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Influence of the insecticide carbofuran on the production and oxidation of methane in a flooded rice soil

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Abstract Applications of a commercial formulation of carbofuran, a carbamate insecticide, at rates of 2 kg and 12 kg active ingredient ha^{-1} to flooded fields planted to rice led to significant inhibition of methane emission. Likewise, laboratory incubation studies showed that carbofuran applied at low rates (5 and 10 μg g^{-1} soil) inhibited the net methane production relative to that of the control, but stimulated it when applied at a rate of 100 μg g^{-1} soil. Interestingly, carbofuran increased the oxidation of methane when applied at low rates and inhibited it when applied at a rate of 100 μg g^{-1} soil.

Key words Methane production · Methane oxidation · Methane emission · Rice soils · Carbofuran

Introduction

Global warming due to increasing concentrations of greenhouse gases such as carbon dioxide, methane and nitrous oxide in the atmosphere is of great concern, as they can trap heat and radiation (Houghton et al. 1995). Among the sources of methane due to anthropogenic activities, ecologically and economically important flooded rice fields are considered as major sources of atmospheric methane (Bouwman 1990). Available data suggest that as much as 80% of the methane produced in the anaerobic soil layers is oxidised in the oxic zones of a flooded soil (Holzapfel-Pschorn et al. 1985; Conrad and Rothfuss 1991). Researches worldwide in recent years have been focused on developing feasible technologies to reduce methane emissions from rice fields without adversely affecting rice yields (Neue et al. 1995). Various options that have been seriously considered as means of reducing methane emissions, mainly from irrigated rice culture, include intermittent flooding and drainage and enhancement of methane oxidation. Alternate flooding and drainage has the advantages of minimising soil reduction processes and maximising the potential level of methane oxidation. Soil amendments with inhibitors of nitrification and gypsum can retard microbial production of methane (Rennenberg et al. 1992).

Insecticides account for 75% of total pesticides used in Indian agriculture. There is evidence that carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate), a carbamate insecticide widely used in rice culture, effects a distinct stimulation of autotrophic ammonium oxidation in flooded rice soils (Ramakrishna et al. 1978; Ramakrishna and Sethunathan 1982). Several autotrophic ammonium oxidisers have been implicated in the oxidation of methane (Bedard and Knowles 1989). We studied the effect of carbofuran on methane production and methane oxidation in flooded rice soil.

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Materials and methods

Experiment A

Methane emission from rice fields treated with carbofuran

The effect of carbofuran on methane emissions, from flooded rice fields was examined. The experiment was conducted on the experimental farm of the Central Rice Research Institute, Cuttack (20 °N, 86 °E) during the wet season of 1995 under irrigated, continuously flooded conditions. Preparation of the field site and management practices were similar to those employed by Adhya et al. (1994). Rice plants (25-day-old seedlings of cv. IR 72) were transplanted at a spacing of 20×15 cm in four plots (5×2 m), separated by levees. Urea (80 kg N ha^{-1}) was applied at the line of transplanting and fields were continuously submerged to a depth of 10±2 cm during crop growth.

A commercial formulation of carbofuran (3% active ingredient, obtained from Nagarjuna Fertilizers, Hyderabad, India) was broadcast in two plots at a rate of 2 and 12 kg active ingredient ha^{-1}, respectively, 47 days after transplanting the rice plants (at the panicle initiation stage). Two plots not treated with carbofuran served as controls. Methane emissions from both the control and carbofuran-treated plots

were monitored at 4, 9, 14, 21, 26 and 30 days after application of carbofuran employing the closed chamber method as described by Adhya et al. (1994) and Parashar et al. (1996). Air samples were drawn into Tedlar sampling bags (AeroVironment, Monrovia) at fixed intervals, of 0, 15 and 30 min between 9.00 and 9.30 a.m., and in the afternoon at the same intervals between 3.00 and 3.30 p.m., and their methane content was analysed by gas chromatography. The average of the morning and evening fluxes, calculated from duplicate measurements made for each treatment at each sampling period, was considered as the flux value for that day.

