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Dissipation dynamics and risk assessment of carbosulfan in brinjal (*Solanum melongena* L.) fruits

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Abstract
The present study was conducted to investigate the persistence behavior and dissipation dynamics of carbosulfan in brinjal fruits to assess its risk in consuming the fruits. The results revealed that the residues were 0.05, 0.12 and 0.27 µg g^{-1} at 15 DAT at the first harvest of the fruits after three rounds of spraying when applied at 250, 500 and 1000 g a.i.ha^{-1} respectively. However, the residues were below detectable limit at all the levels of application at the third harvest of the produce. The residues were below the maximum permissible residue limit (MRL) of 0.1 µg g^{-1} at the recommended dose of 250 g a.i. ha^{-1} even at the first harvest itself making it as a suitable insecticide for managing brinjal pests without any risk.

Keywords: Carbosulfan, residues, brinjal fruits, eggplant, safe harvest, *Solanum melongena*

1. Introduction
Vegetables, an essential component of the human diet, have taken third place along with fruit crops in taking over the pesticide load next to cotton and rice [1]. Eggplant (*Solanum melongena*), or aubergine, is a species of nightshade, grown for its edible fruit, which finds its place in all kind of cuisines. It is cultivated for its tender fleshy fruit, which is used as a common vegetable for the preparation of various dishes. It is highly productive and finds its place as poor man's crop. It is one of the most important vegetable crops in South East Asia [2] and its worldwide cultivation is more than 1,600,000 ha and production is 50 million Mt [3]. From the time of planting till harvest, brinjal (*Solanum melongena* L.) plant is attacked by more than 36 pests including leaf hopper (*Amrasca biguttula biguttula* Ishida), whitefly (*Bemisia tabaci* Genn), aphid (*Aphis gossypii* Glover) and shoot and fruit borer (*Leucinodes orbonalis* Guen) [4]. Insecticides provide an acceptable solution to overcome these pests, as they are highly effective, rapid in curative action and adoptable to most situations, flexible in meeting changing agronomic conditions and relatively economical. Various insecticides belonging to organochlorines, organophosphates, carbamates and synthetic pyrethroids were recommended earlier to control these pests. 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 insect pests of brinjal [5]. 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 [6, 7]. Since brinjal is one of the prime vegetables widely used for making various delicious dishes, the analysis of residues of carbosulfan in fruits is therefore inevitable to assess its risk. Hence a detailed study was conducted on the dissipation behavior and its risk assessment of carbosulfan in brinjal fruits (*Solanum melongena* L.)

2. Materials and Methods
2.1 Field experiment
A replicated and randomized field experiment was conducted to evaluate the harvest time residues of carbosulfan at Urumandampalayam village, Vellakinaru, Coimbatore, on the brinjal variety CO-2, during the period of Jan - May, 2008, with ten treatments replicated four times and the plot size was $20m^2$. Three sprays of carbosulfan (Marshal 25 EC) @ 250, 500 and 1000 g a.i. ha^{-1} were given separately on the crop at an interval of 14 days. The first spraying was given at 10 days after transplanting using pneumatic knapsack sprayer with the

fluid rate was 500 l ha^{-1} and subsequent applications were made at 14 days interval. It was ensured that the insecticide under investigation had not been used earlier in the experimental plot.

2.2 Sampling
Brinjal fruits (500 g) were collected randomly from each treatment at 0 (1 hour after spraying), 1, 3, 7, 14 and 21days after spraying. The samples were brought from field to laboratory by placing in ice boxes to avoid deterioration of residues. The fruits were cut into small pieces, mixed and a subsample of 50 g was taken for residue analysis and analyzed immediately.

