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Received for review November 22, 1974. Accepted August 1, 1975. This research was supported in part by the Office of Naval Research, Microbiology Program, Naval Biology Project, under Contract No. N00014-67-A-0077-0027, Nr 306-067.

Metabolism of Aldicarb by Five Soil Fungi

Alice Stevens Jones

Pure cultures of five common soil fungi grown in Czapek-Dox Broth were tested for degradation of aldicarb, and three of the five were tested against aldicarb sulfoxide, the major toxic metabolite. The fungi were, in decreasing order of their effectiveness in degrading the pesticide: *Gliocladium catenulatum* > *Penicillium multicolor* = *Cunninghamella elegans* > *Rhizoctonia* sp. > *Trichoderma harzianum*. Although there was considerable variability among the fungi in the amounts of organo- and water-soluble products from both aldicarb and aldicarb sulfoxide, the major organosoluble metabolites were aldicarb sulfoxide and the oxime and nitrile sulfoxides, with much smaller amounts of the sulfones. Aldicarb was stable under sterile conditions, but some breakdown of aldicarb sulfoxide was found in sterile media after the 28-day incubation.

The metabolism of Temik aldicarb pesticide [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] has been studied extensively in plants (Bartley et al., 1970; Andrawes et al., 1973) and in animals (Andrawes et al., 1967; Dorough and Ivie, 1968). Coppedge et al. (1967) reported that aldicarb had a half-life of 9–12 days in soils under laboratory conditions. Other studies of aldicarb in soils (Bull et al., 1970) showed that aldicarb disappeared rapidly from moist soils, but it was relatively stable in dry soils. It was found to be stable in moist or dry sand, with a half-life exceeding 56 days. Spurr and Sousa (1966, 1974) tested aldicarb and its metabolites against pathogenic and saprophytic microorganisms. They found no direct disease control and no adverse effects against the saprophytes tested at dosages considerably higher than the recommended usage levels. They also reported that some of the microorganisms tested appeared to be able to utilize aldicarb as a carbon source.

The studies reported here were initiated to determine the ability of five soil fungi to degrade aldicarb and its major toxic metabolite, aldicarb sulfoxide. The various soil fungi chosen are found frequently in cultivated and forest soils (Hodges, 1962) and are capable of rapid growth in synthetic media. The group included three genera which are early colonizers or decomposers of organic materials, i.e. *Cunninghamella*, *Penicillium*, and *Trichoderma*, and two (*Gliocladium* and *Rhizoctonia*) which are often associated with root surfaces (Dickinson and Pugh, 1974).

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MATERIALS AND METHODS

Radiolabeled aldicarb (methylthio-^{14}C labeled, sp act. 7.1 mCi/mmol) was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. The purity was determined to be 98% by thin-layer chromatography (TLC) with 3:1 methylene chloride–acetonitrile and liquid scintillation counting (lsc). Nonlabeled standards of aldicarb and its known metabolites (Table I) were synthesized and authenticated by Union Carbide Corp., Research and Development Dept., South Charleston, W.Va.

Radiolabeled aldicarb sulfoxide was recovered from the organic extracts of fungal cultures by Florisil column chromatography (Andrawes et al., 1967). The radiochemical purity of the sulfoxide was determined to be 98% by TLC with 6:1 chloroform–methanol and lsc.

For the metabolic studies, flasks capped with aluminum foil and containing 50 ml of Czapek-Dox Broth (Difco Laboratories, Detroit, Mich., pH 7.3) were autoclaved and allowed to cool. Then 0.5 ml of an ethanol solution of [^{14}C]aldicarb was aseptically added to each flask from a