Experiment B

Methane production in flooded soil

An alluvial soil, a Typic Haplaquept (deltaic alluvium), with a sandy, clay loam texture (25.9% clay, 21.6% silt, 52.5% sand; pH 6.2, cation exchange capacity 15 mEq 100 g^{-1}, total C 0.76%, total N 0.09%, electrical conductivity 0.6 dS m^{-1}) from the experimental farm of the Central Rice Research Institute, Cuttack was used for the laboratory incubation studies. Air-dried soil samples (5 g) were placed in 15 ml vacutainer tubes (Becton Dickinson, Rutherford, N.J.) and mixed with water at a 1:1.25 soil:water ratio to provide flooded conditions. Carbofuran was applied to the soil at rates of 0, 5, 10 and 100 μg g^{-1} soil. At periodic intervals after incubation at room temperature (28±2 °C), air sample was withdrawn from the head space of the tubes, after the contents had been thoroughly mixed by vortexing for 1 min to release soil-trapped methane, and was analysed for methane using a Varian 3600 gas chromatograph equipped with a flame ionization detector and a molecular sieve (5 Å) column (2 m×0.3 cm stainless steel), as described previously (Ramakrishnan et al. 1995). High purity argon was used as the carrier gas at a flow rate of 1.0 kg cm^{-2}. Column, injector and detector were maintained at 80 °C, 100 °C and 90 °C, respectively. Under these conditions, the retention time for methane was 1.5 min and the detection limit was 0.5 μg in a 1 ml gas sample. On every sampling day three soil tubes from each treatment were sacrificed for the estimation of methane.

Experiment C

Methane oxidation in soil held at 60% moisture holding capacity

To study methane oxidation, 10-g air-dried soil samples were placed in 120-ml sterile serum bottles, and were held at 60% moisture holding capacity for 24 h in a dark incubator to equilibrate. The samples were then treated with an aqueous solution of carbofuran at concentrations of 0, 5, 10, 50 and 100 μg g^{-1} soil. Examination of the time course of methane oxidation commenced as soon as the bottles had been sealed with black rubber septa and the head space injected with 10 ml methane (5%) in argon (to provide approximately 2200 μmol methane g^{-1} dry soil). Another set of soil samples (carbofuran-treated and untreated) were exposed to acetylene, an inhibitor of methane oxidation (Oremland and Capone 1988), at a final concentration of 1% after addition of methane to ascertain the role of methanotrophs in the disappearance of methane from the head space. Soil samples were incubated at room temperature (28±2 °C) with intermittent shaking on a rotary shaker for a period of 8 h in a 24 h cycle. The head space (0.2 ml) of the serum bottles was analysed for methane by gas chromatography on alternate days until day 10.

Methane oxidation in flooded soil

Air-dried soil samples (10 g) were placed in 120-ml sterile serum bottles and mixed with water at a 1:1.25 soil to water ratio to provide flooded conditions. Carbofuran was applied to the soil at rates of 0, 5, 10, 50 and 100 μg g^{-1} soil. After sealing the serum bottles with black rubber septa and injecting the head space with 10 ml methane (5%) in argon, methane oxidation was monitored by analysing the head space gas (0.2 ml) for methane by gas chromatography until day 10. Three incubation vessels of each treatment were sacrificed on each sampling day. The mean values were analysed for statistical significance using Duncan's multiple range test.

Microbiological analyses

Microbiological analyses of the soil samples were performed as follows. Total populations of aerobic and anaerobic bacteria (heterotrophic) in the soil samples, treated with carbofuran or untreated, were estimated by the standard dilution plate technique using tryptone-yeast extract medium (Rand et al. 1975) and the medium developed by Molongoski and Klug (1976) as described by Brahmaprakash et al. (1985), respectively. The "most-probable-number" technique of Schmidt and Belser (1975) was used to estimate the population of autotrophic oxidisers of ammonium. Methanogens were counted by the five-tube most-probable-number technique at a dilution of 10, using tubes prepared under N_2 and then pressurised with a mixture of carbon dioxide/hydrogen as described by Kaspar and Tiedje (1982). The tubes, incubated at 28±2 °C for 30 days, were examined for the presence of methanogens by detection of methane in the head space. In these soil samples, methane oxidisers showing soluble methane monooxygenase (sMMO) activity were enumerated as described by Graham et al. (1992). Three plates for each dilution were incubated in vacuum desiccators containing a methane (5%)-air mixture, with periodic replenishing, for 30 days in an incubator. The colonies which developed a coloured complex with naphthalene and *O*-dianisidine (tetrazotised) were counted positive as methane oxidisers with sMMO.

