



Determination of Carbofuran Residue in the Samples of Sugarcane (*Sacharum officinarum* L) and Soil of Sugarcane Field

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Abstract

An experiment was carried out at the Regional Sugarcane Research Station, Gazipur in which carbofuran (2 kg AI/ha) was applied in sugarcane field to document the level of carbofuran residue left in soil and plant samples after different days of application (DAA). Plant and soil samples were analyzed by using GCMS-EI. Carbofuran residues were found even at 90 DAA both in soil and plant. In case of soil, the amount of carbofuran residues were 24.84, 3.32, 2.12, 0.59, 0.035, 0.02 and 0.005 ppm at 0, 3, 7, 15, 30, 60 and 90 DAA, respectively. In case of plant samples, the lower residue (0.0035 ppm) was observed at 0 DAA compared to those at 3 DAA (0.075 ppm), 7 DAA (0.035 ppm) and 15 DAA (0.015 ppm). At 60 and 90 DAA, residues were the same (0.002 ppm) while at 30 DAA it was 0.0025 ppm. The highest level of carbofuran residue (0.075 ppm) in plant samples was found at 3 DAA which is lower than FAO/WHO recommended MRL (0.1mg/kg crop).

Keywords: Sugarcane, carbofuran, MRL

1. Introduction

Carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl N-methylcarbamate) was developed by FMC as contact and systemic insecticide in 1969, which is also effective as acaricide and nematicide. Carbofuran has a high acute oral and low dermal toxicity. It is reported to be the most effective insecticide in controlling the major rice pests particularly brown planthopper (*Nilaparvata lugens* Stal) when broadcast to the flooded rice fields as granules or when incorporated to the root zone in paper or gelatin capsules (IRRI, 1975).

In Bangladesh, more than 37,712.20 tons of formulated products of pesticide was sold during

2007 of which 14,849.56 tons were carbofuran (Anon, 2007). Carbofuran is generally used for controlling the insect pests of different crops like rice, jute, sugarcane etc. The application rate of carbofuran is 40 kg/ha in sugarcane (Anon, 1996). Side-effect of carbofuran applications was observed in frogs and birds. Some birds that consumed spilled granules died immediately. Carbofuran applications, as practiced in the region, have cause dramatic effects on many non target organisms. Its use is hazardous to wildlife, and it severely reduces the significance of irrigated rice fields as a feeding habitat for birds, including waders, and other insectivorous organisms (Mullie *et al.*, 2005).

Carbofuran is rapidly taken up by plants through the roots from soil, water or nutrient solution and is translocated mainly into the leaves (Ashworth and Sheets, 1972). The rapidity of carbofuran degradation differed in the various plant species and the main metabolite was identified as 3-hydroxycarbofuran (Talekar *et al.*, 1977a). There are several studies on carbofuran degradation after application. Various factors like temperature, water content, pH etc influence its degradation (Stanovick, 1968; Getzin, 1973; Caro *et al.*, 1973 and Talekar *et al.*, 1977b). Talekar *et al.* (1977b) noted that in subtropical soils, carbofuran was more persistent during the dry and relatively cooler autumn and winter months than in summer. After ten months, only 0.6% of the residues present initially could be recovered as parent compound.

Presently, the farmers are totally dependent on the use of insecticides to control insect pests. All pesticides compounds potentially pose environmental hazards as they are chemically tailored to be toxic. While causing lethal effect to target pests, these chemicals may also evoke acute and chronic toxic effect to non – target organisms. An intensified use of insecticides can cause a serious public health hazard especially in the form of residues in food (Mansingh. *et al.*, 1996). MacIntyre *et al.* (1989) reported that low level exposure of food products containing insecticide residues over time might cause cancer, teratogenesis, genetic damage and suppression of the immune system. Though it is not documented, it is apprehended that pesticide residue in soil could pose serious threat to soil microbes. The present investigation therefore, conducted to understand the persistent nature of carbofuran both in soil and sugarcane plant. Efforts have also been made to determine plant uptake of the aged residues of carbofuran and to assess the amount and degradation rate of residue of carbofuran in soil.

2. Materials and Methods

Carbofuran (Furadan 5G) was applied @ 2 kg/ha in a replicated field trial of sugarcane conducted at the Regional Sugarcane Research Station (RSRS) of BSRI, Gazipur during December

2006 to December 2007. The purity of Furadan 5G was tested at the pesticide analytical laboratory of BARI and was found to be 100% pure. Soil (500g) and sugarcane samples (500g) from replicated plots were collected at 0, 3, 7, 15, 30, 60 and 90 days after application (DAA). The standard of carbofuran was obtained from Sigma-Aldrich Laborchemikalien, GmbH P.O. Box-100262, D-30918, Seelze, Germany via Bangladesh Scientific Pvt. Ltd., Dhaka, Bangladesh. The purity of this standard was above 99.6 percent.

