little, if any, ETU on edible crops (<0.05 ppm).

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Persistence and Biodegradation of Carbofuran in Flooded Soils

K. Venkateswarlu, T. K. Siddarame Gowda, and N. Sethunathan*

The persistence of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) in four soils was studied in the laboratory with special reference to flooded conditions. After thin-layer chromatographic separation of residues, carbofuran in the soil samples was converted to its phenol by alkaline hydrolysis and then assayed colorimetrically following diazotization. More rapid degradation of carbofuran occurred in soils under flooded conditions than under nonflooded conditions. Carbofuran degraded rapidly between 20 and 40 days after flooding in most soils including an acid sulfate saline soil, Pokkali, capable of attaining near neutral pH upon flooding; but the insecticide persisted in another acid sulfate saline soil, Kari, perhaps due to its exceedingly low pH of 4.2, even after several weeks of flooding. Heat treatment of soils prior to incubation increased the persistence of carbofuran under flooded conditions. Moreover, a bacterium, isolated from flooded soil by an enrichment technique, decomposed carbofuran in a mineral salts medium. These studies indicate that microorganisms are involved in the degradation of carbofuran in flooded soils.

Brown planthoppers (*Nilaparvata lugens* Stal) cause direct damage to the rice crop by sucking the plant sap leading to a symptom known as "hopperburn" (PANS, 1970). In addition, it acts as a vector of grassy stunt virus disease. Recently, buildup of brown planthoppers particularly in areas where intensive cropping of rice is practiced has caused concern in India (Kulshreshtha et al., 1974) and in several southeast Asian countries.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) appeared to be the most effective insecticide in controlling rice brown planthoppers when broadcast in the flooded rice field soils as granules or when incorporated to the root zone in paper or gelatin capsules (IRRI, 1975). Although carbofuran has received attention in recent years because of its broad spectrum insecticidal and nematicidal properties, its fate in flooded soil ecosystem is little understood. This paper reports the relative persistence of carbofuran in Indian rice soils under flooded and nonflooded conditions and the role of biodegradation in its loss from flooded soils. The soils included two unique acid sulfate saline soils from coastal areas of Kerala, South India where carbofuran is used extensively to control the brown planthoppers in rice.

Laboratory of Soil Microbiology, Central Rice Research Institute, Cuttack-753006, Orissa, India.

MATERIALS AND METHODS

Persistence of Carbofuran in Different Soils under Flooded Conditions. The persistence of carbofuran was studied in four rice soils under flooded conditions. The soils included two unique acid sulfate saline soils from Kerala locally known as Kari and Pokkali. Some pertinent properties of the soils are listed in Table I. The soils were air-dried and ground to pass through 2-mm sieves.

Twenty grams of each soil contained in test tubes (25 × 200 mm) were treated with 1 mg of technical grade carbofuran (99.5% purity, FMC Corporation, Middleport, N.Y.) in 0.1 mL of methanol. After 24 h to allow evaporation of methanol, the soils were flooded with 25 mL of distilled water and incubated for 40 days. Carbofuran residues in two replicate soil samples were estimated at 20-day intervals.

Table I. Characteristics of the Soils Used

Characteristics	Soils			
	Alluvial	Laterite	Acid sulfate, saline (Pokkali)[a]	Acid sulfate saline (Kari)[a]
Location	Central Rice Research Institute, Cuttack-6, Orissa	Pattambi, Kerala	Tellichery, Kerala	Vechoor, Kerala
pH[b]	6.2	5.0	4.2	3.0
Electrical conductivity[c]	0.6	0.2	8.5	15.0
Organic matter (%)	1.61	3.25	8.21	26.41
Total nitrogen (%)	0.09	0.09	0.24	0.36

[a] These unique acid sulfate saline rice soils from Kerala are known by the local names Pokkali and Kari. [b] 1:1.25 soil-water slurry. [c] Electrical conductivity (mmhos/cm) of saturation extract of the soil.

Persistence in Flooded and Nonflooded Soils. The relative persistence of carbofuran in the alluvial soil was studied under flooded and nonflooded conditions. Carbofuran (1 mg) was added to 20-g soils in 0.1 mL of methanol. After 24 h, soils in one set of tubes were flooded with 25 mL of distilled water while the soils in another set were moistened with 2 mL of water to maintain a 50% water holding capacity for nonflooded conditions. Periodically, carbofuran in two replicate samples was estimated after solvent extraction. Prior to solvent extraction, the nonflooded soil sample in each tube was flooded with 23 mL of distilled water to provide the same soil–water ratio as in flooded soils.