Results and discussion

In the experiment to examine the influence of applied carbofuran on methane emission from flooded field plots planted to rice (cv. IR 72), carbofuran applied at rates of 2 and 12 kg active ingredient ha^{-1} caused a significant reduction in the flux of methane as compared to that in the untreated plots (Table 1). On the 9th day after application of carbofuran (56 days after transplanting), methane emis-

Table 1 Effect of commercial formulation of carbofuran on methane emission from flooded rice fields. Carbofuran was broadcast on the 47th day after transplanting (panicle initiation stage). Values in parentheses indicate the days after transplanting. In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test (*a.i.* active ingredient)

Treatment	Methane flux mmol m^{-2} h^{-1} Days after application of carbofuran					
	4 (51)	9 (56)	14 (61)	21 (68)	26 (73)	30 (77)
Control	1.51[a]	1.60[a]	1.59[a]	0.40[a]	0.31[a]	0.26[a]
+Carbofuran at 2 kg a.i. h^{-1}	1.30[b]	0.47[a]	0.79[b]	0.16[b]	0.17[b]	0.14[b]
+Carbofuran at 12 kg a.i. h^{-1}	0.22[c]	0.87[a]	0.89[b]	0.31[a]	0.22[b]	0.16[b]

Table 2 Populations of total aerobic and anaerobic (heterotrophic) bacteria, methane-oxidizing bacteria with soluble methane monooxygenase (*sMMO*), autotrophic ammonium oxidisers and H_2-CO_2 utilising methanogens in soil samples treated with carbofuran at different concentrations. Microbiological analyses of the flooded soil samples for methane production were performed after 40 days of incubation

Carbofuran ($\mu g\ g^{-1}$ soil)	Total aerobic bacteria[a] ($\times 10^5$ cfu g^{-1} soil)	Total anaerobic bacteria[b] ($\times 10^5$ cfu g^{-1} soil)	Methanotrophs with sMMO[c] ($\times 10^5$ cfu g^{-1} soil)	Autotrophic ammonium oxidisers[d] ($\times 10^3$ MPN g^{-1} soil)	H_2-CO_2 utilising methanogens[e] ($\times 10^5$ MPN g^{-1} soil)
0	81	810	2.0	0.7	0.6
5	80	205	2.1	1.9	0.2
10	77	230	2.1	2.6	0.1
100	72	910	1.9	2.2	2.8

[a] Plates were incubated under aerobic conditions at 28±2 °C
[b] Plates were incubated in an anaerobic jar
[c] Incubated under the atmosphere of a methane (5% in argon)-air mixture
[d] MPN (most-probable-number) tubes were incubated under aerobic conditions
[e] MPN tubes were incubated under the anaerobic conditions (nitrogen atmosphere)

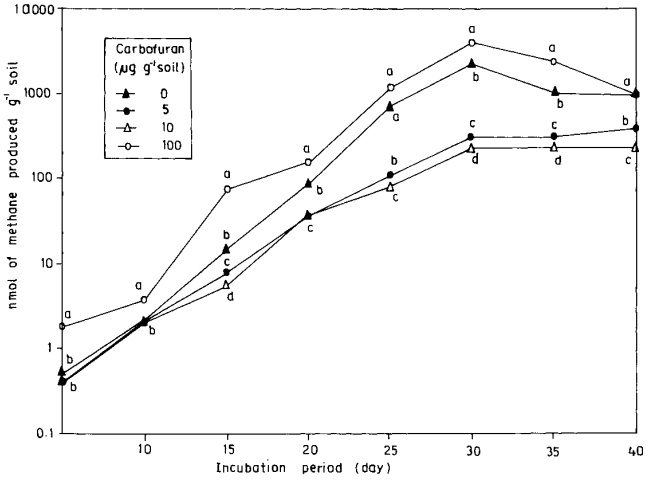

Fig. 1 Effect of carbofuran on net methane production in flooded soil. For each sampling date, means followed by the same letter are not significantly different at the 5% level when compared by Duncan's multiple range test

