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Residues of Endosulfan and its Metabolites in a Rice Field of Madurai, India

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Abstract : Thirty-day-old rice seedlings, IR-20 variety were transplanted into the experimental plots and were sprayed with endosulfan (35% EC) after 52 days at the rate of 0.64 kg a.i/ha. Residues of endosulfan in the plant, soil, and water were found to decrease steadily upto 15 days. A second application of the pesticide was made on the 31st day and the plant was harvested on 56th day after the first application. The residue level on hay and grains was 0.7 μg/g. This level is seven times higher than FAO/WHO-prescribed tolerance level of 0.1 μg/g. Metabolites of endosulfan were traced out in plants, soil, and water during the pre- and post-harvest period.

Key words : endosulfan, metabolites, residues

INTRODUCTION

Endosulfan [6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9 methano-2,4,3 benzodioxathiepin-3-oxide] is a commonly used pesticide to control insects such as cabbage worm, peach tree borer, and several species of aphid and leafhopper which attacks the rice under supervised trial. The distribution and dissipation of this compound have been studied under the tropical climate of South India. Lack of informations on the residues in the consumer products and the soil residues of endosulfan made the author to take up this study.

MATERIALS AND METHODS

Crop and Dosage of Pesticide Used

IR-20 rice variety was chosen for this study. Plots of 20 m². were laid in random block design. Among six plots, three of them were treated and the remaining were kept as control.

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Endosulfan 35% EC formulation (M/s.Excel Industries Ltd., Bombay) in water (3 L) was uniformly sprayed using a back-pack sprayer when the plants were 52 days old at the recommended dose of 0.64 kg a.i./ha. A second spraying was done when the milky white grains were formed (31 days after the first spray application).

Sampling

The following samples were collected at different time intervals (Table 2). Rice plants (*ca.* 200 g), soil (*ca.* 1000 g) and field water (*ca.* 1000 m L) were collected from different parts of each plot, tagged in polyethylene bags, and brought to the laboratory. After mixing and quartering 25 g of plant (25 g of grain), 100 g of soil and 100 mL of water were taken for the residue analysis.

EXTRACTION, CLEAN UP AND ANALYSIS

Recovery

Standardization of extraction procedures and residue level estimations were carried out with the various samples used by the spiking technique. All samples except soil gave recoveries of