Persistence in Autoclaved and Nonautoclaved Soils. The microbial role in carbofuran loss from flooded soils was determined by comparing the relative rate of its degradation in autoclaved and nonautoclaved soil samples. Three soils, viz., alluvial, laterite, and Pokkali, were chosen. Twenty-gram portions of each soil spread as a thin layer were moistened and then autoclaved three times at 121 °C for 1 h at 1-day intervals. Technical grade carbofuran (1 mg) was introduced in 0.1 mL of methanol to autoclaved and nonautoclaved soils. The soils were flooded with 25 mL of sterile distilled water 24 h after addition of the insecticide. The residues in two replicate soil samples were assayed at 20-day intervals.

Bacterial Degradation of Carbofuran. To confirm the role of microorganisms in the degradation of carbofuran in flooded soil, an enrichment culture was prepared by adding 1 mg of the technical grade insecticide to 20-g soils under flooded conditions at 15-day intervals. After five additions of carbofuran, one loopful of the soil suspension was transferred to a carbon- and nitrogen-free sterile mineral salts medium (K_2HPO_4, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $FeSO_4 \cdot 7H_2O$, 0.001 g; distilled water, 1 L) supplemented with an aqueous solution of carbofuran, previously passed through a Millipore filter (pore size, 0.45 μm), to provide a concentration of 20 μg/mL of medium. Within 10 days after transfer, the mineral solution turned turbid, indicating bacterial proliferation utilizing carbofuran as the energy source. One loopful of this bacterial suspension was streaked on to a mineral agar medium supplemented with carbofuran. A bacterium, capable of growing with carbofuran as its energy source, was isolated by this method. The ability of this bacterium to degrade carbofuran as sole carbon source was tested as follows. An aqueous solution of Millipore-filtered carbofuran (360 μg) was introduced under aseptic conditions to 20-mL portions of sterile mineral salts solution contained in 50-mL Erlenmeyer flasks. The media were then inoculated with one loopful of the bacterial suspension in sterile distilled water. Uninoculated media served as controls. After specified periods of incubation at 30 °C in a BOD incubator, the residues from the medium were extracted three times with 30-mL portions of chloroform–diethyl ether (1:1) mixture. The solvent was evaporated at room temperature, and the residues, redissolved in 2 mL of methanol, were analyzed after separation by TLC.

Extraction of Carbofuran from Soils. For extraction of residues from soils, the contents of each tube were transferred to 250-mL Erlenmeyer flasks with 50-mL portions of chloroform–diethyl ether (1:1) and then stirred in a wrist action shaker for 30 min. After centrifugation at 7000 rpm for 10 min, the supernatant was transferred to separatory funnels and the lower fraction was collected in a beaker. The residues in the soil and the water portion remaining after solvent extraction were extracted two times with 40-mL portions of chloroform–diethyl ether. The solvent fractions were pooled, and after evaporation of the solvent, the residues were redissolved in 2 mL of methanol for analysis after separation by TLC.

Thin-Layer Chromatography (TLC). The residues dissolved in methanol were spotted along with carbofuran standard on chromatoplate coated with silica gel G, 300 μm thick. The plates were developed with ether–hexane (3:1) for a distance of 15 cm and air-dried. The authentic compound was located by spraying the chromatoplate with 1% potassium permanganate (Dorough, 1968).

Colorimetry. The method used for estimation of carbofuran was essentially the same as described by Mithyantha and Perur (1974) with some modifications and involved alkaline hydrolysis of carbofuran to its phenol, followed by diazotization. After separation of carbofuran residues in the samples by TLC, the silica gel areas of the samples corresponding to authentic carbofuran were scraped off carefully and transferred to a test tube. Carbofuran in the silica gel was allowed to react with 1.25 mL of 0.3% sodium nitrite solution, 1.25 mL of 0.2% sulfanilic acid in 1 N HCl, and 2.5 mL of 4 N NaOH in a boiling water bath for 20 min. Silica gel was removed by centrifugation, and the supernatant was made up to 10 mL prior to colorimetric analysis in a Spectronic 20 (Bausch & Lomb) at 490 nm.

RESULTS AND DISCUSSION

Recovery of Carbofuran from Soils. The standard curve for carbofuran, when eluted from the chromatoplates and analyzed colorimetrically after alkaline hydrolysis and diazotization, was linear over a range of 2 to 15 mg/L concentration and formed the basis in calculating the amount of the insecticide in the soil samples. Carbofuran applied to the soils was extracted with chloroform–diethyl ether and then analyzed colorimetrically after separation by TLC and subsequent conversion to its phenol. With the complex extraction and analytical procedures used, the recovery of carbofuran immediately after its application to different soils ranged from 56.5 to 73.5%. Recovery for different soils followed the order, alluvial > laterite > Kari = Pokkali. The slightly lower recoveries of carbofuran from organic matter rich soils Kari and Pokkali may, at