2.1. Extraction, separation and clean-up of carbofuran

The methodology prescribed by William and George (2005) with necessary modification was adopted for extraction, separation and clean-up of the sample.

Field collected sugarcane and soil samples (≥ 250 g) were taken for extraction. Sugarcane sample was grounded thoroughly with the meat grinder (Handmixer M-122, Bamix, Switzerland) but for soil this was not needed. For both, a sub sample of 20 g was taken into a wide mouth jar. Then 100 ml of methanol was added to it. Sodium sulphate (Na_2SO_4) was also added with the sample until water was removed from the sample. The mixture was then macerated with high-speed homogenizer (Ultraturax, IKA T18 basic, Germany) for 2 minutes. The homogenized material was then poured into 250 ml conical flask and placed into shaker (Orbital Shaking Incubator, Rexmed, Sweden) for 2-3 hours continuous shaking. After shaking, the slurry was filtered through a Buchner funnel with suction. The flask and filter cakes were rinsed with 8-10 ml of methanol each.

The filtrate was then transferred into 250 ml round bottom flask and was dried to 5-7 ml by evaporation using a rotary vacuum evaporator (Laborota-4001, Heidolph, Germany). The concentrated filtrate was then transferred into 500 ml separatory funnel making 10 ml volume with methanol. Around 20 ml hexane was added with 10 ml filtrate to the separatory funnel and

was shaken vigorously for 3-5 minutes and eluted into vial after keeping undisturbed for 3-5 minutes. The eluate was then centrifuged at 1200 rpm for 5 minutes (Laboratory Centrifuges, Sigma-3K30, Germany). After centrifuge, the supernatant was collected for injection.

2.2. Detection and quantification of carbofuran residue in samples

The residue was measured by GC-MS QP2010 (Shimadzu) with EI mode. The capillary column condition was AT-1, length 30m, ID 0.25mm and film thickness 0.25 μ m. Helium was used as carrier and make up gas. The parameters set for GC and MS are given below:

[GC-2010]:

Column Oven Temp. - 50.0°C, Injection Temp. - 250.00°C, Injection Mode- Splitless
Flow Control Mode - Linear Velocity.

Oven Temp. Program:

Rate	Temperature (°C)	Hold Time (min)
-	50.0	1.00
	20.0 140.0	0.00
	10.0 220.0	0.00

[MS-QP2010]:

Ion Source Temp. - 200.00°C, Interface Temp. - 280.00°C, Solvent Cut Time - 5.00 min, Ionization Mode- EI, Start Time- 5.00 min, End Time - 13.50 min, ACQ Mode- SIM
Ch1-m/z - 164.00

A standard calibration curve was obtained by injecting different concentrations of standard carbofuran solution. The injected volume of supernatant was 1 μ l. Each peak was characterized by its retention time. Sample results were quantitated in ppm automatically by the GC software, which represented the concentration of the final volume injected and from this, concentration in the sample was obtained by back calculation. The sensitivity of the equipment for carbofuran detection was 1 ppb as the limit of detection (LOD).

3. Results and Discussion

The results of the analysis of carbofuran in soil samples have been summarized in Table 1. The results revealed that significant amount of carbofuran residue was found in soil sample even at 90 DAA. The quantities of residue were 24.84, 3.33, 2.12 and 0.59 ppm at 0, 3, 7 and 15 DAA, respectively. While soil samples of 30, 60 and 90 DAA contained 0.035, 0.02 and 0.005 ppm, respectively.

Table 1. Quantity of carbofuran residue estimated from soil samples.

Days after application (DAA)	Application rate (kg/ha)	Sample weight (g)	Total volume prepared (ml)	Injected volume (μ l)	Conc. obtained in final volume (ppm)	Residue of carbofuran in soil (ppm)
0	2	20	10	2	49.69	24.84
3	2	20	10	2	6.63	3.32
7	2	20	10	2	4.24	2.12
15	2	20	10	2	1.18	0.59
30	2	20	10	2	0.07	0.035
60	2	20	10	2	0.04	0.02
90	2	20	10	2	0.01	0.005

Table 2: Quantity of carbofuran residue estimated from sugarcane samples

Days after application (DAA)	Application rate (kg/ha)	Sample weight (g)	Total volume prepared (ml)	Injected volume (μ l)	Conc. obtained in final volume (ppm)	Residue of carbofuran in sugarcane (ppm)
0	2	20	10	2	0.007	0.0035
3	2	20	10	2	0.15	0.075
7	2	20	10	2	0.07	0.035
15	2	20	10	2	0.03	0.015
30	2	20	10	2	0.005	0.0025
60	2	20	10	2	0.004	0.002
90	2	20	10	2	0.004	0.002

This result agrees with the observation of Stanovick (1968) who observed 20 to 35% of carbofuran remaining intact after 40 days of flooding, except in a relatively acid soil (p^H 4.2) from which 77% unchanged parent compound was recovered. The results of the analysis of carbofuran in sugarcane sample have been summarized in Table 2.

