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Effect of Carbofuran and Hexachlorocyclohexane on N_2O Production in Alluvial Soils

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There is concern over the increasing concentrations of greenhouse gases, CO_2, CH_4 and N_2O in the atmosphere. Current atmospheric concentration of N_2O is around 310 ppbv, but it is increasing at 0.2-0.3% year^{-1} (Conrad 1996). Global warming potential of N_2O is about 250 times greater than that of CO_2. Agricultural soils heavily fertilized with nitrogen account for about 10-20% of the anthropogenic emissions of N_2O to the atmosphere (Mosier 1994). Projected increases in the use of nitrogen fertilizers and biofertilizers to meet the global demand for food (Hammond 1990) can lead to increased N_2O emissions.

N_2O is produced in agricultural soils essentially by microbially mediated nitrification and denitritication reactions (Cicerone 1989). In oxic agricultural soils, N_2O emissions from nitrification of ammonium-based fertilizers can be significant (Bremner and Blackmer 1978). In wetland soils where oxygen concentration is limited, denitrification is the important means of N_2O production (Smith 1990). However, predominantly anaerobic soils have a great potential for further reduction of N_2O to N^2 (Duxbury and McConnaughey 1986). Many factors governing nitrification and denitrification in soils include soil water content, temperature, concentration of nitrate or ammonium, available organic carbon and pH. Land use and agricultural practices such as fertilizer management and plant protection practices can also influence N cycling in agricultural soils (Houghton and Skole 1990; Henault et al. 1998). There are instances of stimulation of nitrogenase activity by carbofuran (Patnaik et al. 1994) and inhibition of nitrification by hexachlorocyclohexane (HCH) (Ray et al. 1980) in soils even when applied at recommended levels. Application of carbofuran stimulated autotrophic oxidation of ammonium in simulated oxidized surface layer of a flooded soil (Ramakrishna et al. 1978; Ramakrishna and Sethunathan 1982). In a more recent study (Kumaraswamy et al. 1998), carbofuran, applied at 5 µg g^{-1} soil, effected a stimulation of methane oxidation in soil samples held at 60% water holding capacity. The effect of xenobiotics on microbial production of N_2O in soils is, however, little understood although N_2O is an intermediate formed during nitrification in oxic soils and during nitrification-denitrification sequence in flooded rice soils. In the present study, we report the effect of carbofuran and HCH on N_2O production in soil samples held under

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60% water holding capacity. Also, the effect of repeated applications of carbofuran to flooded soil on N_2O production was examined.

MATERIALS AND METHODS

In a study to determine the effect of carbofuran on N_2O production in unamended and ammonium sulfate amended soil samples, soil cores were collected from the 0-5 cm layer at the experimental farm of the Central Rice Research Institute, Cuttack. Soil is a Typic Haplaquept (deltaic alluvium) with a sandy clay loam texture (pH 6.2, organic carbon 0.76%, total N 0.09%). Before use, soil was air-dried and sieved, (<2 mm). Portions (10 g) of the soil were placed in 120 mL sterile serum bottles and were mixed with either sterile distilled water or aqueous solution of ammonium sulfate at 25 µg g^{-1} soil. Soil samples, without or with added ammonium sulfate, were then treated with an aqueous solution of 75% carbofuran (Rallis India Ltd., Bangalore, India) to provide concentrations of 5, 50 and 100 µg active ingredient g^{-1} soil. Likewise, soil samples were treated with a suspension of 32% HCH (Hindustan Insecticides Ltd., Pune, India) in acetone at 2, 5 and 10 µg g^{-1} soil. In all the cases, the volume of acetone/ aqueous solutions, added to provide different levels of insecticides to the soil, was 1 mL. Unamended soil samples receiving acetone or sterile distilled water alone served as controls. After keeping the serum bottles open overnight for acetone to get evaporated, the soil in each bottle was thoroughly mixed, closed with black rubber septa and incubated at 20 ± 2°C for 40 days. The concentration of N_2O in the headspace gas of incubation vessels was analysed in Hewlett Packard gas chromatograph fitted with electron capture detector and a Porapak N column with the carrier gas (95% argon and 5% methane) flow at a rate of 30 mL min. The column was maintained at 50°C. Under these conditions, the retention time for N_2O was 1.9 min.

