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Studies on the Fate of Ready-Mix Formulation of Endosulfan and Cypermethrin in Tea

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Abstract Ready-mix formulation consists of two insecticides—endosulfan (35 %) and cypermethrin (5 %). To understand the dissipation pattern of the insecticides on made tea from the north-east region of India, it was applied twice in 10 day interval @ 1,000 mL/ha (350 g a.i. endosulfan + 50 g a.i. cypermethrin) and 2,000 mL/ha (700 g a.i. endosulfan + 100 g a.i. cypermethrin) along with untreated control. The made tea samples were collected at 0, 1, 3 and 7 days interval after last application. The dissipation rate of endosulfan and cypermethrin followed first order kinetics. The calculated half-life values were found in the range between 1.7 to 2.1 days for endosulfan and 1.0 to 1.1 days for cypermethrin.

Keywords Endosulfan · Cypermethrin · Dissipation · Half-life · Residue

India is one of the largest producers and exporter of tea (*Camellia sinensis* L.), accounting for 27 % of the world output, followed by China (25 %), Sri Lanka (10 %) and Kenya (9 %). It is cultivated mainly in the north-eastern and southern part of the country, the former representing about 75 % of the total production. In the recent past, tea importing countries such as Germany and others are becoming more health-conscious and are gradually lowering their maximum residue limit (MRL) values in pesticide residues in tea (Bhattacharyaya et al. 1995). In the north-east region of India, tea crops are threatened by more than 100 pests of which the red spider mite is a major one (Bhattacharyaya et al. 1995; Chowdhury et al. 1995; Samanta et al. 1995; Somchoudhury et al. 1993). For control, many miticides are available on the market of which tetradifon was banned and use of ethion and dicofol was restricted due to residue problems in tea, especially for export, so there is a need in respect to appropriate acaricides. Recently a trend has been observed in India, i.e. the use of ready-mix formulations for controlling the various insects in tea gardens.

A ready-mix formulation of 35 % endosulfan [6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide; (Fig. 1)], an organochlorine insecticide, and 5 % cypermethrin [(RS)α-cyano-3-phenoxybenzyl (IRS, 3RS, IRS, 3SR)—3-(2,2 -dichlorovinyl)—2,2,dimethylcyclo- propanecarboxylate; (Fig. 2)], a synthetic pyrethroid, has been introduced in India, effective to control various pests of tea (Bhattacharyaya et al. 1995; Gajbhiye et al. 1989; Samanta et al. 1995). Based on the above information, a two-season (pre-monsoon 2002 and monsoon 2002) field study of this ready-mix formulation was conducted in north-east India for determination of the dissipation pattern as well as the residue level of endosulfan and cypermethrin in tea.

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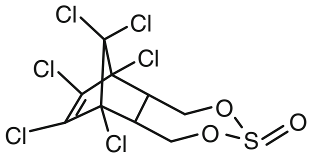

Fig. 1 Endosulfan

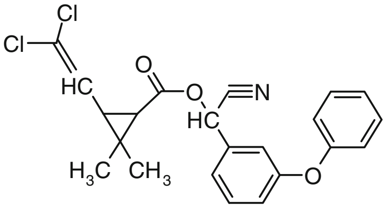

Fig. 2 Cypermethrin

Materials and Methods

A field trial was conducted at Kamalpur Tea Estate, Siliguri, Darjeeling, West Bengal, during 2002–2003. During the field trial, the maximum/minimum temperature for premonsoon and monsoon were 33.8°C/18.8°C and 33.6°C/23.0°C respectively and average relative humidity for premonsoon as 73 % & for monsoon 92 %.

The tea bushes (variety: mixed clones, e.g., TV-19, TV-23 etc.) were replicated thrice in a randomized block design (RBD) in plots with sizes of 100 m^2. Hundred bushes per treatment were taken and the planting of bushes was double hedge type.

