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Dissipation Dynamics of Carbosulfan 25 EC in Water

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Abstract – **Dissipation dynamics of carbosulfan was studied in three different type of water samples (acidic, alkaline and neutral pH) following application of carbosulfan @ 5 and 10 µg g^{-1} levels. The residues progressively declined with time and reached below detectable level (<0.01 µg g^{-1}) at 45 days after application in acidic and alkaline pH, but persisted up to 60 days in neutral pH. However, more than 95% of carbosulfan degraded within 30 days after incubation irrespective of pH. The half-lives of carbosulfan in different pH waters varied from 7.18 to 7.51 days for neutral pH, from 5.70 to 6.29 days for acidic pH and from 4.42 to 5.24 days for alkaline pH. Hence, carbosulfan is a comparatively safer insecticide and will not contaminate water sources, especially in alkaline pH water sources.**

Keywords – **Water, Acidic Ph, Alkaline Ph, Neutral Ph, Carbosulfan Residues, Dissipation, Persistence, Degradation, Half-Life.**

I. Introduction

Insecticides are mostly applied as foliar spray to the crops. It may reach the soil through spilling while spraying which in turn runoff to the water surface. An insecticidal chemical once released into the soil is subjected to physical and biochemical processes, which determine its persistence. Persistent insecticides bring in problems of pollution of ground water, plants, food etc. Hence any insecticide reaching soil should be degraded within a considerable period of time. The reactions such as adsorption, leaching, volatilization, chemical decomposition and microbiological pathways contribute to the degradation of the applied chemical. It is important to understand the degradation and persistence of pesticides in soil and water in order to maximize pesticide efficacy while minimizing any detrimental effects they may have on the ecosystem.

Degradation of pesticides refers to the breakdown of pesticides within the environment. The degradation may occur through photodegradation, chemical degradation or biodegradation. In some cases, there is a complete mineralization of the pesticides whereas in other cases only a partial degradation takes place. This may potentially lead to an accumulation of metabolites, which sometimes are more toxic, *i.e.* more hazardous, than the mother-compound [1]. Sometimes the pesticides are not degraded even though they have proven to be biodegradable. This may, as mentioned above, be due to different environmental factors affecting the activity of the degrader organisms; essential nutrients may be missing, environmental conditions may be unsuitable, or the concentration of the pesticide may be too high or too low [2].

All chemicals are susceptible to photodegradation to some extent. The degree of photodegradation will depend on the intensity of the sunlight and the time of exposure. However, many pesticides move relatively quicker into the soil and are thus no longer exposed to sunlight and therefore not susceptible to photodegradation [3]. Chemical degrada--tion is due to reactions of the pollutant with e.g. water, oxygen or other chemicals [4]. Biodegradation refers to the degradation of the pesticides by organisms; most often microorganisms like bacteria and fungi, but in some cases, plants may be involved in the degradation as well [5]. The degradation rates are affected by soil properties viz., pH and temperature. Lower degradation rates have been observed with a pH above 6.5 and higher rates with pH below 5 [3] However, the effect of pH will depend on the compound being degraded and the organisms responsible for the degradation, and studies showed a more rapid degradation in soils with higher pH [6]. The temperature of the soil also influences degradation rates; the rate of most reactions catalyzed by enzymes tends to double for every 10 ^{o}C increase in temperature (between 10 and 45 ^{o}C). An increase in soil temperature will thus lead to an increase in degradation rate [4].

Degradation and persistence of pesticides can be affected by a wide variety of factors including properties of the pesticides (water solubility, volatility, polarity), properties of the water (pH, temperature, soil composition), and resistance to degradation (biological and chemical), rainfall and aquatic life.

Carbosulfan, (2, 3 - dihydro- 2, 2- dimethyl- 7- benzo-furnanyl (di-n-butyl amino) thio methyl carbamate) a relatively new methyl carbamate insecticide is reported to be effective against sucking and chewing insect pests. Carbosulfan (Fig 1) is a derivative of carbofuran and acts as cholinesterase inhibitor. It is widely used for the control of soil dwelling insecticides and foliar pests in agricultural and horticultural crops and also acts as nematicide. Keeping this in view, a comparative study was undertaken on the dissipation dynamics of the insecticide, carbosulfan and their residues in water with three levels of PH *ie.* Acidic PH, alkaline PH and neutral PH.

