table 3 Dissipation of deltamethrin, endosulfan and malathion residues on capsicum fruits

Interval (Days)	Residue± SD (mg/kg)					
	Deltamethrin		Endosulfan		Malathion	
	0.0028%	0.0056%	0.05%	0.1%	0.05%	0.1%
0	0.667 ± 0.275	0.937 ± 0.109	3.900 ± 0.485	5.694 ± 0.664	2.026 ± 0.524	4.078 ± 0.443
1	0.358 ± 0.065	0.679 ± 0.043	2.687 ± 0.209	3.933 ± 0.163	1.351 ± 0.134	2.258 ± 0.148
3	0.186 ± 0.022	0.353 ± 0.067	1.181 ± 0.125	2.332 ± 0.091	0.703 ± 0.032	1.100 ± 0.045
5	0.097 ± 0.030	0.189 ± 0.062	0.653 ± 0.042	0.998 ± 0.011	0.553 ± 0.090	0.939 ± 0.054
7	BDL	0.068 ± 0.033	0.279 ± 0.029	0.771 ± 0.011	0.107 ± 0.012	0.205 ± 0.017
10	BDL	BDL	0.069 ± 0.041	0.117 ± 0.006	BDL	BDL
Correlation coefficient (r)	- 0.989	- 0.994	- 0.998	- 0.983	- 0.958	- 0.965
Regression equation (Y)	0.666-0.125 X	0.002-0.158 X	0.61-0.172 X	0.809-0.160X	0.334-0.164 X	0.585-0.165 X
RL_{50}	2.41 days	1.9 days	1.75 days	1.88 days	1.83 days	1.83 days

BDL- Below determination limit ; RL_{50}- Residues half life.

Table 4 Effect of capsicum fruit processing on deltamethrin, endosulfan and malathion residues

Treatment	Interval (Days)	Mean residues ± SD (mg/kg)					
		Deltamethrin		Endosulfan		Malathion	
		0.0028%	0.0056%	0.05%	0.1%	0.05%	0.1%
Unprocessed	0	0.667 ± 0.245	0.937 ± 0.109	3.900 ± 0.458	5.964 ± 0.664	2.026 ± 0.524	4.078 ± 0.443
	3	0.186 ± 0.022	0.353 ± 0.067	1.146 ± 0.613	2.332 ± 0.091	0.703 ± 0.032	1.100 ± 0.045
	7	BDL	0.068 ± 0.033	0.295 ± 0.011	0.771 ± 0.011	0.107 ± 0.012	0.205 ± 0.017
Washing	0	0.473 ± 0.192	0.592 ± 0.065	2.816 ± 0.429	4.558 ± 0.547	1.519 ± 0.392	3.014 ± 0.357
	3	0.130 ± 0.015	0.216 ± 0.034	0.761 ± 0.085	1.731 ± 0.071	0.527 ± 0.024	0.825 ± 0.033
	7	BDL	BDL	0.217 ± 0.010	0.505 ± 0.016	0.078 ± 0.011	0.151 ± 0.008
Steaming	0	0.304 ± 0.124	0.420 ± 0.048	2.021 ± 0.516	3.392 ± 0.372	1.033 ± 0.268	1.916 ± 0.076
	3	0.088 ± 0.006	0.083 ± 0.008	0.541 ± 0.063	1.253 ± 0.055	0.258 ± 0.016	0.477 ± 0.071
	7	BDL	BDL	0.134 ± 0.008	0.278 ± 0.123	BDL	0.087 ± 0.006
Washing+	0	0.082 ± 0.018	0.099 ± 0.009	1.203 ± 0.18	1.788 1 ± 0.200	0.506 ± 0.131	0.577 ± 0.047
Steaming	3	BDL	BDL	0.227 ± 0.032	0.860 ± 0.136	0.140 ± 0.00	0.077 ± 0.016
	7	BDL	BDL	0.013 ± 0.008	0.151 ± 0.077	6BDL	BDL

BDL- Below determination limit.

residues on 0 day was calculated to be 0.0117 and 0.0190 mg/kg body weight/day at single and double doses in water washed capsicum fruits, indicated 195.00 and 316.66% higher intake of residues as compared to ADI (0.006 mg/kg body weight/day), respectively. Dietary intake of endosulfan was also above ADI in 3rd day washed fruits, which receded to safer level after 7th day of endosulfan treatment.

The study concluded that the residues of endosulfan persisted for longer period (10 days) compared to deltamethrin and malathion (7 days) on capsicum fruits. The household processes like washing, steaming and washing+ steaming have been found as effective practices in removing the residues of these pesticides, thus making consumption of coloured capsicum as salad or as a component of fast food recipes safe. The steaming of the fruits after washing further enhanced degradation of endosulfan resulting in significant reduction of residues, thereby providing no health risk to the consumers from 3rd day onwards of insecticide application.

The analytical method validation was confirmed with fortification and recovery studies for different insecticides. The recovery determinations were considered satisfactory, when they were between 70 and 120% thus supported the analytical method employed in the study (Trevisan *et al.* 2005). Interpretation of residue results of three pesticides, viz. deltamethrin, endosulfan and malathion exhibited that their rate of decrease follows a first-order kinetics reaction. The residues decreased with time and within every fixed time interval, the decrease was a constant ratio from the amount already present at the beginning of the interval, i.e. the rate of decrease in residues at any time is directly proportional to the amount of the residues at that time, which is the sign of first-order kinetics.

