Microbial Degradation of Oxadiazon by Soil Fungus *Fusarium solani*

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Degradation of oxadiazon [2-*tert*-butyl-4-(2,4-dichloro-5-isopropoxyphenyl-Δ^2-1,3,4-oxadiazolin-5-one (**1**)], a preemergent soil-applied herbicide, by a soil fungus, *Fusarium solani* (Mortius) Sacc., has been investigated. *F. solani*, isolated from soil by enrichment culture technique, degraded the herbicide **1** by cometabolic process. Eleven metabolites have been isolated, out of which three metabolites ($\mathbf{M_1}$, $\mathbf{M_2}$, and $\mathbf{M_3}$) were characterized by spectroscopic evidence. Metabolites $\mathbf{M_1}$ and $\mathbf{M_3}$ are reported for the first time as metabolites of oxadiazon. The main metabolic pathways involve oxadiazoline ring cleavage without dechlorination reaction.

Keywords: *Degradation; cometabolism; metabolic pathway; oxadiazon; Fusarium solani*

INTRODUCTION

Oxadiazon [2-*tert*-butyl-4-(2,4-dichloro-5-isopropoxyphenyl-Δ^2-1,3,4-oxadiazolin-5-one, **1** (Figure 3)], a preemergent soil-applied herbicide introduced in 1969, has been used for controlling annual grasses and broadleaf weeds in rice, soybean, cotton, orchards, onion, peanut, potato, and ornamentals. The persistence of oxadiazon in/on soil is very high, since less than 25% of the applied herbicide **1** disappeared after 175 days under both moist (75% field capacity) and flooded conditions (Ambrosi et al., 1977). Li and Wong (1980) also found that 73–96% of the applied oxadiazon remained after 144 days in three soils duly saturated with water or flooded. Adsorption, translocation, and metabolism of the herbicide **1** in rice and other plants have also been studied (Ishizuka et al., 1974, 1975; Hirata and Ishizuka, 1975; Bingham et al., 1980; Achhireddy et al., 1984; George, 1982). The major metabolites isolated in rice plant were carboxylic acid, phenolic, alcohol, and dealkylated derivatives of **1** (Ishizuka et al., 1975). Oxadiazoline ring cleavage yielded a product identified as 1-(2,4-dichloro-5-isopropoxyphenyl)-1-(methoxycarbonyl)-2-trimethylacetyl hydrazine (DIMTH) from rice plant (Hirata and Ishizuka, 1975). Phenolic, carboxylic acid, and dealkylated derivatives of oxadiazon were also identified as metabolites of oxadiazon in soil (Ambrosi et al., 1977), but there appears to be no evidence of oxadiazoline ring cleavage and dechlorinated products of the herbicide **1** in/on soil.

A survey of literature revealed that the fate of oxadiazon (**1**) by soil microorganism has not been studied adequately. Furthermore, no microbial strain could be isolated that could effectively utilize oxadiazon as a sole source of carbon or nitrogen (Finkel'shtein et al., 1984), and they also found that 6 of 32 cultures, studied so far, affect about 20% transformation of **1** within 20–30 days. It was therefore thought worthwhile to undertake the *in vitro* degradation study of oxadiazon by *Fusarium solani* (Mortius) Sacc., an important soil-borne fungus, the results of which form the subject matter of the present paper.

EXPERIMENTAL PROCEDURES

Chemicals. Technical grade oxadiazon (85.9%) was purified by recrystallization first from benzene and then from ethanol (99.2%, mp 89–90 °C). Analytical oxadiazon (99.7%) was obtained from the U.S.EPA for comparison. All organic solvents used in the entire study were of analytical grade and freshly distilled prior use. All inorganic chemicals were of laboratory grade and used as obtained.

Analyses. *Chromatography. 1. TLC.* Thin layer chromatography was performed for isolation, identification, and purification of metabolites, on glass plates (20 cm × 20 cm) precoated with 0.5 mm silica gel G (Qualigens, India, TLC grade) using four different solvent systems: A, *n*-hexane–benzene (1:1 v/v); B, *n*-hexane–acetone (95:5 v/v); C, *n*-hexane–acetone (8:2 v/v); D, benzene–ethyl acetate (95:5 v/v). Iodine was used as chromogenic reagent for visualizing the spots.

2. GLC. Routine gas–liquid chromatographic estimation of oxadiazon was done on a Hewlett-Packard Model 5890A gas chromatograph coupled with a Hewlett-Packard 3392A integrator equipped with Ni^{63} electron capture detector (ECD). The column used for analysis was a glass column (6 ft × 2 mm i.d.) packed with 5% DC-200 on Chromosorb WHP (80–100 mesh).

