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Effect of Moisture Regime, Temperature, and Organic Matter on Soil Persistence of Carbosulfan

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Carbosulfan [2,3-dihydro-2,2-dimethyl-7-benzofuranyl (di-n-butyl)aminosulfenyl) methylcarbamate] is one of the promising carbamate insecticides currently recommended for use in agriculture in India. Recently, we found that carbosulfan is readily hydrolysed to carbofuran in flooded soils, and this hydrolysis is more pronounced at 35°C than at 25°C (Sahoo et al. 1990). Microorganisms were implicated in its rapid loss from flooded soils, because of its more rapid degradation in nonsterilized soil than in sterilized soil. The present study is concerned with the effect of moisture regime (nonflooded versus flooded), temperature, and organic matter on soil persistence of carbosulfan.

MATERIALS AND METHODS

Two soils from rice fields, an alluvial soil (pH 6.2, 1.6% organic matter, 0.09% total nitrogen from the experimental farm of the Central Rice Research Institute, Cuttack (CRRI), India) and a laterite soil (pH 7.2, 0.6% organic matter, 0.04% total nitrogen from Sukinda, India)were used. Technical grade carbosulfan (89.6% purity) was obtained from Rallis India Limited, Bangalore, India.

The soils were air-dried, ground, and sieved through a 2-mm mesh screen before use. To study the effect of moisture regime on the persistence of carbosulfan in soils, 20-g portions of sieved, alluvial and laterite soils were placed separately in sterile, glass test tubes (200 x 25 mm) and maintained in flooded and nonflooded (60% water-holding capacity) conditions by adding adequate sterile, distilled water (25 mL in flooded and 3.6 mL (alluvial) and 2.8 mL (laterite) in nonflooded soils). After 10 days of incubation at 28 $\pm$ 2°C, technical grade carbosulfan in 0.1 mL of acetone containing 1 mg of active ingredient of the insecticide was added to both flooded and nonflooded soils. Duplicate samples were removed at periodic intervals for extraction and colorimetric analysis of

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carbosulfan and its hydrolysis product, carbofuran, after separation by thin-layer chromatography (TLC).

In another experiment, persistence of carbosulfan was studied in sterile and nonsterile soils (alluvial and laterite) held at 25 and 35°C under nonflooded conditions. Portions (20 g) of sieved alluvial and laterite soils were placed separately in sterile, glass test tubes (200 x 25 mm), and adequate sterile, distilled water was added to each tube to provide 60% water-holding capacity. One set of test tubes of both soil types was sterilized by autoclaving at 121°C for 1 h for three consecutive days. The nonflooded autoclaved and non-autoclaved soils were then incubated at 25 $\pm$ 1°C and 35 $\pm$ 1°C in a B.O.D. incubator. After 10 days of incubation, 0.1 mL of carbosulfan in acetone containing 1 mg of active ingredient was added to each tube as described earlier. At periodic intervals, duplicate samples were extracted and analysed for carbosulfan and its hydrolysis product, carbofuran, by gas-liquid chromatography (GLC).

To study the effect of organic matter on the persistence of carbosulfan, 20-g portions of sieved alluvial soil in sterilized, glass test tubes (200 x 25 mm) were amended with 5% (w/w) rice straw as organic matter. Soil samples without rice straw served as control. Sterile, distilled water (25 mL) was added to all the tubes to maintain a flooded condition. After 10 days of incubation at 28 $\pm$ 2°C, 0.1 mL of carbosulfan in acetone (containing 1 mg of active ingredient) was added to separate sets of test tubes. At periodic intervals duplicate samples were removed for extraction and colorimetric analysis of carbosulfan and carbofuran after separation by TLC.

For GLC analysis, residues were extracted in ethyl acetate and analysed in a Varian model 3400 gas chromatograph as described earlier (Sahoo et al. 1990). For TLC, the total contents (soil and water) in each tube were transferred to a 250-mL Erlenmeyer flask. The residues were extracted thrice by shaking the soil and water with 50, 40 and 30 mL of ethyl acetate, respectively. After each extraction, the ethyl acetate fraction was pooled into a beaker and allowed to evaporate at room temperature. After complete evaporation of ethyl acetate, the residues were dissolved in 1 mL of methanol; 200 µL of the solution was spotted on a silica-gel G plate. The silica gel plates were then developed in a solvent system of hexane and diethyl ether (4:3 v/v). The authentic compound on the chromatoplate was located by spraying of 1 N methanolic NaOH followed by a 0.1% methanolic solution of p-nitrobenzene diazoniumfluoroborate. The silica gel portions of the samples corresponding to the same mobility as the authentic compound were scraped into test tubes and then treated with 2 mL of 1 N methanolic NaOH followed by 1 mL of 0.01% methanolic p-nitrobenzene diazonium-fluoroborate solution. The contents in each tube were centrifuged and the absorbance of the supernatant was measured in a Beckman spectrophotometer at 520 nm. The amount of the residues was calculated by comparing the absorbance value with the standard curve. The pH of soil samples were determined at regular intervals at a 1:1.25 soil-water ratio.