After sampling the headspace for estimation of N_2O, 50 mL of 2 M KCl was added and the incubation vessels were shaken for 30 min. The suspension was allowed to settle for 30 min and then filtered through a Whatman no. 42 filter paper. Nitrate in the 2 M KCl extracts was determined after treating with phenol 2,4-disulphonic acid and 6 N NH_4OH followed by calorimetric determination (Jackson 1967). Triplicate incubation vessels of each treatment were sacrificed at each analysis.

In another experiment, 100-g portions of air-dried sieved alluvial soil (pH 7.5, organic carbon 0.60%, total N 0.034%) from the experimental farm of the Indian Agricultural Research Institute, New Delhi were placed in plastic beakers (9 cm x 9 cm) and then flooded with 125 mL of water. After 10 days of submergence, soil samples were repeatedly treated with carbofuran in 0.1 mL of acetone at 5 µg g^{-1} soil at today intervals in a staggered manner, so that at 30 days after flooding, soil samples received 0, 1, 2 and 3 applications of the insecticide. At 40 days after flooding, water was decanted from the beakers and after 24 h, soil cores from plastic beakers were transferred to sterilized 120 ml-serum bottles with the help of 13 mm plastic tube. Soil samples of each treatment (receiving 0, 1, 2 and 3 applications of the insecticide) were treated with aqueous solution (1.5 mL) of carbofuran to provide a concentration of 50 µg g^{-1} soil. Soil samples receiving an equal amount of sterile distilled water served as respective control. Serum bottles were then closed with rubber septa and then incubated at 30 ± 2°C. At regular intervals, concentration of N_2O in the headspace of the serum bottles was estimated by gas chromatography as described earlier.

RESULTS AND DISCUSSION

Addition of carbofuran at 5, 50 and 100 µg g^{-1} soil to soil samples without ammonium sulfate, held at 60% water holding capacity, was neither stimulatory nor inhibitory to N_2O production, in terms of the increase in the concentration of N_2O in the headspace of serum bottles, during 40-day incubation (Table 1). The net increase in N_2O concentration in the headspace over that of atmospheric concentration ranged only between 30 and 50 ppbv in both control and carbofuran-treated soil samples without ammonium sulfate. But, carbofuran effected a distinct stimulation of N_2O production in soil samples amended with ammonium sulfate irrespective of its concentration.

Table 1. Effect of commercial formulation of carbofuran on N_2O production in the alluvial soil samples with or without ammonium sulfate

Carbofuran added (µg g^{-1} soil)	Net N_2O produced µg g^{-1} soil	
	Without ammonium sulfate	With ammonium sulfate (25 µg g^{-1} soil)
0	0.61	0.60
5	0.66	4.86
50	0.61	1.98
100	0.66	4.08

The headspace concentration of N_2O showed a 2- to 8-fold increase (over that of control without carbofuran) in soil samples treated with ammonium sulfate and carbofuran. Evidently, stimulatory effect of carbofuran on N_2O production in soil samples held at 60% water holding capacity was noticed only when applied in combination with ammonium sulfate. Carbofuran is known to stimulate nitrification, in terms of nitrate formed, in flooded soil (Tirol et al. 1981), in simulated oxidized surface of a flooded soil (Ramakrishna et al. 1978) and in rice rhizosphere soil suspension (Ramakrishna and Sethunathan 1982). But, at higher concentrations (50 and 100 µg g^{-1} soil) of carbofuran, nitrification to nitrate in soil samples amended with ammonium sulfate was inhibited (Table 2) as reported earlier in rice rhizosphere soil suspension (Ramakrishna and Sethunathan 1982). Moreover, in soil samples held under 60% water holding capacity, as in the present study, there was no stimulation of nitrification to nitrate (past N_2O) by carbofuran at low concentrations (Table 2), in contrast to the earlier reports of stimulation by carbofuran in flooded rice soils. It is worthwhile to mention here that carbofuran even at 100 µg g^{-1} soil stimulated N_2O production in soil samples amended with ammonium sulfate, albeit inhibition of nitrification to NO_3^-.