The ready-mix formulation of endosulfan and cypermethrin as liquid formulation was commercially supplied (M/S Excel Crop Care Ltd., Mumbai, India). It was applied using a knapsack sprayer twice in 10 day intervals at 1,000 mL/ha (350 g a.i. endosulfan + 50 g a.i. cypermethrin) and 2,000 mL/ha (700 g a.i. endosulfan + 100 g a.i. cypermethrin) in 500 L/ha water separately in different plots of tea. Control plots were maintained simultaneously.

For sampling green tea leaves (two leaf and a bud, 1 kg) were plucked randomly from each treatment at 0 (2 h after spray), 1, 3 and 7 days after the last application. The green tea leaf samples were processed to tea (CTC, 100 g) at Kamalpur Tea Estate factory following standard manufacturing methods.

Analytical standards of α-endosulfan (99.5 %), β-endosulfan (99.5 %), endosulfan sulfate (99.5 %), cypermethrin (99.5 %) and the commercial grade ready-mix formulation were supplied by M/S Excel Crop Care Ltd., Mumbai, India. The percent purity of the insecticides was checked by GC-ECD. Anhydrous sodium sulfate was heated to 140°C overnight prior to use. All reagents were of analytical grade. All organic solvents used were distilled before use and water used was double glass distilled. The samples were extracted immediately after collection; if this was not possible, the samples were stored at −18°C.

The employed analytical methods for determination of endosulfan and cypermethrin were validated in this laboratory with some modifications in the clean up procedure (Bhattacharyaya et al. 1995; Gajbhiye et al. 1989; Samanta et al. 1995).

For extraction of endosulfan residue, 20 g made tea was taken for analysis from each treatment after quartering. The samples were blended in a Remi automix blender for 2 min with 100 mL of acetone and filtered through a Buchner funnel. The remaining portion in the blender was re-extracted twice with 50 mL acetone each and filtered.

The combined extract was concentrated to 50 mL in a rotary vacuum evaporator at 40°C under reduced pressure. The extracted materiel was transferred into a 500 mL separatory funnel with addition of 100 mL saturated sodium chloride solution. Then it was partitioned thrice with n-hexane (100 + 50 + 50 mL and the phases were allowed to separate. The organic layer was passed through anhydrous sodium sulphate (10 g) which was placed on a funnel (diameter 7.5 cm; height 7.5 cm) and the flow rate was 20 mL/min. The hexane fraction was concentrated in a rotary vacuum evaporator and the volume was made to about 100 mL in a conical flask. The extract was dried over anhydrous sodium sulphate and 2 g of activated charcoal was added to the concentrated hexane extract. The conical flask was shaken thoroughly for about 30 min and allowed to stand. Then the content was filtered through Whatman no. 42 filter paper and the filtrate was collected in a 250 mL conical flask. The charcoal present on the filter paper was washed carefully with a solvent mixture of n-hexane:acetone (9:1, v/v) to bring the total volume to 250 mL. The combined filtrate was concentrated in a rotary vacuum evaporator at 40°C and the volume was made up to 10 mL with distilled n-hexane for gas-chromatographic analysis.

For extraction of cypermethrin, 20 g made tea was blended in a blender (Remi Automix, India) for 2 min with 150 mL of a mixture of acetonitrile:water (6.5:3.5, v/v) and filtered through Buchner funnel under suction.

The filtrate was concentrated in a rotary vacuum evaporator at 40°C. The concentrated extract was transferred to a 1 L separatory funnel and diluted with 250 mL of 5 % sodium chloride solution, and extracted thrice (100 + 50 + 50) mL with a mixture of hexane:ether (8:2, v/v). The combined organic fractions were collected over 100 g anhydrous sodium sulphate and concentrated in a rotary vacuum evaporator at 40°C. The concentrated extract was reconstituted with 5 mL hexane and chromatographed over a column (column length: 25 cm with reservoir: 50 mL; internal diameter: 3 cm) packed with silica gel (10 g) and 2 g anhydrous sodium sulphate at the top as adsorbent. The column was first flushed with 50 mL hexane which was discarded. The further eluate obtained with

200 mL of a mixture of hexane:ethyl acetate (9.5:0.5, v/v) was concentrated and volume was made up to 10 mL with n-hexane for gas chromatographic analysis.