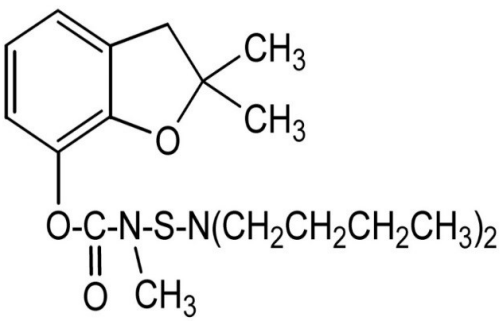

Fig 1. Structural formula of carbosulfan

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II. Materials and Methods

A. *Sampling*

The three-different type of water samples (acidic, alkaline and neutral pH) collected were added with 5 and 10 µg g-1 levels of carbosulfan technical grade and incubated in the laboratory. The samples were taken for analysis at 1, 3, 7, 15, 30, 45, 60, 75, 90 and 105 days after treatment.

B. *Extraction, Clean up and Estimation*

Fifty ml of the treated water sample was taken in a 500 ml separating funnel mixed with 10 ml of saturated sodium chloride solution. The residues were extracted with 25 ml of dichloromethane in hexane (15:85) shaking vigorously for 2-3 minutes and releasing the pressure intermittently. The layers in the separating funnel were allowed to separate by keeping the separating funnel undisturbed. The lower aqueous layer was collected in one litre conical flask and the upper organic layer was collected in a 250 ml conical flask. The aqueous layer was transferred to another one litre separating funnel and extracted again with 2 x 50 ml of dichloromethane in hexane. The organic layer at every step of extraction was pooled in a 250 ml conical flask. The extract with hexane was dried in about 50 g anhydrous sodium sulphate. The hexane layer was concentrated to 2-5 ml using rotary vacuum evaporator at 40oC.

C. *Derivatisation*

The condensed extract was dissolved in 1 ml of reactant solution (1.0 g of 1- fluoro 2, 4-dinitrobenzene in 100 ml of acetone) and transferred quantitatively to stoppered 25 x 190 mm test tubes [7]. With this, 15 ml of phosphate buffer solution (phosphate buffer of pH 11 - 25.0 g of Na_2HPO_4 was dissolved in 2480 ml of distilled water and 20 ml of 1 M NaOH solution) was added, mixed well and kept in the water bath and the temperature was maintained at 50°C for 30 minutes. After 30 minutes the test tubes were removed from the water bath, cooled and transferred to a 60 ml separatory funnel. The content was extracted twice with exactly two 25 ml portions of n-hexane and the organic layer was collected and the hexane extract was condensed and retained for final determination.

D. *Operation Parameters*

The carbosulfan residues were dissolved in 10 ml of hexane for final injection (2 µl) in Chemito model 2685 Gas Chromatography (GC) equipped with 63Ni electron capture detector (ECD) and fitted with a glass column (3% OV 17) of 1.8 m x 2.0 mm I.D. The operating conditions were as follows: injector port (255°C), detector (280°C), oven temperature (235°C), column temperature (300oC), carrier gas (nitrogen) 20 ml min-1. Under these operating parameters, the residue of carbosulfan was detected at 8.0th minute (retention time).

E. *Recovery Studies*

The analytical method of carbosulfan was standardized with the known purity standards in gas chromatography. Recovery studies were conducted to assess the validity of the present method. Fifty ml of the three different water samples were fortified at 0.5, 1.0 and 1.5 µg g^{-1} of carbosulfan technical grade (92.5% purity) solution. The residues were extracted and estimated as per the method mentioned above. The analytical method of carbosulfan was standardized with the known purity standards in gas chromatography. Recovery studies were conducted to assess the validity of the present method.

F. *Determinability*

The minimum detectable level or sensitivity of the Gas Chromatography was 0.1 ng for carbosulfan. The lower quantitative limits (LOQ) of GC for brinjal fruits considering 50g weight was 0.002 µg with a final volume of the extract being 2 ml and injection volume of 2µl. Values below this level are reported as below detectable level (BDL).

G. *Data Analysis*

The insecticide degradation pattern was analyzed by applying seven transformation functions [8, 9]. The half-life was calculated using Pesticide Residue Half Life Calculator software developed by Department of Soil Science, Tamil Nadu Agricultural University, Coimbatore and best fit degradation model was determined. The safe waiting period was worked out as per the formula given below ([10].

$$\text{Safe waiting period (TMRL)} = \frac{\log K_2 - \log (\text{MRL/tolerance})}{\log K_1}$$