2.1.1 Extraction, clean up and estimation
For the analysis of carbosulfan, a representative sample of 50 g fruit was extracted with 100 ml of 9:1 methanol: pH 8 buffer (4.925 g of K_2HPO_4 + 0.465g of KH_2PO_4) in a Waring laboratory blender, then filtered with Büchner funnel and the extract was condensed in a rotary vacuum flash evaporator (BüchiRoto vapor R-14 ®). The condensed extract was transferred to a separating funnel, mixed with 250 ml of saturated sodium chloride solution and extracted thrice with 50 ml of dichloromethane. The dichloromethane extract was combined and evaporated to near dryness through rotary vacuum flash evaporator (BüchiRoto vapor R-14®). For column clean up, glass columns (50 x 1.5 cm I.D.) were used. The tip of the chromatographic column was plugged with cotton wool and filled with anhydrous sodium sulphate to a height of 2 cm followed by 8 cm of Florisil®: charcoal mixture (8 g of deactivated Florisil® + 1.5 g of acid washed charcoal) and overlaid with 2 cm layer of anhydrous sodium sulphate. The Florisil® was activated at 135 ºC for six hours in a hot air oven and cooled in a desiccator. The cooled Florisil® was deactivated with 4 per cent water. The deactivated Florisil® was used as fresh. The condensed dichloromethane extract was loaded onto the column and eluted with a mixture of 9:1 hexane and ethyl acetate. About 100 ml of eluent was collected and condensed to near dryness.

2.2 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[8]. 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 ºC 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.

2.3 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 ^{63}Ni 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 ^{0}C), detector (280 ^{0}C), oven temperature (235 ^{0}C), column temperature (300 ^{0}C), carrier gas (nitrogen) 20 ml min^{-1}. Under these operating parameters, the residue of carbosulfan was detected at 8.0th minute (retention time).

2.4 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. Ten gram of fruits was fortified with 0.1, 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.

2.5 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).

2.6 Data Analysis
The insecticide degradation pattern was analysed by applying seven transformation functions [9, 10]. The half-life was calculated using Pesticide Residue Half Life Calculator software developed by Department of Soil Science, Tamil Nadu Agricultural University, Coimbatore [11] and best fit degradation model was determined. The safe waiting period was worked out as per the formula given below [12].

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

3. 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 in brinjal fruits. 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, which is in accordance with earlier reports that the mean recovery value of carbosulfan was 91.00 and 97.30 per cent in potato plant [13]. The analyses of orange homogenates spiked with carbosulfan between 0.03 and 0.25 µg g^{-1} of carbosulfan exhibited recoveries between 66 and 98 per cent [14]. The standard chromatograms of carbosulfan at different levels of spiking are provided in Fig 3.
The initial deposit of carbosulfan at the recommended dose @ 250 g a.i. ha^{-1} is 11.29 µg g^{-1} at 1hour after spraying, which dissipated to 8.89 and 3.43 µg g^{-1} in 1st and 3rd day registering 21.25 and 69.61 percent dissipation, 0.92 to 0.05 in 7th and 14th day with 91.8 and 99.55 per cent dissipation and reached below detectable limit at 21st DAT (Table 2). At double the recommended dose @ 500 g a.i. ha^{-1}, the initial deposit of 19.75 mg kg^{-1} at 1hour after spraying dissipated to 13.2, 6.09, 1.09 and 0.121 µg g^{-1} with a dissipation percentage of 33.16, 69.16, 94.47 and 99.38 at 1, 3, 7, 14th DAT and reached below detectable on the 21st day. The largest dose of carbosulfan *ie.* 1000 g a.i. ha^{-1}, recorded an initial deposit of 34. µg g^{-1}, which dissipated to 21.57, 10.27, 2.96 and 0.27 mg kg^{-1} with a dissipation percentage of 36.68, 69.85, 91.31, 99.20 per cent and reached below detectable limit on 21st DAT recording

100 per cent dissipation (Fig 4). The carbosulfan residues extracted in brinjal fruits immediately after application was 11.29, 19.75 and 34.07 µg g^{-1} respectively for 250, 500 and 1000 g a.i.ha^{-1}. It was quite expected that higher levels of application resulted in higher amount of initial deposits on brinjal fruits. The quantity of carbosulfan after the expiry of 14 days was 0.05, 0.121 and 0.27 µg g-1 for the three doses of carbosulfan. The present study was in accordance with earlier reports that carbosulfan residues was detected till 7 DAS and the detected quantity of residue was above MRL up to 3 DAS in brinjal[15]. Similar findings were previously conveyed that the residues of carbosulfan persisted in brinjal fruits only between 3-5 days at the dosages of 187.5, 375.5 g a.i ha^{-1} [16]and that carbosulfan residues deteriorated beyond 3 DAT for the dosage of 1.5 mlÂ·L^{-1} rate and 10 DAT for 3.0 mlÂ·L^{-1} rate [17]. Reports beforehand revealed that traces of carbosulfan residues (0.01 µg g^{-1}) was detected in brinjal leaves after 20 days of application [18]. Such findings was also conveyed previously that the residues of betacyfluthrin in brinjal fruits declined progressively and became non detectable after seven days [19]. Carbofuran (a key metabolite of carbosulfan) when applied on brinjal crop by following single (1.0 kg a.i. ha^{-1}) and split application (2.0 kg a.i. ha^{-1}) resulted carbosulfan residues ranged from 58-60 ppb and 50-60 ppb at the first and third harvest respectively on brinjal fruits [20]. The residues of carbosulfan in roots and shoots of chickpea persisted beyond 90 days but residues only occurred in green seeds at trace to non-detectable levels [21].