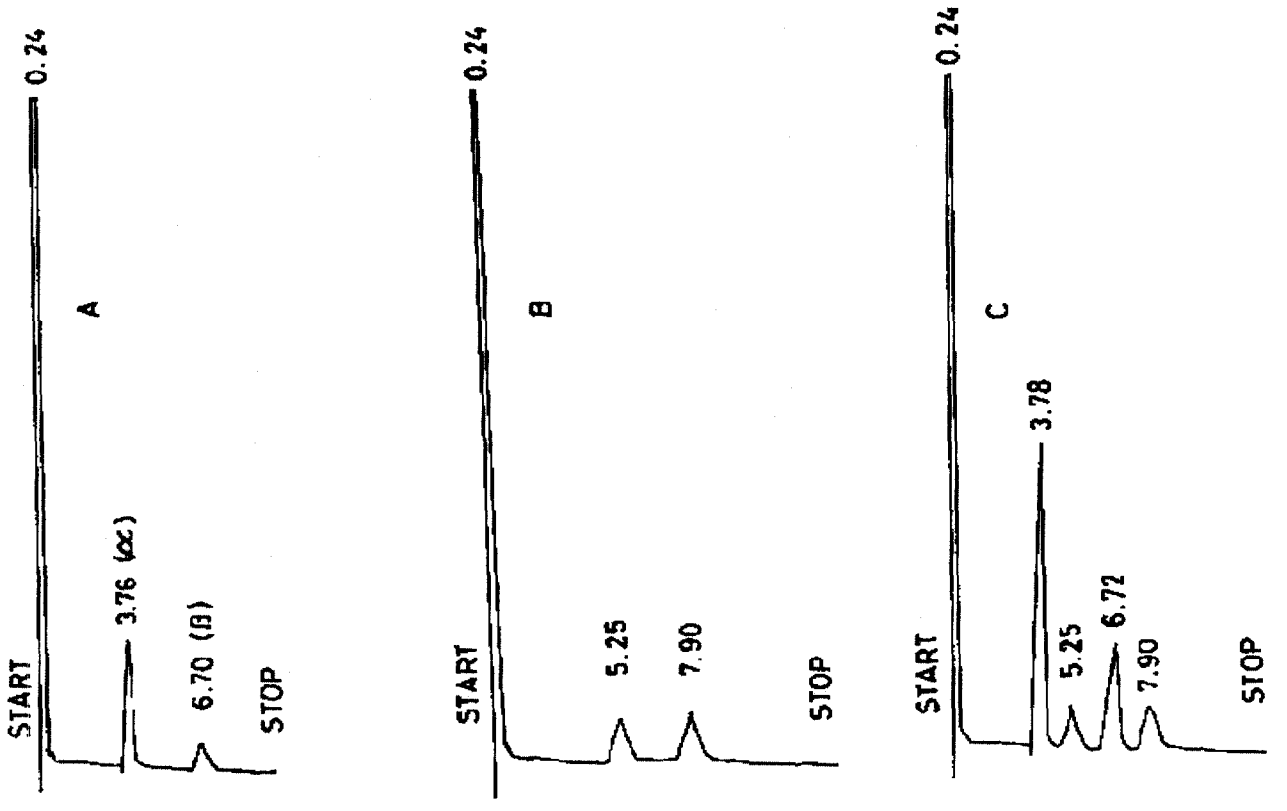

Fig. 1. Gas Chromatographic profiles of endosulfan and rice plant samples.

A - Standard endosulfan 400 pg

B - Rice plant extract

C - Rice plant extract spiked with endosulfan

80% or higher. The recovery from soil showed only 60%.

Plant Leaves / Grains

Twentyfive grams of leaves / grains were cut into small pieces, homogenized with 150 mL of hexane/acetone (41:59 v/v) and filtered. The filtrate was extracted with 80 mL portions of hexane, using 2% sodium chloride for breaking emulsions. The extracts were passed over anhydrous sodium sulfate and concentrated in 3 mL hexane, and it was loaded onto a Florosil column (2.2 cm id x 30 cm and 100~120 mesh, Sigma Chemical Co., USA), topped with 2 cm of anhydrous sodium sulfate. After washing the column with hexane, the pesticide residues were eluted with 6% of diethyl ether in hexane[10], concentrated to 2 mL, and 10 μL was analyzed by Gas Liquid Chromatography(GLC).

Soil

Soil samples (100 g) were extracted with 200 mL of hexane /acetone (41:59 v/v) in a soxhlet extractor for 12 hrs. After filtration, 70 mL water was added and the mixture was extracted with hexane (80 mL) three times. The hexane extract was passed through anhydrous sodium sulfate and then concentrated[10]. The residue was dissolved in 2 mL hexane and 10 μL was taken up for GLC analysis.

Water

Water samples (100 mL) were extracted three times with 150 mL chloroform. The organic extract was passed through anhydrous sodium sulfate, evaporated to dryness, and made up to 2 mL hexane. From this 10 μL was taken up for GLC analysis.

Table 1. Recoveries and minimum detection limits of endosulfan and its metabolites in plant, grain, soil and water

Compound	Recovery (%)				Minimum Detection limit (ng)
	Plant	Grain	Soil	Water	
α-endosulfan	82	83	60	81	0.2
β-endosulfan	88	87	61	85	0.2
endosulfan ether	98	97	62	94	0.2
endosulfan alcohol	97	98	61	92	4.0
endosulfan lactone	95	96	61	93	0.2
endosulfan sulfate	90	91	60	89	2.0

Gas Liquid Chromatography

The residues were estimated using a Hewlett Packard (USA) model 5890A Gas Liquid Chromatographic system with column 180 cm×0.4 cm, 5% OV-25 on W HP (100~120 mesh), Detector ^{63}Ni ECD with 250℃, Column temperature 225℃, Injector temperature 240℃ and Nitrogen flow rate 30 mL/min.