3. HPLC. High-performance liquid chromatography was carried out for isolation and purification of metabolites on a Waters Associates (Milford, MA) high-performance liquid chromatograph with a U6K injector, M45 and 6080A dual pumps run by a model 720 system controller, and a Lambda Max Model 440 LC spectrometer set at 254 nm. Reversed phase separation was achieved on a 15 cm × 4.6 mm i.d. Supelcosil LC-18 (bonded octadecylsilane) column of 5 μm particle size from Supelco Inc. (Bellefonte, PA). The elution of the column was done by methanol–water (1:1 v/v) solvent mixture at a flow rate of 0.5 mL min^{-1}.

Spectroscopic Analyses. 1. IR. Infrared spectra were measured in KBr pellets using a Perkin-Elmer Model 1310 spectrophotometer.

2. ^1H NMR. ^1H nuclear magnetic resonance spectra were recorded on a General Electric QE-300 spectrometer (300 MHz)

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0021-8561/95/1443-2964$09.00/0 © 1995 American Chemical Society

with a Fourier transform system using $CDCl_3$ as solvent and TMS as an internal standard.

3. GC–MS. GC–MS spectra of the isolated metabolites of oxadiazon were obtained on a Hewlett-Packard Model 5890A Series II GC equipped with a Hewlett-Packard Model 5970A quadruple mass spectrometer and a Hewlett-Packard Model 9885 M disk drive with a DB-1 cross-linked methyl silicon capillary column (30 m × 0.25 mm i.d. and 0.25 μm film thickness), J&W Associates, San Francisco, CA) with splitless injection. Oven temperature was programmed from 100 to 265 °C at the rate of 10 °C min^{-1} with the initial temperature held for 3 min; the injection temperature was 230 °C. Helium (He) was used as carrier gas. Low-resolution electron impact mass spectra (EI-MS) were taken at an ionization potential of 70 eV, and the source block temperature was maintained at 150 °C, whereas chemical ionization (CI-MS) data were recorded at 60 °C with methane and D_2O used as reagent gas.

Isolation of Organisms and Growth Conditions. The fungus was isolated from rice field of alluvial soil of West Bengal, India [District Seed Farm, BCKV, Kalyani; sand, 46.37%; silt, 30.15%; clay, 23.48%; organic matter, 1.32%; CEC (mequiv 100 g^{-1}) 13.23; pH 7.2] with no previous history of oxadiazon application. Two grams of fresh and air-dried soil (80 mesh) was added to 100 mL of sterilized mineral nutrient solution containing 0.3 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of $CaCl_2 \cdot 2H_2O$, 0.2 g of $FeSO_4 \cdot 7H_2O$, 1.0 g of NH_4Cl, 2.0 g of KH_2PO_4, 3.0 g of Na_2PO_4, and 0.001 g of $(NH_4)_6MoO_{24} \cdot 4H_2O$ of distilled water, and oxadiazon (1), dissolved in a minimum volume of methanol, was added aseptically in a 250 mL conical flask; the final concentration of oxadiazon was 500 ppm. The flask was then incubated at 28 ± 1 °C. After 1 month, 10 mL of soil–water (nutrient) solution was pipetted from the flask and again added aseptically in another 250 mL of similar sterilized nutrient solution with oxadiazon (500 ppm) and incubated at 28 ± 1 °C for another 2 months in total darkness. Microorganisms were isolated from each flask by streaking an inoculum from the soil–water nutrient solution on an agar plate containing herbicide (1, 250 ppm) at 2 month intervals. After 5 days of incubation at 28 ± 1 °C, the selected colonies were transferred to the culture tubes containing nutrient solution with oxadiazon (250 ppm). Four Gram-positive bacteria and only one fungus were isolated; the fungus was characterized as *F. solani* (Mortius) Sacc. (345650).

For degradation study the fungus was then grown in modified Czapek Dox nutrient medium containing 3.0 g of $NaNO_3$, 1 g of KH_2PO_4, 0.5 g of $MgSO_4$, 9.5 g of KCl, 0.01 g of $FeSO_4 \cdot 7H_2O$, and 4 g of dextrose L^{-1} of distilled water (final pH 6.8) and taken in a 500 mL Erlenmeyer flask. After sterilization, oxadiazon (1) in minimum methanol was added aseptically to maintain the final concentration of oxadiazon of 500 ppm, and the flask was inoculated with the fungus followed by incubation at 28 ± 1 °C in total darkness.