The first harvest of brinjal fruits after spraying was on 14th day, where a very meager amount of 0.05, 0.121 and 0.27 mg kg $^{-1}$ of residues was detected, which is below the maximum permissible limit at the recommended dose of 250 g a.i. ha^{-1}. Thus the brinjal fruit harvested after spraying carbosulfan were found to be safe with no risks at the recommended dose. This is in accordance with previous findings who reported residues of carbosulfan detected for different doses *viz.*, 250, 500 and 1000 g a.i.ha^{-1} were below detectable limit in the brinjal fruits sampled for residues at first and third harvest [22]. The fruit samples from third harvest did not reveal its presence at the minimum detection limit of 0.002 mg kg^{-1}. The results indicated that carbosulfan residues detected in brinjal fruits both at the first and third harvest were well below the prescribed limit for the different doses applied *viz.*, 250, 500 and 1000 g a.i. ha^{-1}. In case of higher doses of carbosulfan (500, 1000 g a.i. ha^{-1}) though more amount of residues persisted in the fruits at first harvest, the residues dissipated quickly and reached to below detectable level at third harvest.

The degradation pattern of carbosulfan was fit into different models and the best-fit model was chosen based on the highest R^2 values. The modified regression equation (R^2) was calculated for arriving the best fit of the degradation of carbosulfan in brinjal fruits. Irrespective of the doses of carbosulfan, the degradation in brinjal fruits uniformly followed first order function and seemed to fit first order kinetics [16]. The carbosulfan degradation kinetics in brinjal fruits revealed that the intercept *ie.*, initial deposit (a) and the slope *ie.*, the degradation reaction rate (b) increased with the increased dose of carbosulfan treatment (Table 3 and 4). The half-life of carbosulfan increased with the increase in levels of carbosulfan. The carbosulfan half-life in brinjal fruits ranged from 1.78 to 2.03 days. The safe waiting period for harvest being 12.36, 14.19, and 16.89 days for doses of carbosulfan at 250, 500 and 1000 g a.i.ha^{-1} (Fig 5). Hence it may be recommended to harvest the brinjal fruits after allowing a waiting period of 15 days, which is in confirmation with the safe waiting period of 12-30 days reported earlier [22, 23]. This results are also in accordance with earlier workers [24] reporting the safe waiting period for harvest of carbosulfan at 250, 500 and 1000 g a.i.ha^{-1} were 12.36, 14.19 and 16.89 days respectively, which was readily in agreement with previous workers [25]. Such an observation was earlier reported that the safe waiting period for harvest of brinjal fruits when deltamethrin was applied @10-15 g a.i.ha^{-1} was 5 days and the initial deposits dissipated within 5 days of application [26]. This was also in accordance with the previous findings that the half-life of fluvalinate in brinjal fruits was 7 days [27].

Table 1: Recovery percentage of carbosulfan in brinjal fruits

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

Table 2: Degradation and persistence of carbosulfan in/on brinjal fruits

DAT	Carbosulfan @ 250 g a.i.ha^{-1}		Carbosulfan @ 500 g a.i.ha^{-1}		Carbosulfan @ 1000 g a.i.ha^{-1}	
	Mean ± SD	%D	Mean ± SD	%D	Mean ± SD	%D
0	11.29±1.413	ND	19.75±2.472	ND	34.07±4.265	ND
1	8.89±1.417	21.25	13.20±2.105	33.16	21.57±3.439	36.68
3	3.43±0.354	69.61	6.09±0.609	69.16	10.27±1.060	69.85
7	0.92±0.134	91.8	1.091±0.159	94.47	2.96±0.430	91.31
14	0.05±0.004	99.55	0.121±0.009	99.38	0.27±0.021	99.20
21	BDL	100.00	BDL	100.00	BDL	100.00

