Bull. Environm. Contam. Toxicol. 21,328-333 (1979)

Effect of Benomyl and Its Hydrolysis Products, MBC and AB, on Nitrification in a Flooded Soil

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Benomyl [methyl-1-(butyl-carbamoyl)-2-benzimidazole carbamate] is extensively used in agriculture as a systemic fungicide for controlling a broad spectrum of phytopathogenic fungi including rice pathogens. Benomyl is transformed readily to methyl-2-benzimidazole carbamate (MBC) and 2-aminobenzimidazole (AB) in soils (HELWEG 1973a, BAUDE et al. 1974) and in water (KILGORE and WHITE 1970). The major breakdown product, MBC has also fungicidal properties (PETERSON and EDGINGTON 1969, CLEMENS and SISLER 1969).

Side-effects of benomyl on nitrification in soils have been investigated by several workers (HELWEG 1973a and b, GOWDA 1973, VAN FASSEN 1974, GOWDA et al. 1976, 1977); but reports are conflicting. GOWDA (1973) and HOFER et al. (1971) reported inhibition of nitrification by benomyl at 15 to 1000 ppm levels in nonflooded soils. There are also reports that nitrification is little affected (HELWEG 1973a) or slightly enhanced (VAN FASSEN 1974) by applications of benomyl to nonflooded soils at concentrations ranging from 10 to 1000 ppm. Recently, GOWDA et al. (1976, 1977) noticed stimulation of heterotrophic nitrifiers by benomyl in simulated oxidized surface of a flooded soil at a concentration of 5000 ppm which was toxic to autotrophic nitrifying bacteria. In this study, we report the effects of benomyl and its breakdown products, MBC and AB on nitrification in simulated oxidized surface of a flooded soil and in cultures of autotrophic nitrifying bacteria.

MATERIALS AND METHODS

Benomyl, MBC and AB. Commercial formulations of benomyl and MBC, Benlate 50% W.P. and Derosal 20% respectively, were obtained from Hoechst Pharamaceuticals Ltd., Bombay. Technical grade benomyl, MBC and AB were gifted by E. I. Du Pont de Nemours and Co., Delaware, U.S.A.

Soil. An alluvial soil (pH 6.0, organic matter 1.35%, total nitrogen 0.09%) from the experimental farm of the Central Rice Research Institute, Cuttack was used in this study.

Simulated oxidized surface of the flooded soil. The oxidized surface of the flooded soil was simulated in the laboratory by placing 10 g soil in a 250-ml Erlenmeyer flask to give a thin

0007-4861/79/0021-0328 $01.20

layer of soil and then flooded with 20 ml distilled water (GOWDA et al. 1977).

Nitrification in benomyl-, MBC- and AB-amended soils. The commercial formulations of benomyl and MBC, viz. Benlate and Derosal respectively, and technical grade AB were used in this study. Benomyl and its breakdown products were incorporated into 10 g soils at the rates of 10, 100 and 1000 ppm active ingredient with respect to soil together with 500 ppm of ammonium sulfate. The soils were then flooded with 20 ml of distilled water. Soils receiving only ammonium sulfate served as controls. At 10-day intervals, the nitrate in two replicate soil samples was extracted and analyzed colorimetrically.

Nitrification in cultures of autotrophic bacteria. Ammonium-oxidizing bacterium, Nitrosomonas sp. was the same as used in an earlier study (GOWDA et al. 1977) while nitrite-oxidizing bacterium, Nitrobacter agilis was obtained from Dr. N. Walker, Rothamsted Experimental Station, England. To test the effect of benomyl and its breakdown products on autotrophic nitrification, technical grade benomyl, MBC and AB were dissolved in methanol and then introduced into sterile 100-ml Erlenmeyer flasks in 1-ml portions to provide a final concentration of 10 and 100 ppm in 40-ml media. After evaporating off the methanol at room temperature, the sterile mineral salts media specific for Nitrosomonas sp. and N. agilis containing ammonium sulfate and sodium nitrite as nitrogen source respectively (GOWDA et al. 1977) were dispensed in 40 ml portions to the flasks. After equilibration for 24 h in a Gallenkamp orbital shaker, the ammonium- and nitrite-containing media were inoculated with 7 to 10 day old cultures of Nitrosomonas sp. and N. agilis, respectively. Media without benomyl and its breakdown products served as controls. After desired period of incubation with nitrifiers at room temperature, nitrite in the media was assayed to determine the amount of nitrite formed in Nitrosomonas medium or lost from Nitrobacter medium.