The residual half-life values reflected the slower rate of

Table 5 Dietary exposure of insecticides through consumption of washed and washed+ steamed coloured capsicum

	Conc. (%)	Intake	Residues intake mg/kg/day (days after treatment)					
			Washing			Washing +Steaming		
			0	3	7	0	3	7
Deltamethrin	0.0028	DI	0.0020	0.0005	0.00	0.0003	0.00	0.00
		% ADI	20.00	5.00	0.00	3.00	0.00	0.00
	0.0056	DI	0.0025	0.0009	0.00	0.0004	0.00	0.00
		% ADI	25.00	9.00	0.00	4.00	0.00	0.00
Endosulfan	0.05	DI	0.0117	0.0032	0.0009	0.0050	0.0010	0.0001
		% ADI	195.00	53.33	15.00	83.33	16.67	1.67
	0.1	DI	0.0190	0.0072	0.0021	0.0076	0.0036	0.0006
		% ADI	316.66	120.00	35.00	126.67	60.00	10.00
Malathion	0.05	DI	0.0063	0.0022	0.0003	0.0021	0.0006	0.00
		% ADI	21.00	7.33	1.00	7.00	2.00	0.00
	0.1	DI	0.0126	0.0034	0.0006	0.0024	0.0003	0.00
		% ADI	42.00	11.33	2.00	8.00	1.00	0.00

DI-Daily intake of insecticide; ADI-Acceptable daily intake CADI of deltamethrin-0.01, endosulfan-0.006 and malathion-0.03 mg 1 kg body weight/day

decomposition of deltamethrin deposits as compared to endosulfan and malathion. Pyrethroids being photo stable and strong lipophilic in character, dissipation of deltamethrin has been arrested by the waxy surface of capsicum fruits. Malathion was got moved easily into the inner tissues of capsicum fruits, resulting into enzymatic break down of its residues (Menzer and Dauterman 1970). Endosulfan also disappeared relatively faster from plant surfaces owing to its high vapour pressure (Maier-Bode 1968). Antonious *et al.* (1998) have also reported faster dissipation of endosulfan with its half life (RL_{50}) of 2 days on pepper leaves. Higher residues were reported in greenhouse-grown vegetables and fruits, when compared to the cultivation in open-field for endosulfan in tomatoes, peppers and cucumbers (Aguilera-del *et al.* 1997). However, higher residues of acephate were found in polyhouse grown tomato leaves in comparison to open field but observed same persistence period in both conditions (Trevisan *et al.* 2005).

The household processes, such as washing, peeling, and cooking often lead to significant decline in the amount of pesticide residues (Schattenberg *et al.* 1996). Tap water washing was found to dislodge 36.25 - 42.76% deltamethrin residues from contaminated okra fruits (Dikshit *et al.* 2001, Singh *et al.* 2004). Washing of 0 and 3 day old endosulfan treated capsicum fruits resulted in reduction of 23.58-33.60 per cent residues. These results are in agreement with those of Kumari (2008), who reported 27, 34 and 36 per cent reduction of endosulfan initial deposits on washing of brinjal, cauliflower and okra fruits, respectively. Patyal *et al.* (2004) reported a significant relief from endosulfan sprayed apple fruits washed with water. Among three pesticides studied, endosulfan and malathion were found to provide least relief compared to deltamethrin. The results indicated that water solubility of pesticides did not play a significant role in removing the insecticides residues as has also been observed for endosulfan, malathion and permethrin on different produce (Krol *et al.* 2000). Tap water washing of different vegetables has also been reported to dislodge the mancozeb residues up to 25.68% (Sharma *et al.*1994). Coloured capsicum is generally consumed as salad or partially heated for different fast food preparations. The heat involved in cooking procedure and the type of system may increase degradation and volatilization of pesticide residues. Our studies confirmed degradation of different insecticides residues belonging to synthetic pyrethroids, organo chlorines and organophosphorus groups of insecticides by steaming capsicum fruits. Our findings are in close agreement with other study, who reported that cooking of okra fruits provided 64.13-79.69% relief from endosulfan residues (Sharma *et al.*2003). Dikshit *et al*. (2001) reported 50.0-70.0 and 61.8% initial deposits reduction of different insecticides after washing followed by cooking of brinjal and sponge gourd fruits, respectively. Washing and steaming of chickpea grains completely removed the deltamethrin residues from an initial load of residues of 0.051 ppm (Lal and Dikshit 2001). Cooking followed by washing has also been reported an effective process in reducing mancozeb residues from cabbage, knol-khol, okra and brinjal fruits (Sharma *et al.*1994), whereas, preparation of jam after apple fruit cooking in pressure cooker resulted in significant reduction of endosulfan residues (Patyal *et al.*2004).

The residue risk was worked out on the basis of ADI of the deltamethrin, endosulfan and malathion, due to non fixation of MRLs for the respective, insecticides by the Codex and FSSAI on coloured capsicum. Coloured capsicum is an important component of most of the Indian fast food recipes, added mostly as steamed. The steaming of capsicum fruits after washing though was found to dislodge endosulfan residues, yet intake was more (126.67 %) than the ADI on the day of insecticide application. However, these residues receded below the ADI (0.0001-0.0036 mg/kg body weight/ day) on day 3 onwards. The said household processing was found very effective in lowering down the deltamethrin and malathion residues. The dietary intake of deltamethrin and malathion was 3-4 and 1-8% of their respective ADIs.

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