A gas chromatograph (Hewlett-Packard, Model 5890) equipped with ^{63}Ni electron capture detector was coupled to a data processor (Chemito 5000; supplied by Chemito Instruments, Nasik, Maharstra, India). The column used for analysis of endosulfan and cypermethrin was a DB-5 fused silica megabore (J&W, USA), 30 m × 0.53 mm i.d., 1.5 µm film thickness. The operating mode is split less.

The operating parameters of the gas chromatograph were as follows: oven temperature isothermal 200°C (for α and β-endosulfan, endosulfan sulphate) or 260°C (for cypermethrin); injector temperature 275°C; detector temperature 320°C; flow rate of carrier gas ((N_2) 14 mL/min; Make up gas flow 30 mL/min; Total gas flow 44 mL/min; limit of quantification (LOQ) 0.01 ppm (for α and β-endosulfan, endosulfan sulphate and cypermethrin); limit of detection (LOD) 0.005 ppm (for α and β-endosulfan, endosulfan sulphate and cypermethrin).

A stock solution of 1 ppm of each insecticide (analytical grade, 99.5 %; supplied by M/s Excel Industries Ltd., Mumbai, India) was prepared in acetone (AR grade) as an external standard. One microliter each of 1 ppm of analytical grade α and β-endosulfan, endosulfan sulphate and cypermethrin was injected into the gas chromotograph using the above mentioned parameters. The retention times of α-endosulfan, β-endosulfan, endosulfan sulfate and cypermethrin were 8.85, 12.73, 16.78 and 7.74 min, respectively. The retention times of each of the insecticides in made tea were compared with those of the external standards and the data were recorded.

The recovery experiment was carried out by fortifying made tea with 5, 2 and 1 ppm α-endosulfan and cypermethrin and 5, 1 and 0.5 ppm β-endosulfan and endosulfan sulphate. Average recoveries of α-endosulfan, β-endosulfan and endosulfan sulfate was found to be in the range of 85 %–91 % and in the case of cypermethrin, the range was 93 %–96 %. The dissipation data were subjected to regression analysis (Hoskins 1961) for computing residual half-lives (Gunther and Blinn 1955).

Results and Discussion

The residue data of endosulfan and cypermethrin in made tea at different day intervals were presented in the Tables 1, 2. The corresponding dissipation rates, regression equations and half-life values have been calculated on the basis of residue data. Interestingly, the results show that the residues of endosulfan and cypermethrin in made tea decline progressively with time irrespective of dose and season. It is evident from the Tables 1, 2 that the initial deposits of endosulfan 2 h after spraying were found in the range of 1.1–4.1 ppm irrespective of dose and season while in case of cypermethrin the corresponding values were 0.9–2.0 ppm. Afterwards, there is a sharp decline in endosulfan and cypermethrin for both doses. Seven days after application, more than 90 % of the endosulfan is dissipated, and cypermethrin is below detection limit, irrespective of dose and season. No residue was detected in untreated control samples throughout the study. The dissipation rate followed first order kinetics irrespective of dose and season. The calculated half-lives values ($T_{1/2}$) were found to be in the range of 1.7–2.1 days for endosulfan and 1.0–1.1 days for cypermethrin irrespective of dose and season.

The calculated half-lives of endosulfan and cypermethrin in made tea in both seasons were low compared to the half-lives of endosulfan and cypermethrin in other vegetables and crops. The lower values of the residues of both pesticides might be due to several factors viz., irradiation, temperature, pH, moisture (Agnihothrudu and Muraleedharan 1990; Chen et al. 1987; Crosby et al. 1972; Jaggi et al. 2001; Kumar et al. 2004; Miller and Donaldson 1994, Miller and Zepp 1983), growth dilution factor etc. (Bisen

Table 1 Persistence of Endosulfan in made tea for consecutive two seasons at recommended and double the recommended dose