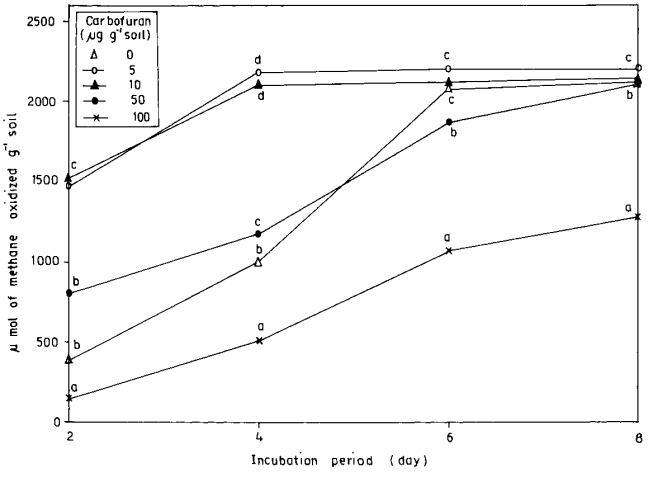

Fig. 2 Effect of carbofuran on methane oxidation in soil samples held at 60% moisture holding capacity. The concentration of methane added to the headspace air was 2200 µmol g^{-1} soil, and methane oxidation in terms of the decrease in the concentration of methane in the headspace of the incubation vessel was monitored. For each sampling date, means followed by the same letter are not significantly different at the 5% level when compared by Duncan's multiple range test

sion from untreated plots was 1.60 mmol CH_4 m^{-2} h^{-1} as compared to 0.47 and 0.87 mmol CH_4 m^{-2} h^{-1} in plots treated with carbofuran at rates of 2 and 12 kg active ingredient ha^{-1}, respectively. Almost a two- to three-fold decrease in methane emissions compared to those of the untreated field was noticed following the application of carbofuran. It is interesting to note that the application of carbofuran, even at a representative field application rate of 2 kg active ingredient ha^{-1}, caused a distinct reduction in methane flux.

In the laboratory-incubation study, on methane production (experiment B), the soil samples treated with carbofuran at rates of 5 and 10 µg g^{-1} soil accumulated substantially less methane under flooded conditions than the controls during a 30-day incubation period (Fig. 1). In contrast, addition of carbofuran at a concentration of 100 µg g^{-1} soil effected a distinct stimulation of methane production compared to that of the control. This finding is contrary to the commonly held belief that the chemically induced inhibition of microbially mediated processes is more pronounced at higher than at lower concentrations of the chemical applied (Wainwright 1979).

Microbiological analyses showed that the population of total aerobic (heterotrophic) bacteria was not altered by the addition of carbofuran, but that the total amount of anaerobic bacteria in soil treated with carbofuran at a rate of 100 µg g^{-1} soil was higher than that of controls, or samples treated with either 5 or 10 µg g^{-1} soil (Table 2). Interestingly, carbofuran exerted a positive influence on the population of autotrophic ammonium oxidisers at all the test concentrations used, as reported previously (Ramakrishna and Sethunathan 1982). But, when carbofuran was applied at a rate of 100 µg g^{-1} soil, the population of methanogens increased, while at lower rates (5 or 10 µg g^{-1} soil) there was no effect on the size of this population.

Table 3 Influence of carbofuran on the populations of total aerobic (heterotrophic) bacteria, methane-oxidizing bacteria with soluble methane monooxygenase, and autotrophic ammonium oxidisers. Microbiological analyses of the soil samples for methane oxidation were performed after 10 days of incubation

Carbofuran ($\mu g\ g^{-1}$ soil)	Total aerobic bacteria[a] ($\times 10^6$ cfu g^{-1} soil)	Methanotrophs with sMMO[b] ($\times 10^4$ cfu g^{-1} soil)	Autotrophic ammonium oxidisers[c] ($\times 10^4$ MPN g^{-1} soil)
0	123	430	0.3
5	142	1680	22.0
100	106	290	26.0

[a] Plates incubated under aerobic conditions at 28±2 °C
[b] Plates were incubated under the atmosphere of methane (5% in argon)-air mixture at room temperature
[c] MPN tubes were incubated under aerobic conditions