RESULTS AND DISCUSSION

Recoveries and Minimum Detection Llimits

A typical separation of the mixture of α and β isomers is shown in Fig. 1 (with spiked plant samples). Metabolites of endosulfan such as endo ether, endo alcohol, endo lactone and endo sulfate were resolved in the respective retention time 1.75, 2.25, 4.34, and 8.94 minutes. The minimum detection limit, the recoveries of endosulfan and its metabolites in the spiked samples such as plant, grain, soil, and water are shown in the Table 1.

The recovery was in the order of 80% and above except soil where the recovery was 60% even on immediate recovery. The time lapse before extraction decreased the recovery. Residue levels in different samples are given in Table 2. All values are the average of three independent measurements.

Plants

The initial deposit on the plant leaves was about 6.86 μg /g and this level has dropped by about 10 fold on 9th day and the residue level was increased to 0.78 μg/g on 15th day. This may be due to the translocation of residues from the soil to the plant system. A second spraying was made at the milky white stage of the grains (*i.e.* 113 days after seedling). The initial deposit was 2.55 μg/g. By the time of harvest the residues were present at the level of 0.72 μg/g which is seven times higher than the FAO/WHO-prescribed tolerance limit of 0.1 μg/g.

Table 2. Deposit levels of endosulfan (α+β) in rice fields after different intervals of its application

Sampling* time (days)	Plant	Grains	Soil	Water
Just after spray	6.86±2.83	...	0.124±0.007	0.050±0.040
1	2.18±0.77	...	0.200±0.020	0.003±0.000
3	1.23±0.40	...	0.050±0.004	0.009±0.002
9	0.52±0.38	...	0.020±0.006	0.002±0.000
15	0.78±0.24	...	0.090±0.006	0.040±0.008
31 (II Spray)	2.55±1.15	1.96±0.57	0.250±0.040	0.040±0.000
46	0.75±0.64	0.28±0.05	0.020±0.010	Field dried
56 (harvest)	0.72±0.07	0.70±0.04	ND	Field dried

All values are μg/g or μg/mL.

* Average weather conditions: maximum temperature 32±2℃; minimum temperature 21±2℃; relative humidity 82±2%, rainfall: 11.2 mm (7th day of spray); 6.0 mm (8th day of spray); 3.4 mm (24th day of spray); 1.2 mm (25th day of spray), ND-Not Detected.

Grains

The plants received the second spray at the milky white stage at that time the deposit level was 1.96 μg/g. During the harvesting time the residue level was 0.7 μg/g again and it is seven times higher than the FAO/WHO tolerance limit.

Soil

As mentioned earlier, the recovery of endosulfan from soil was extremely low. This depends upon the soil type. The experimental soil is black rich in organic matter. The initial deposit of endosulfan on the soil was in the range of 0.12 to 0.2 μg/g. After 24 hrs the residue level decreased slowly to 0.09 μg/g on the 15th day. After the second spray the residue detected was 0.25 μg/g. During the harvest period low levels have been found.

Table 3. Endosulfan metabolites in rice fields

	Sampling time* (Days)	E. ether	E. lactone	E. alcohol	E. sulfate
	Just after Spray	0.02±0.001			
P	1				
L	3				0.03±0.004
A	9	0.01±0.001	0.010+0.001		
N	15	0.02±0.003			
T	31 (II)	0.03±0.007			
	46	0.02±0.003	0.003+0.001		
	56	0.01±0.002			
	Just after Spray				
G	1				
R	3				
A	9				
I	15				
N	31 (II)	0.003±0.002			
S	46	0.010±0.004	0.002±0.0004		
	56	0.120±0.080	0.034±0.0200		
	Just after Spray	0.070±0.010	0.050±0.0090		
	1	0.320±0.008	0.050±0.009		3.65±0.10
S	3	0.270±0.170	0.020±0.004		
O	9	0.260±0.011	0.001±0.0006	1.55±0.09	
I	15	0.230±0.090	0.0002±0.000		0.001±0.0002
L	31 (II)	0.470±0.240	0.002±0.0004		0.050±0.016
	46				
	56	0.0100±0.005	0.002±0.0004	0.98±0.08	
	Just after Spray	0.0110±0.001	0.001±0.0007		
W	1	0.0020±0.0004	0.0004±0.000		0.004±0.0001
A	3	0.0001±0.000	0.0003±0.0001		0.004±0.0001
T	9		0.0020±0.000		
E	15		0.0003±0.000		
R	31 (II)				
	46	Field dry			
	56	Field dry			

All values are μg/g, μg/mL.