Season	DAT	Recommended dose (350 g a.i. endosulfan + 50 g a.i. cypermethrin)			Double the recommended dose (700 g a.i. endosulfan + 100 g a.i. cypermethrin)		
		Residue in ppm (M ± SD)	Dissipation (%)	Regression equation [Half life (days)]	Residue in ppm (M ± SD)	Dissipation (%)	Regression equation [Half life (days)]
Pre-monsoon (2002)	0	2.2 ± 0.3	–	Y = 3.3–0.2X (2.0)	3.5 ± 0.3	–	Y = 3.5–0.1X (2.1)
	1	1.3 ± 0.2	42.00		2.3 ± 0.2	35.00	
	3	0.55 ± 0.1	75.11		0. 9 ± 0.2	74.00	
	7	0.2 ± 0.1	92.00		0.3 ± 0.1	90.00	
Monsoon (2002)	0	1.1 ± 0.2	48.00	Y = 3.3–0.2X (1.7)	4.1 ± 0.2	–	Y = 3.5–0.2X (1.9)
	1	0.6 ± 0.1	75.00		2.3 ± 0.5	44.00	
	3	0.1 ± 0.02	87.0		0. 9 ± 0.2	78.00	
	7	0.1 ± 0.04	95.00		0.3 ± 0.1	92.00	

a.i. active ingradient, *DAT* day's after treatment, *M* mean of three replicates

Table 2 Persistence of cypermethrin in made tea for consecutive two seasons at recommended and double the recommended dose

Season	DAT	Recommended dose (350 g a.i. endosulfan + 50 g a.i. cypermethrin)			Double the recommended dose (700 g a.i. endosulfan + 100 g a.i. cypermethrin)		
		Residue in ppm (M ± SD)	Dissipation (%)	Regression equation [Half life (days)]	Residue in ppm (M ± SD)	Dissipation (%)	Regression equation [Half life (days)]
Pre-monsoon (2002)	0	1.0 ± 0.2	–	Y = 3.0–0.3X (1.0)	1.9 ± 0.2	–	Y = 3.3–0. 3X (1.1)
	1	0.6 ± 0.2	46		0.9 ± 0.3	52	
	3	0.1 ± 0.02	87		0.3 ± 0.03	85	
	7	BDL	–		BDL	–	
Monsoon (2002)	0	0.9 ± 0.2	–	Y = 3.0–0.3X (1.0)	2.0 ± 0.3	–	Y = 3.3–0.3X (1.0)
	1	0.5 ± 0.1	43		1.0 ± 0.1	50	
	3	0.1 ± 0.02	87		0.3 ± 0.02	87	
	7	BDL	–		BDL	–	

a.i. active ingradient, *DAT* day's after treatment, *M* mean of three replicates, *BDL* below detectable level

and Ghosh Hajara 2000, Chen and Wan 1988) which causes rapid degradation and/or dissipation of endosulfan and cypermethrin. The lower values in made tea may be attributed to three factors i.e., evaporation, degradation and codistillation during processing (Cabras et al. 1998).

In the present investigation it was observed that the doses or seasons did not show any effect on the half-lives of endosulfan and cypermethrin. The half-lives of endosulfan were about 2 day in pre monsoon and 1.7–1.9 days in monsoon while the corresponding value of cypermethrin was about 1 day in both seasons, in accord with previous studies where the value for endosulfan was 0.6–1.2 days (Samanta et al. 1995) and for cypermethrin in the range of 0.1–0.2 days (Bhattacharyaya et al. 1995) and 1.7–1.8 days (Gajbhiye et al. 1989). The initial value of endosulfan in made tea was found in the range of 2.2–4.1 ppm and the initial value of cypermethrin was 0.9–2.0 ppm, well below the MRL value of endosulfan (30 ppm) and cypermethrin (20 ppm) in made tea fixed by the Codex Alimentarious Commission (FAO/WHO 2006). More than 90 % of endosulfan residue was dissipated at the 7th day after application, in case of cypermethrin the residue reached below detection limit. This is in line with the normal plucking schedule.

In the present investigation it was observed that the ready-mix formulation of endosulfan and cypermethrin is safe to use in tea fields. The present findings established that there is no need to omit one round of plucking when ready-mix formulation of endosulfan and cypermethrin is being applied in the tea garden under the climatic conditions of the North-East region of India.

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