III. Results and Discussion

The linearity of the calibration curve was established in the range of 0.1 to 1.5 µg g^{-1} (4 levels) with a correlation coefficient (R^2) of 0.994, 0.981 and 0.992 in neutral, acidic and alkaline water samples. The recovery studies revealed a satisfactory recovery of carbosulfan residues (Table 1) with mean per cent recoveries of 95.77, 100.17, 101.63 and 102.67 percent and the Relative Standard Deviation (RSD) of 8.66, 6.82, 5.64 and 5.91 when samples were spiked at 0.1, 0.5, 1.0, and 1.5 µg g^{-1}, respectively for neutral pH water samples. The recovery of carbosulfan residues in acidic pH water samples (Table 2) were also acceptable with mean per cent recoveries of 94.93, 97.50, 101.50 and 101.20 percent and the Relative Standard Deviation (RSD) of 6.40, 5.67, 6.04 and 5.41 when samples were spiked at 0.1, 0.5, 1.0, and 1.5 µg g^{-1}, respectively. A pleasing recovery of carbosulfan residues was noticed in alkaline Ph water samples with mean per cent recoveries of 99.50, 96.10, 99.43 and 101.67 percent and the Relative Standard Deviation (RSD) of 7.21, 6.87, 6.03 and 6.63 when samples were spiked at 0.1, 0.5, 1.0 and 1.5 µg g^{-1}, respectively (Table 3).

Table 1. Recovery percentage of carbosulfan in neutral PH water samples

Spiking level (µg g^{-1})	Recovery per cent (%)				
	R1	R2	R3	Mean ± SD	RSD
0.1	105.2	89.6	92.5	95.77 ± 8.30	8.66
0.5	92.3	104.6	103.6	100.17 ± 6.83	6.82
1.0	99.1	97.6	108.2	101.63 ± 5.74	5.64
1.5	97.4	109.3	101.3	102.67 ± 6.07	5.91

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Table 2. Recovery percentage of carbosulfan in acidic PH water samples

Spiking level (µg g^{-1})	Recovery per cent (%)				
	R1	R2	R3	Mean ± SD	RSD
0.1	101.3	94.3	89.2	94.93 ± 6.07	6.4
0.5	102.4	91.5	98.6	97.50 ± 5.53	5.67
1.0	94.6	103.6	106.3	101.50 ± 6.13	6.04
1.5	96.7	107.3	99.6	101.20 ± 5.48	5.41

The results are in accordance with earlier reports that the mean recovery value of carbosulfan was 91.00 and 97.30 per cent in water [11]. The analyses of water spiked with carbosulfan between 0.03 and 0.25 µg g^{-1} exhibited recoveries between 96 and 98 per cent [12]. The standard chromatogra--ms of carbosulfan at different levels of spiking were displayed in Fig 2.

Table 3. Recovery percentage of carbosulfan in alkaline PH water samples

Spiking level (µg g^{-1})	Recovery per cent (%)				
	R1	R2	R3	Mean ± SD	RSD
0.1	105.6	101.3	91.6	99.50 ± 7.17	7.21
0.5	96.3	102.6	89.4	96.10 ± 6.60	6.87
1.0	103.8	92.6	101.9	99.43 ± 5.99	6.03
1.5	94.2	107.3	103.5	101.67 ± 6.74	6.63