Analysis of ammonium, nitrite and nitrate. In soil incubation studies, nitrogen from the soils was extracted with 50 ml of Morgan's solution (CH_3COONa-CH_3COOH buffer, pH 4.8) as described by SAHRAWAT and RAJENDRAPRASAD (1975). After stirring for 30 min in a wrist action shaker, the contents were filtered through a Buchner funnel with suction and the clear filtrate was analyzed for ammonium by nesslerization (JACKSON 1958), nitrite by diazotization (BARNES and FOLKARD 1951) and nitrate by phenol disulfonic acid method (BREMNER 1965). In pure culture studies with autotrophic nitrifiers, nitrite in the mineral salts medium was assayed colorimetrically by diazotization after appropriate dilution of the medium.

RESULTS AND DISCUSSION

Commercial formulations of benomyl and MBC (Benlate and Derosal respectively) and technical grade AB were used to study their effect on nitrification in simulated oxidized surface of flooded soils. Nitrification proceeded rapidly in unamended soils and in soils amended with 10 and 100 ppm benomyl (Table 1) as judged by the disappearance of ammonium with the concomitant formation of nitrate during 30-day incubation period. But, benomyl at 1000 ppm level drastically inhibited the oxidation of ammonium to nitrate and this inhibitory action was well pronounced even after 30 days.

TABLE 1

Effect of benomyl on nitrification in simulated oxidized surface of a flooded soil

	mg nitrate recovered/10 g soil*		
Benomyl	Incubation (days)		
(ppm)	10	20	30
0	2.0 (0.8)	3.0 (0)	3.8 (0)
10	1.9 (1.0)	2.7 (0)	3.7 (0)
100	1.2 (1.2)	2.4 (0)	3.8 (0)
1000	0.3 (1.7)	0 (1.2)	0 (1.0)

*Figures in parentheses represent the amount of ammonium recovered/10 g soil; Initial amount of nitrate and ammonium recovered/10 g soil was 0.2 mg and 1.3 mg, respectively.

Among the degradation products of benomyl, MBC was relatively non-toxic to nitrification in soils as compared to its parent compound. No appreciable inhibition of nitrification occurred in soils amended with 10 and 100 ppm MBC while at 1000 ppm nitrification was slightly retarded (Table 2).

TABLE 2

Effect of MBC on nitrification in simulated oxidized surface of a flooded soil

	mg nitrate formed/10 g soil		
MBC	Incubation (days)		
(ppm)	10	20	30
0	0.5	2.8	4.4
10	0.5	2.9	4.7
100	0.6	2.8	4.3
1000	0.3	2.0	3.8

AB, also a common degradation product of benomyl, inhibited nitrification in soils at concentrations of 100 and 1000 ppm (Table 3). In terms of nitrate formed, AB was relatively less toxic than the parent compound.

TABLE 3

Effect of AB on nitrification in simulated oxidized surface of a flooded soil

AB (ppm)	mg nitrate formed/10 g soil Incubation (days) 10	20	30
0	2.7	4.0	4.5
10	2.1	2.7	4.6
100	1.9	2.4	2.7
1000	0.3	0.8	1.0

The potential toxicity of benomyl to nitrification was demonstrated also in pure cultures of nitrifying bacteria. Thus, benomyl inhibited the oxidation of ammonium by Nitrosomonas sp. (Table 4) and of nitrite by N. agilis (Table 5) even at a concentration of 10 ppm. Among the degradation products, AB showed toxicity to both groups of nitrifiers, but only at higher concentration of 100 ppm while MBC was virtually innocuous, as in soils, to both groups of nitrifiers irrespective of its concentration (Tables 3 and 4). Undoubtedly, benomyl was far more toxic than its degradation products, MBC and AB at least with respect to autotrophic nitrification.