Table I. Standards Used in Study of Aldicarb Metabolism

Chemical name	Common name
Organosoluble	
2-Methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime	Aldicarb (A)
2-Methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime	Aldicarb sulfoxide (A_1)
2-Methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime	Aldicarb sulfone (A_2)
2-Methyl-2-(methylsulfinyl)propionaldehyde oxime	Oxime sulfoxide (A_1O)
2-Methyl-2-(methylsulfonyl)propionaldehyde oxime	Oxime sulfone (A_2O)
2-Methyl-2-(methylsulfinyl)propionitrile	Nitrile sulfoxide (A_1N)
2-Methyl-2-(methylsulfonyl)propionitrile	Nitrile sulfone (A_2N)
Water soluble	
2-Methyl-2-(methylsulfinyl)propanol	Alcohol sulfoxide (A_1Al)
2-Methyl-2-(methylsulfonyl)propanol	Alcohol sulfone (A_2Al)
2-Methyl-2-(methylsulfinyl)propionamide	Amide sulfoxide (A_1Am)
2-Methyl-2-(methylsulfonyl)propionamide	Amide sulfone (A_2Am)
2-Methyl-2-(methylsulfinyl)propionic acid	Acid sulfoxide (A_1Ac)
2-Methyl-2-(methylsulfonyl)propionic acid	Acid sulfone (A_2Ac)

Table II. Percent Distribution of Recovered Radioactivity from Aldicarb Cultures after Various Periods of Incubation[a]

	7 days		14 days		21 days		28 days	
Culture	Organic	H_2O	Organic	H_2O	Organic	H_2O	Organic	H_2O
Control	96	4	96	4	97	3	97	3
G. catenulatum					65	35	68	32
P. multicolor					70	30	70	30
Rhizoctonia sp.					78	22		
C. elegans					83	17		
T. harzianum	96	4	89	11	91	9	86	14

[a] Average of two replicates.

Table III. Percentage of Total Recovered Radioactivity in Organosoluble Metabolites from 21-Day Aldicarb Cultures[a]

Culture	A	A_1	A_2	A_1O	A_1N	$A_2O + A_2N$
Control	90	3	Trace	1	1	2
C. elegans	Trace	65	1	9	8	Trace
P. multicolor	2	64	Trace	2	2	Trace
T. harzianum	24	62	3	1	1	Trace
G. catenulatum	50	11	Trace	1	2	1
Rhizoctonia sp.	63	10	Trace	2	2	1

[a] Average of four replicates.

hypodermic syringe fitted with a Swinney adaptor containing a 0.25-μm Millipore filter, and 1-ml aliquots were aseptically removed for lsc. The final concentration of aldicarb was 0.05 mg/50 ml of medium, equivalent to about 0.2 lb of active ingredient/acre. This is well below normal rates of field application.

Duplicate flasks were inoculated with laboratory stock cultures of *Cunninghamella elegans*, *Gliocladium catenulatum*, *Penicillium multicolor*, *Rhizoctonia* sp., and *Trichoderma harzianum*. Uninoculated flasks served as controls. The flasks were incubated on a Burrell wrist-action shaker at ambient temperature (25 ± 2°C) for 7, 14, 21, or 28 days.

Flasks containing [^{14}C]aldicarb sulfoxide were prepared as above, inoculated with *C. elegans*, *G. catenulatum*, and *T. harzianum*, and incubated for 28 days.

After incubation, the cultures were homogenized in a Waring blender and centrifuged to remove the mycelial fragments. Samples of the mycelial pellets were suspended in scintillation cocktail and checked for radioactivity. The aqueous supernatants were decanted into 250-ml separatory funnels and extracted 4 times with equal volumes of 1:1 chloroform–acetonitrile. The organic extracts were concentrated to 5 ml on a rotary evaporator at 35°C, and the total radioactivity of the organic and aqueous phases was determined by lsc. The percent recovery of the original ^{14}C was 80%. The aqueous phases were then freeze-dried and the residues taken up in 10 ml of 50% ethanol.

The metabolites in the organic and aqueous phases were characterized by two-dimensional TLC comparison with known standards according to the procedures of Bartley et al. (1970). The radioactive metabolites were detected by autoradiography. In addition, the aqueous phases were analyzed by a second two-dimensional TLC system with benzene–methanol–acetic acid (79:14:7) and ether–formic acid–water (90:7:3). The metabolites from the organic phases were quantitated by scraping spots into scintillation vials for lsc.