DAT - Days after treatment; R - Residues (µg g^{-1});% D - Percentage degradation; ND – Not Detected, BDL – Below Detectable Level

Table 3: Dissipation pattern of carbosulfan in brinjal fruits with statistical parameters

Treatments	a	UCL	LCL	b	UCL	LCL	$T_{(0.5)}$	UCL	LCL	Prediction equation
Carbosulfan 25 EC @ 250 g a.i.ha^{-1}	7.098	7.342	6.855	-0.388	-0.354	-0.422	1.786	1.943	1.629	Y=7.09-0.388X
Carbosulfan 25 EC @ 500 g a.i.ha^{-1}	7.508	7.843	7.172	-0.366	-0.319	-0.413	1.889	2.131	1.647	Y=7.508-0.366X
Carbosulfan 25 EC @ 1000 g a.i.ha^{-1}	8.046	8.198	7.893	-0.340	-0.318	-0.361	2.038	2.166	1.91	Y=8.04-0.340X

a - Intercept; b - Slope; $T_{(0.5)}$ - Half-life ; UCL-Upper Confidence Limit; LCL-Lower Confidence Limit; T Half- Half life

Table 4: Correlation coefficient for carbosulfan in brinjal fruits by different methods of transformation of residue data with statistical parameters

Function	Carbosulfan 25 EC @ 250 g a.i.ha^{-1}		Carbosulfan 25 EC @ 500 g a.i.ha^{-1}		Carbosulfan 25 EC @ 1000 g a.i.ha^{-1}	
	r	r^2	r	r^2	r	r^2
First order	0.997	0.987	0.995	0.990	0.998	0.987
1.5th order	0.898	-25.66	0.945	-0.10.65	0.927	-7.636
2nd order	0.814	-1.427	0.852	-1.497	0.837	-1.677
RF First order	0.814	-1.427	0.932	-0.203	0.926	0.087
RF 1.5th order	0.693	0.075	0.759	0.581	0.736	0.745
RF 2nd order	0.588	-0.932	0.629	-0.926	0.615	-0.945
Inverse PL	0.874	0.984	0.910	0.962	0.878	0.963