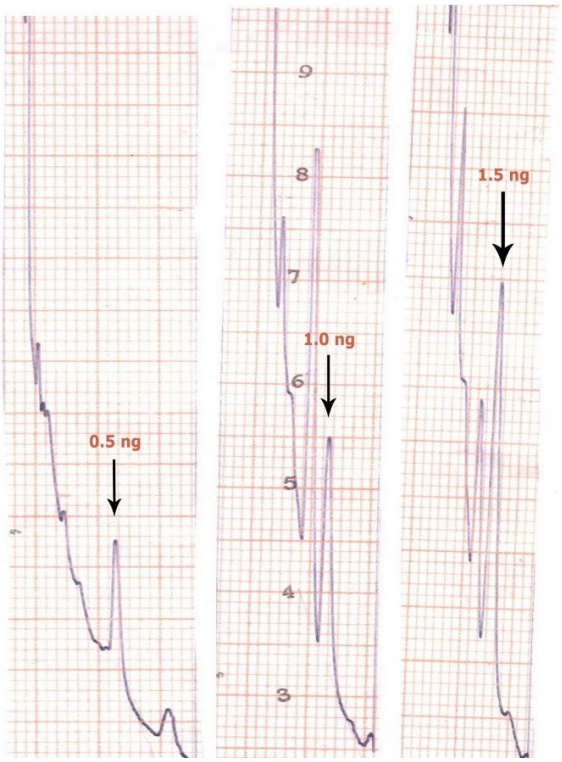

Fig. 2. Standard Chromatogram of Carbosulfan

Table 4. Degradation and persistence of carbosulfan in water

DAT	Neutral pH (7.0)				Acidic pH (5.5)				Alkaline pH (8.5)			
	5 µg g^{-1}		10 µg g^{-1}		5µg g^{-1}		10 µg g^{-1}		5 µg g^{-1}		10 µg g^{-1}	
	R	% D	R	% D	R	% D	R	% D	R	% D	R	% D
1	4.00	20.0	8.46	15.4	3.46	30.8	8.05	19.5	3.61	27.8	7.93	2.07
3	2.83	43.4	6.29	37.1	2.50	50.0	5.63	43.7	2.65	47.0	6.09	39.1
7	1.72	65.6	3.02	69.8	1.32	73.6	2.80	72.0	1.46	70.6	2.61	73.9
15	0.98	80.38	1.80	82.0	0.50	90.0	1.56	84.4	0.68	86.4	1.34	86.6
30	0.24	95.18	0.58	94.2	0.04	99.3	0.29	97.1	0.09	98.2	0.18	98.2
45	0.06	98.78	0.09	99.08	BDL	100	BDL	100	BDL	100	BDL	100
60	BDL	100.0	BDL	100	BDL	100	BDL	100	BDL	100	BDL	100

The degradation and persistence of carbosulfan in water was evaluated by fortifying the three different pH water samples with carbosulfan at 5 and 10 µg g^{-1} and extracting the residue after the expiry of the incubation period at varying intervals (Table 4). The results revealed that the maximum level of carbosulfan at 5 and 10 µg g^{-1} levels in neutral, acidic and alkaline pH water samples was detected on first day, and thereafter degraded to 95 per cent after 30 days of application, irrespective of the pH. The carbosulfan concentrations of 4.00, 3.46, 3.61 and 8.46, 8.05, 7.93 µg g^{-1} were detected at 1 DAA

in water samples of pH 5.5, 7.0 and 8.5 at 5 and 10 µg g^{-1} levels, which further degraded to 0.24, 0.092, 0.037 and 0.58, 0.29 and 0.18 µg g^{-1} at 30 DAA. The 5 µg g^{-1} and 10 µg g^{-1} levels of acidic and alkaline pH samples reached BDL at 45 DAA, whereas in neutral pH, the residues persisted up to 45 days and reached BDL at 60 DAA (Fig. 3).

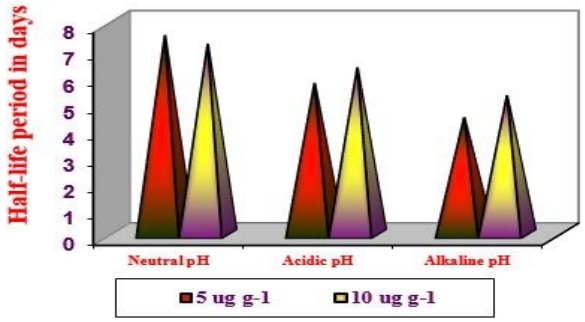

Fig. 3. Half-life period of of Carbosulfan

It is evident from the results that the initial concentration of carbosulfan ranged from 3.46 to 4.00 at 5 µg g^{-1} level and 7.93 to 8.46 at 10 µg g^{-1} level and thereafter carbosulfan degraded up to 95 per cent after 30 days of application. The acidic and alkaline pH water samples reached BDL after 45 days of application, whereas neutral pH reached BDL after 60 days of application. This was in accordance with earlier reports that carbaryl degraded in water within 21 days of treatment. Irrespective of pH levels the half-life of carbosulfan in water ranged from 4.4 to 7.51 days, indicating its low persistence 13]. Earlier attempts in this regard confirmed that carbosulfan was more persistent at neutral than at acidic or alkaline pH [14]. The residues in water samples having acidic and alkaline pH reached below detectable level (<0.002 micro g ml^{-1}) on the 45th day, while in water samples having neutral pH the residues persisted over 45 days and became non-detectable on the 60th day. Previous reports confirmed that none of the ground water samples from paddy growing areas where carbofuran application has been a regular practice for over 20 years, contained any detectable level (< 0.005 mg lit^{-1}) of carbosulfan [15]. The low persistence of carbosulfan in water samples was corroborated in previous studies [16]. The carbamates, carbosulfan and carbofuran have low persistence in water and medium persistence in soil solution of tropical irrigated rice fields [17]. Hydrolysis may be the probable principal factor. Responsible for the low persistence of carbosulfan at low and high pH waters.