Table 2. Concentration of nitrate nitrogen in alluvial soil samples, with or without ammonium sulfate and treated with carbofuran, after 40 days of incubation

Carbofuran added (µg g^{-1} soil)	NO_3^-N (µg g^{-1} soil)	
	Without ammonium sulfate	With ammonium sulfate (25 µg g^{-1} soil)
0	0.54	0.59
5	0.42	0.65
50	0.46	0.34
100	0.76	0.33

The effect of repeated applications of carbofuran on N_2O production in alluvial soil was examined. Flooded soil samples, not previously exposed to carbofuran or pretreated once, twice or thrice with carbofuran (0, 1, 2 and 3 applications), were subsequently incubated with (50 µg g^{-1} soil) or without carbofuran for 20 days. Interestingly, repeated applications of carbofuran to flooded soil samples led to a distinct increase in N_2O production (Table 3). This stimulatory effect of carbofuran in pretreated soil samples was noticed after 1, 2 and 3 applications; but, there was no further stimulation between 2 and 3 applications. It is interesting to note that even soil samples, not previously exposed to carbofuran, effected an increase in N_2O production when subsequently incubated with carbofuran at 50 µg g^{-1} soil for 20 days; but this increase was less pronounced than that in pretreated soil samples. Also of special mention is that soil samples, pretreated with carbofuran (once, twice and thrice) showed increased N_2O production (over that in previously untreated samples) when subsequently incubated not only with carbofuran, but also without carbofuran. Thus, carbofuran stimulated N_2O production in soil samples held at 60% water holding capacity (Table 1) as well as under flooded conditions (Table 3).

The effect of HCH on N_2O production in soil samples (unamended or amended with ammonium sulfate) held at 60% water holding capacity was examined. HCH effected a distinct inhibition in the production of N_2O, both in the presence and absence of ammonium sulfate (Table 4), concomitant with a drastic inhibition of NO_3^- production (Table 5). The inhibitory effect of HCH on the production of N_2O and NO_3^- increased with increase in its concentration. HCH is known to inhibit autotrophic nitrification of the NH_4^+ to NO_3^- (Ray et al. 1980). N_2O

is an intermediate during autotrophic nitrification. Evidently, decreased production of N_2O in HCH-treated soil samples was related to its toxicity to nitrification.

Table 3. Effect of repeated applications of carbofuran to flooded soil on N_2O production

Incubation (days)[2]	Carbofuran added to pretreated soil ($\mu g\ g^{-1}$ soil)	N_2O production ($\mu g\ g^{-1}$ soil)[1]			
		Number of soil pretreatment with carbofuran			
		0	1	2	3
10	0	0.035	0.226	1.257	1.279
	50	0.088	1.487	1.635	1.605
15	**0**	0.090	0.873	1.568	1.944
	50	0.421	2.032	2.380	2.418
20	0	0.578	2.392	3.916	4.168
	50	1.925	3.886	4.688	4.650

[1] Flooded soil samples contained in plastic beakers were untreated or treated once, twice or thrice (0, 1, 2 and 3 applications) with carbofuran at 5 $\mu g\ g^{-1}$ soil.

[2] Soil samples pretreated with carbofuran (0, 1, 2 and 3 applications) were transferred to serum bottles and subsequently incubated with carbofuran at 0 and 50 $\mu g\ g^{-1}$ soil for 20 days

Table 4. Effect of commercial formulation of hexachlorocyclohexane (HCH) on N_2O production in an alluvial soil, unamended or amended with ammonium sulfate

HCH added ($\mu g\ g^{-1}$ soil)	Net N_2O produced ($\mu g\ g^{-1}$ soil)[1]	
	Without ammonium sulfate	With ammonium sulfate
0	2.04	1.78
2	1.23	0.51
5	0.33	0.21
10	0.41	0.33

[1] at 40 days

Table 5. Effect of hexachlorocyclohexane (HCH) on the concentration of nitrate nitrogen in alluvial soil samples

HCH added (µg g^{-1} soil)	NO_3^--N (µg g^{-1} soil)	
	Without ammonium sulfate	With ammonium sulfate (25 µg g^{-1} soil)
0	2.08	2.07
2	0.88	0.21
5	0.22	0.16
10	0.17	0.39

Application of carbofuran to soil samples held under nonflooded and flooded conditions led to a stimulation of N_2O production. In contrast, HCH inhibited the production of both N_2O and NO_3^- in nonflooded soil samples.

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