Table 4 Effect of carbofuran application on production and oxidation of methane in soil samples measured on 4th day of incubation and methane emission from flooded field plots planted with rice cv. IR 72. *n.d.*, Not determined; for other abbreviations see Table 1

Carbofuran	Methane production in flooded soil samples (nmol CH_4 g^{-1} soil day^{-1})	Methane oxidation (μmol of CH_4 oxidised g^{-1} soil day^{-1})		Emission of methane from field plots (mmol CH_4 m^{-2} h^{-1})
		Soil samples held at 60% water holding capacity	Flooded soil samples	
Treatment in soil samples				
0	20.0	239.3	249.3	–
5	4.4	512.3	545.3	–
10	3.2	549.8	526.0	–
50	n.d.	301.3	292.3	–
100	30.0	54.5	126.0	–
Treatment in field plots				
Control	–	–	–	0.945
+2 kg a.i. ha^{-1}	–	–	–	0.505
+12 kg a.i. ha^{-1}	–	–	–	0.445

In order to examine whether the carbofuran-induced inhibition of methane production at low concentrations of the insecticide, vis-á-vis stimulation at high concentrations, was related to its effect on methane oxidation, we examined the degree of methane oxidation in soil samples held at 60% moisture holding capacity and under flooded conditions. Interestingly, methane oxidation proceeded more rapidly at low concentrations of carbofuran (5 $\mu g\ g^{-1}$ soil) than in controls or soil samples amended with high concentrations of carbofuran (100 $\mu g\ g^{-1}$ soil). It was evident that the oxidation of methane was stimulated when carbofuran was applied at a rate of 5 $\mu g\ g^{-1}$ soil, but was inhibited when carbofuran was applied at a rate of 100 $\mu g\ g^{-1}$ soil (Fig. 2). Carbofuran exerted similar effects on methane oxidation in flooded alluvial soil (Fig. 3). Moreover, estimates of the population of methanotrophs showed that carbofuran stimulated the proliferation of methane oxidisers when applied at low concentrations (5 $\mu g\ g^{-1}$ soil) (Table 3). This would explain the inhibition of methane production when the concentration of carbofuran was low vis-á-vis its stimulation when the concentration of carbofuran was high.

Data presented in this study show that when carbofuran was applied at a rate of 100 $\mu g\ g^{-1}$ under flooded conditions, the production of methane was stimulated, but its oxidation was inhibited by this concentration of carbofuran (Table 4). At low concentrations of carbofuran, methane oxidation was stimulated, and this led to a decrease in net methane production compared to that of the control. Our studies show that certain practices used in rice cultivation,

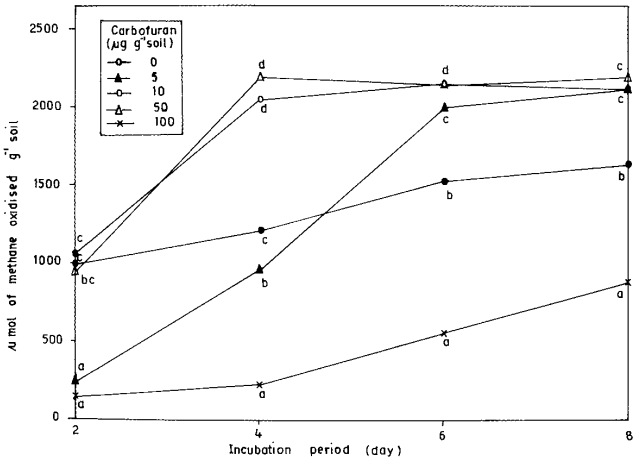

Fig. 3 Methane oxidation in flooded soil samples (1:1.25 soil:water ratio) as affected by the addition of carbofuran. The concentration of methane added to the headspace air was 2200 $\mu mol\ g^{-1}$ soil and methane oxidation in terms of the decrease in the concentration of methane in the headspace of the incubation vessel was monitored. For each sampling date, means followed by the same letter are not significantly different at the 5% level when compared by Duncan's multiple range test.

such as application of carbofuran at low rates, can lead to a reduction in the emission of methane from rice fields.

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