RESULTS AND DISCUSSION

The persistence of carbosulfan in two soil types, alluvial and laterite, was examined under flooded and nonflooded (60% water-holding capacity) conditions. Soil submergence distinctly accelerated the hydrolysis of carbosulfan to carbofuran in both soils (Table 1). Thus, the concentration of carbosulfan decreased to undetectable levels in 8 days in the alluvial soil under flooded conditions; but, under nonflooded conditions, carbosulfan was detected in significant amounts even after 15 days (Table 1). The same trend was noticed also in laterite soil; but the insecticide appeared to be more persistent in the laterite soil than in alluvial soil under both water regimes. Concomitant with the decrease in the concentration of carbosulfan, carbofuran accumulated in both soils, but in greater amounts in flooded soils than in nonflooded soils.

According to an earlier study (Sahoo et al. 1990), degradation of carbosulfan was more pronounced at 35°C than at 25°C and in nonsterilized soil than in sterilized soil under flooded

Table 1. Amount of carbosulfan and its hydrolysis product, carbofuran recovered from an alluvial soil under flooded and nonflooded conditions

	Flooded		Nonflooded	
Days	Carbo-sulfan (μM)	Carbo-furan (μM)	Carbo-sulfan (μM)	Carbo-furan (μM)
		Alluvial soil		
0	96.0$\pm$5.8	0	100.1$\pm$4.9	0
4	28.2$\pm$2.0	55.4$\pm$2.9	57.8$\pm$5.3	34.8$\pm$3.6
8	>2.5	37.5$\pm$0.9	15.4$\pm$2.8	32.8$\pm$4.1
15	>2.5	22.8$\pm$0.7	5.3$\pm$2.4	29.1$\pm$2.0
		Laterite soil		
0	109.7$\pm$0.7	0	94.6$\pm$3.3	0
10	7.9$\pm$0	88.1$\pm$2.3	25.0$\pm$3.9	64.4$\pm$3.4

conditions. In another experiment, we studied the persistence of carbosulfan in alluvial and laterite soils (sterile and non-sterile) under nonflooded conditions at 25 and 35°C. As in flooded soils (Sahoo et al. 1990), more rapid degradation of carbosulfan occurred at 35°C than at 25°C and in nonsterile soil samples than in sterile samples under nonflooded conditions. The concentration (µM) of carbosulfan in sterile and nonsterile soil (alluvial) under nonflooded conditions decreased from 84 at the start to 56 and 33 at 25°C and 45 and 0 at 35°C, respectively after 15 days of incubation. The same trend was noticed in the laterite soil. Moreover, carbofuran formed by primary hydrolysis of carbosulfan, accumulated in greater amounts at 35°C than at 25°C in both nonsterile soils under nonflooded conditions. In sterile soil samples at both temperatures, carbofuran was detected only in negligible amounts. These observations suggest that as in flooded soils (Sahoo et al. 1990), rapid hydrolysis of carbosulfan to carbofuran in nonflooded soils, especially at 35°C, is mediated by microorganisms.

Carbosulfan appeared to undergo faster hydrolysis in alluvial soil than in laterite soil under both flooded (Sahoo et al. 1990) and nonflooded (Table 1) conditions. Hydrolysis of carbosulfan increases with a decrease in pH (Ramanand et al. 1991). The pH of alluvial and laterite soils at flooding was 6.2 and 7.2, respectively, and after 10 days of flooding (when carbosulfan was added), both soils registered a pH of about 7.0. Hence, more rapid hydrolysis of carbosulfan in alluvial soil than in laterite soil cannot be attributed to the differences in the pH of these two soils.