TABLE 4

Effect of benomyl, MBC and AB on nitrification by Nitrosomonas sp.

Treatment	Concentration (ppm)	µg nitrite recovered/ml medium Incubation (days) 6	15
Unamended		3.8	108.0
Benomyl	10	2.0	2.1
	100	1.7	2.0
MBC	10	4.5	146.4
	100	3.0	104.5
AB	10	3.7	146.4
	100	2.8	3.9

TABLE 5

Effect of benomyl, MBC and AB on nitrification by Nitrobacter agilis

Treatment	Concentration (ppm)	µg nitrite recovered/ml medium — Incubation (days) 6	15
Unamended		289.5	0
Benomyl	10	444.2	513.2
	100	444.2	440.5
MBC	10	296.1	0
	100	473.8	0
AB	10	352.0	0
	100	622.5	661.3

Benomyl exerts both inhibitory (GOWDA 1973; this report) and little or slightly stimulatory (VAN FASSEN 1974) effects on nitrification in soils. Such conflicting reports on pesticidal action on soil microorganisms and their activities are not uncommon in the literature. The reported discrepancies with respect to benomyl action on nitrification could at best be ascribed to different soil properties and different test conditions in view of their importance in determining the overall persistence of benomyl in soil environment. According to our studies, the effects of benomyl on nitrification may be related to the differential rates of its conversion to MBC since nitrification was drastically inhibited by benomyl and not at all by MBC both in soils (Tables 1 and 2) and in pure cultures of autotrophic bacteria (Tables 4 and 5).

Recent reports (REDDY et al. 1976) show that large losses of applied ammonium nitrogen can occur in flooded soils because of its high rate of diffusion from the reduced soil layer to the oxidized surface leading to its nitrification in the oxidized surface and eventual loss in volatile forms by denitrification of the products of nitrification in the reduced layer. The results of the inhibitory action of benomyl on nitrification may have applied significance in preventing the nitrogen loss from flooded soils.

ACKNOWLEDGMENTS

We thank Dr. H. K. PANDE, Director for encouragement and Dr. N. WALKER, Rothamsted Experiment Station, England for providing the culture of Nitrobacter agilis. This study was supported, in part, by funds from the Department of Atomic Energy, Government of India and Department of Science and Technology, Government of India.

REFERENCES

BARNES, H., and A. R. FOLKARD: Analyst 76, 599 (1951).

BAUDE, F. J., H. L. PEASE, and R. F. HOLT: J. Agr. Food Chem. 22, 413 (1974).

BREMNER, J. M.: In "Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties." C. A. Black (ed), p. 1179, Amer. Soc. Agron., Madison, Wisconsin (1965).

CLEMENS, G. P., and H. D. SISLER: Phytopathology 59, 705 (1969).

GOWDA, T. K. S.: M. Sc. thesis, Univ. Agr. Sci., Bangalore (1973).

GOWDA, T. K. S., R. SIDDARAMAPPA, and N. SETHUNATHAN: Soil Biol. Biochem. 8, 435 (1976).

GOWDA, T. K. S., V. R. RAO, and N. SETHUNATHAN: Soil Sci. 123, 171 (1977).

HELWEG, A.: Tidsskr. f. Planteavl. 77, 232 (1973 a).

HELWEG, A.: Tidsskr. f. Planteavl. 77, 375 (1973 b).

HOFER, I., T. BECK, and P. WALLNOFER: Z. Pflkrankh, Pfl. Path. Pflschutz. 7, 398 (1971).

KILGORE, W. W. and E. R. WHITE: Bull. Environ. Contam. Toxicol. 6, 1 (1970).

PETERSON, C. A., and L. V. EDGINGTON: J. Agr. Food Chem. 17, 898 (1969).

REDDY, K. R., W. H. PATRICK, Jr., and R. E. PHILIPS: Soil Sci. Soc. Am. J. 40, 528 (1976).

van FASSEN, H. G.: Soil Biol. Biochem. 6, 131 (1974).

SAHRAWAT, K. L., and RAJENDRAPRASAD: Pl. Soil 42, 304 (1975).