FAO/WHO Codex Alimentarius Commission Recommended

MRL of carbofuran : 0.1 mg/kg crop

ADI of carbofuran : 0.01mg/kg body weight (Anon. 1993)

Table 2 shows that carbofuran residue was found in the sugarcane plant sample even at 90 DAA. The amount of carbofuran residue found in plant samples were 0.0035, 0.075, 0.035, 0.015, 0.0025, 0.002 and 0.002 ppm at 0, 3, 7, 15, 30, 60 and 90 DAA, respectively. None of these amounts was above the recommended MRL for carbofuran in crop. This result agrees with the observation of Battu *et al.* (2000). They observed that carbofuran-derived residues were not detected in cane juice. Moreover, FAO/WHO (1979) also reported that the 26 samples of barley, sugarbeet, maize, onion, rape, carrot which were treated with carbofuran at sowing but did not find any detectable residues in any of the samples. Similar result was reported by Anon. (1977), who collected egg plants sample from three locations of Mexico to analyse for

carbofuran and 3-hydroxy-carbofuran. Sampling was done after 7 and 14 days of treatment. In both sampling occasions no residue was detected.

In the present study, the carbofuran residue in plant sample at 0 DAA was 0.0035 ppm, which is much lower than those obtained from samples of 3 and 15 DAA (0.075 and 0.015 ppm). This might be attributed to the reason that carbofuran needed time to be adequately up taken by the plant after its application. Deuel (1975) reported that carbofuran required 96 hours to be up taken by plants. Two supervised trials were carried out in South Africa in which 3 kg ai/ha (registered dosage) and 4.5 kg ai/ha were applied. The residue in sugarcane was lower than limit of determination (0.2 mg/kg) in both experiments (Anon. 1979).

3.1. Trend of degradation

The trends of degradation of carbofuran residue in soil and plant are shown in Figure 1. Figure 1 shows that carbofuran residue in soil degraded steadily up to 15 DAA (86.63-97.62%) but later the rate of degradation was negligible. In case of plant samples, degradation was found to be 53.33% at 7 DAA, which went up to 80% at 15 DAA. From 30 to 90 DAA, the residue degradation ranged from 96.00 to 97.33%. Lack of available information related to the carbofuran degradation limits the results to be discussed.

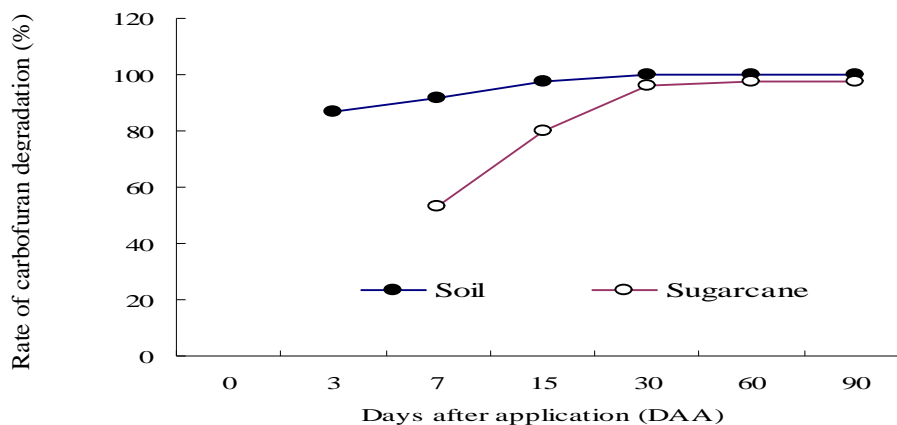


Fig. 1. The trend of carbofuran degradation in soil and sugarcane

4. Conclusions

Protocols for the determination of carbofuran residue from soil and plant samples have been developed. Carbofuran residues were found to be present in soil and plant sample up to 90 DAA. As higher amount of carbofuran residue remained in the soil up to 30 DAA, the future research should be conducted on its impact on soil microbes.

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