* Average weather conditions: maximum temperature 32± 2°C; minimum temperature 21±2°C; relative humidity 82± 2%, rainfall: 11.2 mm (7th day of spray); 6.0 mm (8th day of spray); 3.4 mm (24th day of spray); 1.2 mm (25th day of spray).

Water

The initial deposit was very low (0.05 μg/mL). The residue levels came down, and it was 0.04 μg/mL on 15th day after the first spray application.

Metabolites of Endosulfan

Endosulfan ether, endosulfan lactone were the major metabolites reported in the soil, water and plants (Table 3). Endosulfan sulfate appeared after 24 hrs of the spray was 3.65 μg/g in the soil and the level decreased to 0.001 μg/g on 15th day after spray. Grains were also showed the presence of endo ether and endo lactone upto the harvest period. This report correlates well with the findings of Kullman and Matsumura[8].

Post Harvest Residues

An attempt was made to investigate the post harvest residues particularly in the soil. The results were presented in the Table 4. Different layers of soil showed uniform levels of endosulfan (0.01~0.02 μg/g). Endo ether and endo lactone were the dominant metabolites and high levels of endo alcohol was reported in the first layer (0.96 μg/g) and third layer (1.69 μg /g). First layer also showed the presence of endo sulfate (0.023 μg /g).

Residue Levels on the Leaves

The present investigation of residue levels on leaves in agreement with the following report. When endosulfan 35% EC was sprayed on rice at a rate of 0.22 kg a.i/ha, the residues on leaves was 12 μg/g on the first day and during the harvest period the residue levels on grains was 1.0 μg/g[7]. And the same type study have been made by Ceron *et al.*[1] and Raha *et al.*[9] with different plants. The FAO/WHO[4] prescribed tolerance limit for the rice plant is 0.1 μg/g. It is not advisable for the farmer to enter the field, when the levels on the leaves are very high.

Residues in the Consumer Products

Analysis of hay and grains showed that they were retaining considerable amount of endosulfan residues. This can be rectified by sun drying and parboiling the hay and grains[7].

Residue Levels in Soil

The residue levels in soil on the day of spraying and also the post-harvest period are in close agreement with the findings of Jayaraman and Jebakumar[5,6]. They showed the strong

Table 4. Endosulfan ($\alpha+\beta$) and its metabolites in rice fields - Post harvest period study

Days after harvest	Soil layer cm	Endosulfan ($\alpha+\beta$)	E. ether	E. lactone	E. alcohol	E. sulfate
46	7.5	0.011±0.003	0.015±0.004	0.001±0.0002	0.96±0.01	0.023±0.004
106	7.5	0.010±0.001	0.006±0.0002	0.002±0.0001	0.34±0.008	0.001±0.0002
46	15	0.010±0.001	0.015±0.001	0.001±0.0002	...	...
106	15	0.070±0.010	0.005±0.0002	0.002±0.0004	...	...
46	22.5	0.020±0.005	0.004±0.0003	0.002±0.0004	1.69±0.10	...
106	22.5	0.020±0.004	0.007±0.0004	0.003±0.0005	1.26±0.20	0.001±0.0002

All values are $\mu g/g$.

binding tendency of endosulfan in a model ecosystem experiment and also calculated the percolation rate of endosulfan through soil layers in the order of 5 $\mu g/cm/day$.

Residue Levels in Water

The deposit level in water was in the range of 0.04 ~ 0.05 $\mu g/mL$. Endosulfan has been found in agricultural run off and industrial areas where it is manufactured. The agricultural run off in USA indicated that it followed within 4 days of application (0.35 kg/ha). And residues can be averaged, 16 $\mu g/L$ in the runoff[2]. It may also be mentioned that endosulfan is included as one of the potential compounds for ground water contamination[3]. Therefore endosulfan soil binding and percolation through soil layers require separate deeper study.

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