RESULTS AND DISCUSSION

The distribution of radioactivity between the organic and aqueous phases after various periods of incubation of the cultures and controls containing aldicarb is shown in Table II. In the controls, the ratio remained constant through 28 days. In decreasing order of percentage of water-soluble products, the cultures were: *G. catenulatum* > *P. multicolor* > *Rhizoctonia* sp. > *C. elegans* > *T. harzianum*. Only a trace of radioactivity was found in the mycelial pellets.

TLC analyses of the organic extracts provided additional information on the relative metabolic activity of the fungi. Data from the 21-day cultures are presented in Table III. The stability of the parent compound under sterile con-

Table IV. Percent Distribution of Total Recovered Radioactivity in Organo- and Water-Soluble Metabolites from 28-Day Aldicarb Sulfoxide[a]

Culture	A_1	A_1O	A_2	A_1N	$A_2O + A_2N$	Water-soluble metabolites
Control	72	5	N.D.[b]	3	2	18
G. catenulatum	44	14	Trace	11	3	28
C. elegans	64	5	1	5	N.D.[b]	25
T. harzianum	82	7	1	8	Trace	2

[a] Average of two replicates. [b] N.D. = none detected.

ditions was shown by the high percentage of residual aldicarb (A) found in the controls. The oxidative metabolism of aldicarb to aldicarb sulfoxide (A_1) was most rapid in the *C. elegans* and *P. multicolor* cultures, with *T. harzianum* showing an appreciable conversion as well. *G. catenulatum* had one of the higher percentages of residual aldicarb. The major organosoluble metabolites found were aldicarb sulfoxide, oxime sulfoxide (A_1O), and nitrile sulfoxide (A_1N), with only small amounts of the corresponding sulfones (A_2O and A_2N). This production indicates that the metabolic pathway in these fungi is similar to that found in higher plants and animals.

The reported toxic metabolites of aldicarb are aldicarb sulfoxide and aldicarb sulfone (A_2) (Union Carbide Corp., 1970; Spurr and Sousa, 1974). The detoxification potential of the fungi was determined by calculating the total toxic compounds remaining at the end of the incubation period. The order was: *G. catenulatum* (61%) > *P. multicolor* (66%) = *C. elegans* (66%) > *Rhizoctonia* sp. (73%) > *T. harzianum* (89%). This is very similar to the order found among the fungi in the production of water-soluble products.

The major water-soluble metabolites found were the alcohol and amide sulfones. Moderate amounts of the alcohol and amide sulfoxides were also found, as were small amounts of the acids. In addition, a poorly defined spot which remained at the origin in all TLC systems was found in all cases. Incubation of the samples for 24 hr with β-glucosidase and β-glucuronidase (Worthington Biochemical Corp., Freehold, N.J.) did not alter the TLC pattern. These findings suggest that the metabolites were not present as glucose or glucuronic acid conjugates. Inadequate separation of metabolites in the solvent systems used prevented quantitation of the water-soluble metabolites.

The distribution of recovered radioactivity from the cultures with [^{14}C]aldicarb sulfoxide is shown in Table IV. The degradative potential of the fungi was, in decreasing order: *G. catenulatum* > *C. elegans* > *T. harzianum*. Less degradation of aldicarb sulfoxide occurred in the *T. harzianum* cultures than in the controls. The metabolic pattern with aldicarb sulfoxide was similar to that found with aldicarb.

These results indicate that the ability to metabolize aldicarb and its sulfoxide is common in soil fungi. Bull et al. (1970) reported that aldicarb disappeared rapidly from moist soils but not from dry soils or sand. They concluded that volatilization was a major factor in the disappearance of aldicarb from the soils studied. The results of the study reported here suggest that microbial activity could also explain their results. Microbial activity would be minimal in dry soils and sand and would increase with increased moisture content up to field capacity in soils but not in sand.

ACKNOWLEDGMENT

This work is the result of a cooperative agreement between the National Institutes of Environmental Health Sciences and the USDA Forest Service. The author is indebted to Janet Guthrie for technical assistance.

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Received for review April 24, 1975. Accepted September 2, 1975. Mention of proprietary products and manufacturers in this paper is for identification only and does not consitute endorsement by the U.S. Department of Agriculture to the exclusion of other products that may be suitable.