RF- Root Function; Inverse PL- Inverse Power Law

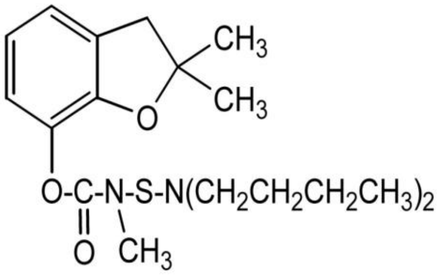

Fig 1: Structural formula of carbosulfan

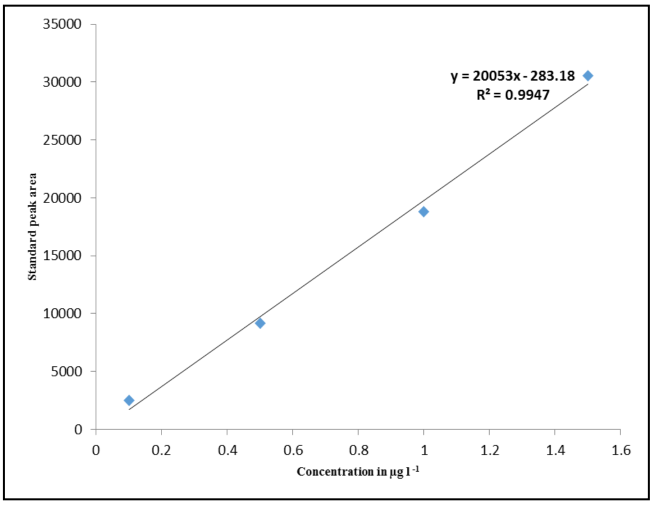

Fig 2: Linearity calibration curve of carbosulfan in brinjal fruits

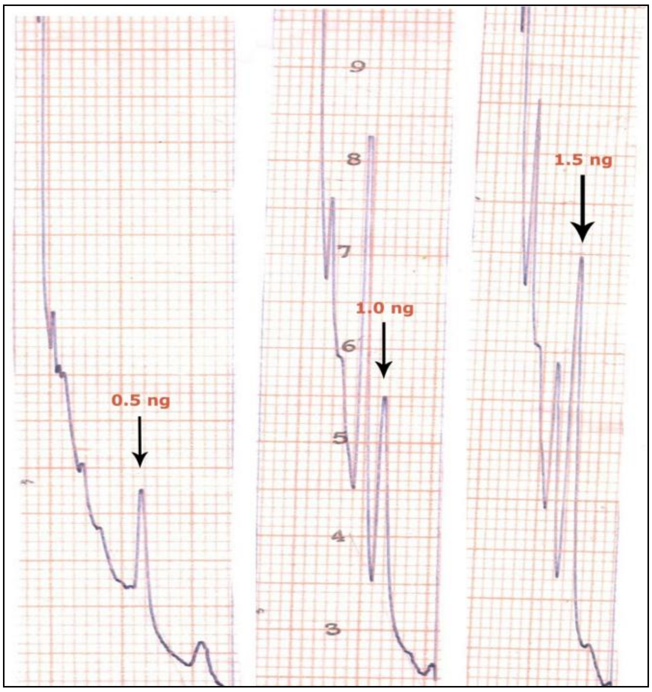

Fig 3: Standard chromatograms of carbosulfan

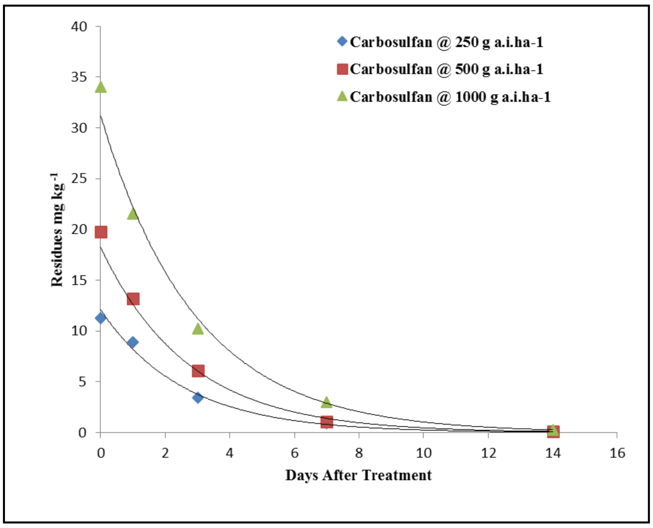

Fig 4: Dissipation of carbosulfan residues in brinjal fruits

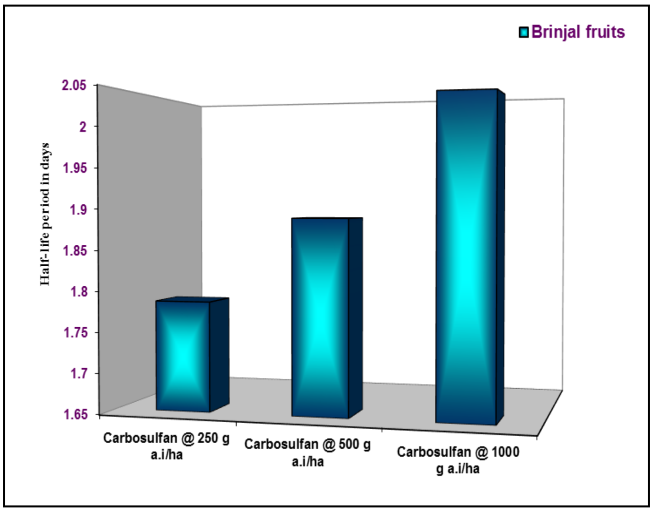

Fig 5: Half-life period of carbosulfan in different doses

4. Conclusion

The present study provided adequate information to the farmers for safe harvesting period of the brinjal sprayed with carbosulfan under agroclimatic conditions. The results indicated that carbosulfan residues detected in brinjal fruits both at the first and third harvest were well below the prescribed limit for the different doses applied *viz.*, 250, 500 and 1000 g a.i. ha^{-1} and hence the carbamate chemical, carbosulfan is well recommended for managing the brinjal pests without any harm to the environment.

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