In contrary, investigations on the persistence of carbofuran in soil treated with carbofuran and irrigated with water at the salinity levels of 0, 10, 40 and 90 m eq l^{-1} revealed that the insecticide was found to persist for over four months from the soils of all the treatments, degrading the residues @ 15, 30, 35 and 46 days half-life values respectively for the four salinity levels [18].

The pesticide persistence in an environment can be characterized by the pesticide half-life in such a compartment. The half-life is a measure of the time required for the pesticide concentration to be reduced to half the original value throughout biological or chemical degradati--on processes. Some pesticides are found in a specific compartment as a transformation product of another pesticide originally applied or by transport from a compartment to another. In these cases, the original concentration, necessary for the half-life calculation, is obtained by means of a special transformation kinetics that cannot be described by a first-order kinetics [19].

The computed modified regression coefficient revealed that in all treatments carbosulfan degradation followed first order function (Table 5). Previous studies revealed that carbosulfan degradation followed first order function [20]. The intercept (a), slope (b) and half-life (T0.5) and their upper and lower limits are presented in Table 6. The half-life values ranged from 7.18 to7.51 for neutral pH, from 5.7 to 6.29 for acidic pH and from 4.42 to 5.24 for alkaline pH. The outcome of the study was in agreement with earlier reports that carbosulfan was immediately transformed to carbofuran through hydrolysis, with a half-life of 3 days (78 h) in paddy water [21]. Similar results were previously obtained i.e., 5.8 days [22]. The half-life of carbosulfan was found to be 3 days in some reports [23] meanwhile other studies determined values of 22, 26 and 18 days [24].

IV. Conclusion

The dissipation of carbosulfan in water in the present investigation confirmed the lower persistence nature of the carbosulfan in the environment, but intensive research on monitoring the bioaccumulation of this chemical on the soil micro flora and the aquatic biota will improve the superiority of the chemical among the new molecules in the pesticide market.

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Table 5. Dissipation pattern of carbosulfan in water with statistical parameters

Water	Dose (µg g^{-1})	a	LL	UL	b	LL	UL	$T_{(0.5)}$	LL	UL	Prediction equation
Neutral pH	5	5.945	6.118	5.773	-0.092	-0.084	-0.997	7.510	8.123	6.906	Y = 5.945-0.092X
	10	6.685	7.059	6.310	-0.096	-0.080	-0.112	7.180	8.389	5.978	Y = 6.685-0.960X
Acidic pH	5	5.899	6.183	5.610	-0.121	-0.103	-0.139	5.706	6.573	4.839	Y = 5.899-0.120X
	10	6.649	7.000	6.290	-0.110	-0.087	-0.132	6.290	7.590	4.980	Y = 6.649-0.110X
Alkaline pH	5	6.090	6.320	5.850	-0.156	-0.141	-0.172	4.420	4.860	3.980	Y = 6.090-0.156X
	10	6.718	7.076	6.350	-0.127	-0.104	-0.150	5.242	6.420	4.437	Y = 6.718-0.127X

a - Intercept b - Slope $T_{(0.5)}$ - Half-life LL - Lower limit UL - Upper limit

Table 6. Correlation coefficient for carbosulfan water by different methods of transformation of residue data with statistical parameters

Soiltype	Dose (µg g^{-1})	Order of functions													
		1st order		1.5th order		2nd order		RF1st order		RF 1.5thorder		RF 2nd order		nverse function	
		r	r^2	r	r^2	r	r^2	r	r^2	r	r^2	r	r^2	r	r^2
Neutral pH	5	**0.996**	**0.970**	0.939	-5.80	0.823	-2.30	0.970	0.884	0.829	-	0.679	-2.58	0.839	0.678
	10	**0.985**	**0.952**	0.886	-27.58	0.756	-1.79	0.956	0.895	0.769	-8941	0.610	-1.87	0.829	0.715
Acidic PH	5	**0.951**	**0.961**	0.797	-5.26	0.653	-171.0	0.865	0.670	0.665	-122.3	0.511	-3.53	0.687	-0.81
	10	**0.906**	**0.896**	0.716	-132.7	0.590	-4.04	0.803	0.518	0.576	-	0.449	-2.28	0.624	-.003
Alkaline pH	5	**0.981**	**0.987**	0.886	0.156	0.754	-15.40	0.920	0.858	0.708	-7.004	0.613	-21.13	0.759	0.018
	10	**0.883**	**0.859**	0.691	-197.7	0.576	-4.12	0.774	0.445	0.552	-	0.436	-2.363	0.593	-0.124

RF- root function r - regression co-efficient r^2 – modified r^2