Table II. Persistence of Carbofuran in Different Soils under Flooded Conditions

Incubation, days	μg of carbofuran recovered/g of soil[a]			
	Alluvial	Laterite	Pokkali	Kari
0	735	643	565	570
20	410	493	420	540
40	148	202	200	440

[a] Mean of two replicates. Carbofuran added to 20 g of soil, 1 mg.

best, be attributed to its greater adsorption to complex organic matter in these soils. Although sorption behavior of carbofuran in these soils is not known, studies with parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate) and lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane) tend to show almost irreversible nature of sorption of these insecticides in Kari and Pokkali soils (Wahid, 1976). Also, according to earlier reports (Siddaramappa and Sethunathan, 1975; Gowda and Sethunathan, 1976), initial recoveries of γ- and β-hexachlorocyclohexane and endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*endo*-5,8-dimethanonaphthalene) were relatively low in Kari and Pokkali soils.

Persistence in Flooded Soils. The data on the persistence of carbofuran in four soils under flooded conditions are given in Table II. The degradation of carbofuran in soils was relatively slow until 20 days after flooding; but between 20 and 40 days of flooding, appreciable degradation occurred except in Kari soil. Only 20 to 35% of carbofuran remained intact after 40 days of flooding in alluvial, laterite and Pokkali soils as compared with exceedingly high recovery of over 77% from Kari soil during the same period. The persistence of carbofuran in soils followed the order: Kari > Pokkali > laterite > alluvial. Likewise, γ- and β-hexachlorocyclohexane (Siddaramappa and Sethunathan, 1975) and endrin (Gowda and Sethunathan, 1976) showed extremely long persistence in Kari soil despite favorable conditions of flooding in contrast to the reported instability of these chlorinated hydrocarbon insecticides in predominantly anaerobic flooded conditions (Sethunathan, 1973). More rapid degradation of carbofuran in Pokkali soil than in Kari soil, despite several common characteristics, could be largely ascribed to the remarkable differences in the pH attained by these soils following flooding. Both Kari and Pokkali soils are acidic at the time of flooding; but, the pH of Pokkali soil increases, as in most acid soils (Ponnamperuma, 1972), and reaches near neutrality within 2 weeks after flooding, whereas Kari soil is characterized by highly acidic conditions with a pH rise from 3.0 to 4.2 even after 40 days of flooding. Presumably, extreme acid conditions in Kari soil seem to be unfavorable for carbofuran degradation since, according to an earlier report (Caro et al., 1973), persistence of carbofuran in nonflooded soils was increased by more acid conditions. In yet another study, Getzin (1973) found that carbofuran degraded seven to ten times faster in an alkaline soil (pH 7.9) than in acid or neutral soils (pH 4.3 to 6.8).

The data on the overall persistence of carbofuran in the alluvial soil are in close agreement with bioassay results obtained in the Entomology Division of our institute employing green leafhoppers (Kalode et al., 1970) and brown planthoppers (Ramamohan-Rao, 1976). When green leafhoppers and brown planthoppers were allowed to feed on rice plants for different periods after application of carbofuran to the alluvial soil under flooded conditions, mortality of 100% for both insects occurred until 20 to 30 days. Its insecticidal efficiency declined by the end of 40 days, presumably as a result of a rapid decrease in carbofuran residues in the soil as revealed by chemical analysis in the present study. Moreover, studies at International Rice Research Institute (IRRI, 1975) revealed that carbofuran concentration in rice roots ranged from 0.230 to 0.277 mg/kg fresh weight at 5, 10, and 20 days after application of 3% granules at 2 kg of active ingredient/ha to the soil; but the concentration dropped to 0.058 mg/kg at the end of 40 days. Similarly, carbofuran residues in leaf blade and stem declined to low levels between 20 and 40 days. Rapid degradation of carbofuran from flooded soils between 20 and 40 days would, perhaps, explain relatively low carbofuran residues in plant tissues during this period.

Table III. Carbofuran Recovered from an Alluvial Soil under Flooded and Nonflooded Conditions

Incubation, days	μg of carbofuran recovered/20 g of soil[a]	
	Flooded	Nonflooded
0	625	625
20	475	565
40	155	464

[a] Mean of two replicates. Carbofuran added to 20 g of soil, 1 mg.

Table IV. Carbofuran Recovered from Autoclaved (A) and Nonautoclaved (NA) Soils under Flooded Conditions

Incubation, days	μg of carbofuran recovered/20 g of soil[a]					
	Alluvial		Pokkali		Laterite	
	NA	A	NA	A	NA	A
0	683	663	605	645	580	648
20	453	550	473	620	490	633
40	170	490	228	530	215	470

[a] Mean of two replicates. Carbofuran added to 20 g of soil, 1 mg.