Table 2. Degradation of carbosulfan in flooded alluvial soil amended with rice straw

Days after carbosulfan addition	Rice straw-amended[1]		Unamended[2]	
	Carbosulfan[3]	Carbofuran[4]	Carbosulfan[3]	Carbofuran[4]
0	38.1±6.6	75.9±3.6	92.0±2.6	12.2±1.4
2	0	40.2±6.3	52.2±6.8	19.0±8.1
4	0	36.8±1.1	21.7±0	60.3±9.5
8	0	25.3±2.3	3.5±0	34.1±1.8
15	0	5.0±0.5	0	20.1±1.6

[1] Carbosulfan was added to the flooded soil 10 days after the addition of rice straw.

[2] Carbosulfan was added to unamended soil 10 days after flooding.

[3] µM carbosulfan recovered.

[4] µM carbofuran formed from carbosulfan.

Alluvial soil has a higher orgnic matter content than the laterite soil (1.6% versus 0.6%). Possibly, accelerated hydrolysis of carbosulfan in the alluvial soil is related to its higher organic matter content. Whether addition of an organic source such as rice straw to a soil would accelerate the hydrolysis of carbofuran was therefore examined. Interestingly, all the added carbosulfan (131 µm) disappeared within 2 days after its addition to the soil preflooded for 10 days with rice straw (5% w/w) (Table 2). During the same period, the concentration of carbosulfan decreased to only 40% of the original level in flooded soil not amended with rice straw. The amount of carbofuran recovered was about 40 µM and 19 µM from rice straw-amended and unamended soil, respectively. Evidently, carbofuran was not recovered in stoichiometric amounts during hydrolysis of carbosulfan, possibly due to the soil binding of the former or its subsequent metabolism. The addition of organic matter increases the microbial activity and hastens the drop in redox potential of a flooded soil (Ponnamperuma 1972). The pH of rice straw-amended soil was 7.30 as compared to the pH value of 7.46 in unamended soil at 10 days after flooding (when carbosulfan was added to the soils). Since carbosulfan is known to be stable at higher pH, accelerated hydrolysis of this insecticide in flooded rice straw-amended soil is due to reasons other than pH.

Interestingly, substantial hydrolysis of carbosulfan was noticed especially in rice straw-amended soil even at 0 time sampling. Evidently, this hydrolysis proceeded during extraction of the residues from the soil. It may be mentioned that soil used in this study was flooded for 10 days with rice straw before the addition of carbosulfan. It is well known that decomposition of rice straw under flooded conditions can lead to the accumulation of organic acids, acetic acid in particular (Chandrasekaran and Yoshida 1973, Rao and Mikkelsen 1977). We have evidence that significant hydrolysis of carbosulfan can occur even in autoclaved water extract of flooded rice straw-amend soil (A. Sahoo, unpublished data). Possibly, interaction between the organic acids or other products of anaerobic decomposition of organic matter (added and native) effected the instantaneous hydrolysis of carbosulfan during extraction. This is supported by the data on exceptionally rapid hydrolysis of carbosulfan when it was shaken with rice straw-amended soil preflooded for 10 days (Table 3). Within 240 min of shaking with rice straw-amended soil, almost all the added carbosulfan was hydrolysed. Even, flooded soil without rice straw hydrolysed carbosulfan to some extent, possibly due to the interaction between reduction products of soil organic matter and carbosulfan. Anaerobic decomposition of soil organic matter in flooded soil can also lead to the accumulation of organic acids and other reduction products (Ponnamperuma 1972), but to a less extent than in rice straw-amended soil.

It is concluded that soil submergence stimulated the degradation of carbosulfan and addition of rice straw to flooded soil further accelerated it.

Table 3. Degradation of carbosulfan on its shaking with alluvial soil, preflooded with and without rice straw for 10 days

Equilibration period (min)	Rice straw-amended[1]		Unamended[2]	
	Carbo-sulfan[3]	Carbo-furan[4]	Carbo-sulfan[3]	Carbo-furan[4]
5	33.2±1.0	55.3±2.2	87.3±0	0
12	11.8±0	61.9±2.2	30.7±1.1	31.1±0.6
240	0	23.7±3.3	19.7±3.9	33.2±3.1

1 Alluvial soil flooded with rice straw for 10 days was shaken with carbosulfan.

2 Alluvial soil flooded for 10 days without rice straw was shaken with carbosulfan.

3 µM carbosulfan recovered.

4 µM carbofuran formed from carbosulfan.

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