The recent intensified use of carbofuran in areas comprised of Kari soil to control the increasing population of brown planthoppers should be viewed with caution because of its extreme stability in this soil. Carbofuran is relatively more water soluble than several organochlorine and organophosphate insecticides. Its water solubility and a lag of about 20 days in its degradation in most soils also raise the problem of environmental contamination through residues in surface runoff waters from flooded rice fields, particularly in areas comprised of Kari and related highly acidic soils.

More rapid degradation of carbofuran occurred in soils under flooded conditions than under nonflooded conditions (Table III). More than 75% of carbofuran was decomposed in flooded soils at the end of 40 days as compared with 26% loss in nonflooded soils. Similarly, certain organophosphorus and organochlorine insecticides are known to decompose more rapidly under flooded conditions than under nonflooded conditions, attributed largely to the participation of anaerobic (facultative and/or obligate) microorganisms in flooded soils (Sethunathan, 1973).

Microbial Degradation. The role of microorganisms in carbofuran loss from Pokkali, alluvial, and laterite soils under flooded conditions was studied by comparing its relative stability in autoclaved and nonautoclaved soil samples. These soils were chosen because of their capacity to degrade carbofuran (Table II). In all three soils, autoclaving of the soils prior to the insecticide incorporation increased the persistence of carbofuran to a great extent, indicating microbial participation in its degradation (Table

Table V. Degradation of Carbofuran by a Bacterium Isolated from Flooded Alluvial Soil

Incubation, days	μg of carbofuran recovered/20 mL of incubation medium[a]	
	Uninoculated	Inoculated
0	320	320
10	295	250
20	278	150
40	223	trace

[a] Mean of two replicates. Carbofuran added to 20 mL of medium, 360 μg.

IV). During a 40-day incubation period, about 62 to 75% carbofuran was decomposed in nonautoclaved soils as compared with only 18 to 27% loss in autoclaved soils. The slow rate of degradation until 20 days followed by more rapid loss between 20 and 40 days in nonautoclaved soils is indicative of microbial involvement in carbofuran degradation.

More direct evidence for microbial role in carbofuran degradation in flooded soil was provided when a bacterium, isolated from a flooded soil by enrichment culture technique, degraded carbofuran under static conditions (Table V). At the end of 40 days after inoculation with the bacterium, carbofuran residues in 20 mL of incubation medium decreased from 320 μg to negligible levels; in uninoculated media, during the same period, no appreciable degradation of the insecticide occurred. Carbofuran was supplied to the bacterium as sole source of carbon and nitrogen.

In a recent study (Getzin, 1973), liberation of $^{14}CO_2$ from ring labeled [^{14}C]carbofuran in a nonflooded soil system indicated microbial degradation since microorganisms are implicated in ring cleavage of organic molecules. Similarly, in nonflooded soils, more rapid mineralization of ^{14}C-carbonyl-labeled carbofuran to $^{14}CO_2$ occurred in nonsterile conditions (Williams et al., 1976). Among the microorganisms isolated from carbofuran-amended soils, actinomycetes were particularly active in converting carbofuran to CO_2. The data on the increased persistence of carbofuran in sterile soils (Table IV) and its degradation by a bacterium isolated from flooded soils (Table V) lead to the conclusion that, as in nonflooded soils, microorganisms participate in the rapid loss of carbofuran in flooded soils.

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Fate of Carbofuran and Its Metabolites on Strawberries in the Environment

Thomas E. Archer,* James D. Stokes, and Royce S. Bringhurst

Three varieties of strawberries, Day-Neutral, Tioga, and Tufts, were sprayed once with carbofuran (Furadan 4-Flowable) at the rates of (4 and 8 oz of active ingredient)/acre during fruit maturation. The berries and leaves were sampled, and the residues of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) and its metabolites were determined. The combined residues on the berries never exceeded the tolerance level of 0.5 ppm nor did the carbamate fraction exceed 0.2 ppm 6 days after application. In some instances, the amounts of the metabolites 3,7-diol and 3-oxocarbofuran increased until 7 days after application but then decreased in amounts to harvest. Reduction of residues probably resulted from dilution by plant growth and volatilization from the plant tissue surfaces.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) has been registered for use as an insecticide or nematicide on various crops, alfalfa, field corn, peanuts, rice, sweet peppers, sugarcane, bananas, tobacco (Furadan, 1974), and more recently to control root weevils, *Brachyrhinus*, *Nemocestes*, *Perifelinus*, and *Sciopithes* spp. on strawberries. The registered formulation, Furadan 4-Flowable (64 oz of active ingredient (a.i.)/gal), is applied at 2.6 to 5.1 fluid oz per 1000 linear feet of row once in a 10- to 12-in. band over the rows, spaced 42 in. apart, after last harvest but before Oct 1. The established tolerance for carbofuran and its carbamic and phenolic metabolites

Departments of Environmental Toxicology and Pomology, University of California, Davis, California 95616.