Rate of Degradation and Fungal Biomass. To study the rate of degradation of oxadiazon (1) by *F. solani*, fungal cultures were incubated with the modified Czapek Dox nutrient medium with the herbicide (1) in 21 250 mL conical flasks each containing 40 mL of the medium over the period from 1 to 21 days. The herbicide (1) concentration in each flask amounted to 500 ppm, and the standard fungal spore suspension obtained from agar slant was added to each medium. The cultures were then incubated for 0, 2, 4, 6, 12, 17, and 21 days in the dark at 28 ± 1 °C. After desired time intervals, three flasks were harvested separately, and the content of each flasks was centrifuged at 1500 rpm; 20 mL of supernatent was then extracted with an equal volume of ethyl acetate for routine GC and TLC analyses. Similarly, another set of experiments was also performed with the nutrient medium containing oxadiazon (500 ppm) in the absence of dextrose. In both cases (with and without dextrose) the control flasks contained fungal mycelium in the nutrient medium without oxadiazon and oxadiazon in the nutrient medium without fungal inocula. No degradative products were detected in uninoculated controls. Again, in all cases for mycelial dry weight (biomass of fungal culture) determination, triplicate samples of the culture were harvested separately by suction filtration through a Whatman No. 1 filter paper and washed three times with equal volumes (75 mL) of distilled water. Then the filter papers containing mycelium were dried overnight in the oven at 80 °C and weighed.

Table 1. Rate Kinetics of Oxadiazon Degradation by *F. solani*

presence of dextrose	$Y = 92.24 - 2.54X$
	$r = -0.9694$
	$r^2 = 0.9397$
absence of dextrose	$Y = 99.05 - 1.51X$
	$r = -0.9786$
	$r^2 = 0.9577$

Metabolite Isolation. To produce sufficient quantities of oxadiazon (1) metabolites, 20 500 mL Erlenmeyer flasks, each containing 100 mL of sterilized nutrient medium with dextrose (4 g L^{-1}), were treated with oxadiazon (500 mg L^{-1}) in minimum methanol and inoculated with the fungus. After 15 days of incubation at 28 ± 1 °C, the contents of all the flasks were combined and filtered under vacuum to separate the mycelia from the medium. The mycelia were then washed with 10 × 20 mL of deionized distilled water. The aqueous phase was then extracted with an equal volume of ethyl acetate, and the mycelia were extracted with 500 mL of methanol for 5 h. Both organic extracts were combined, passed through anhydrous Na_2SO_4, and concentrated to 2–5 mL with the help of rotary vacuum evaporator at 50 °C. The concentrated organic extract was then subjected to column chromatography over a silica gel (Qualigens, India, 60–120 mesh) column (75 cm × 2.5 cm i.d.). The column was then successively eluted with 250 mL each of *n*-hexane and mixtures of 0.5, 2.0, 5, 10, and 20% acetone in *n*-hexane; these are designated fractions 1–6, respectively.

RESULTS AND DISCUSSION

The effect of sugar (dextrose) on the disappearance of oxadiazon (1) by the isolated soil fungus *F. solani* and corresponding fungal biomass are presented in Figure 1. The rate of degradation of the herbicide **1** by *F. solani* as well as fungal biomass seemed to be affected by the presence of dextrose (Table 1). There was no noticeable decrease in the amount of oxadiazon, as more than 70% of the applied herbicide (1) remained unchanged even after 3 weeks of incubation in the absence of dextrose. Similar results were also observed by Finkel'shtein et al. (1984). On the other hand, in the presence of sugar (dextrose) *F. solani* transformed more than 58% of oxadiazon within 21 days and yielded greater biomass. Similarly, in the absence of dextrose the fungal biomass increased steadily up to 8 days of incubation, after which time no remarkable growth was observed. Simultaneously, in the presence of dextrose the fungal biomass increased rapidly within the first 12 days and remained almost constant for the following days of incubation (Figure 1). The results thus indicated that *F. solani* could not effectively utilize oxadiazon as a sole source of carbon and, therefore, it can be assumed that the mechanism involved for the transformation of oxadiazon could be attributed to cometabolism.

GC–MS and TLC of six different column eluates (fractions 1–6) indicated the presence of 11 metabolites of oxadiazon (1) which were not found in controls. The chromatographic behavior TLC-R_f and GC–MS retention time (GC–R_t) of the isolated metabolites from different column eluates (corresponding fraction) are presented in Table 2. Among these 11 metabolites, 2 metabolites, designated $\mathbf{M_2}$ and $\mathbf{M_3}$, were characterized on the basis of their IR, ^1H NMR, and mass spectral data. Except for compounds $\mathbf{M_2}$ (estimated yield 16%) and $\mathbf{M_3}$ (estimated yield 19%), all other metabolites (an estimated yield of ≃2%) were isolated only in a small

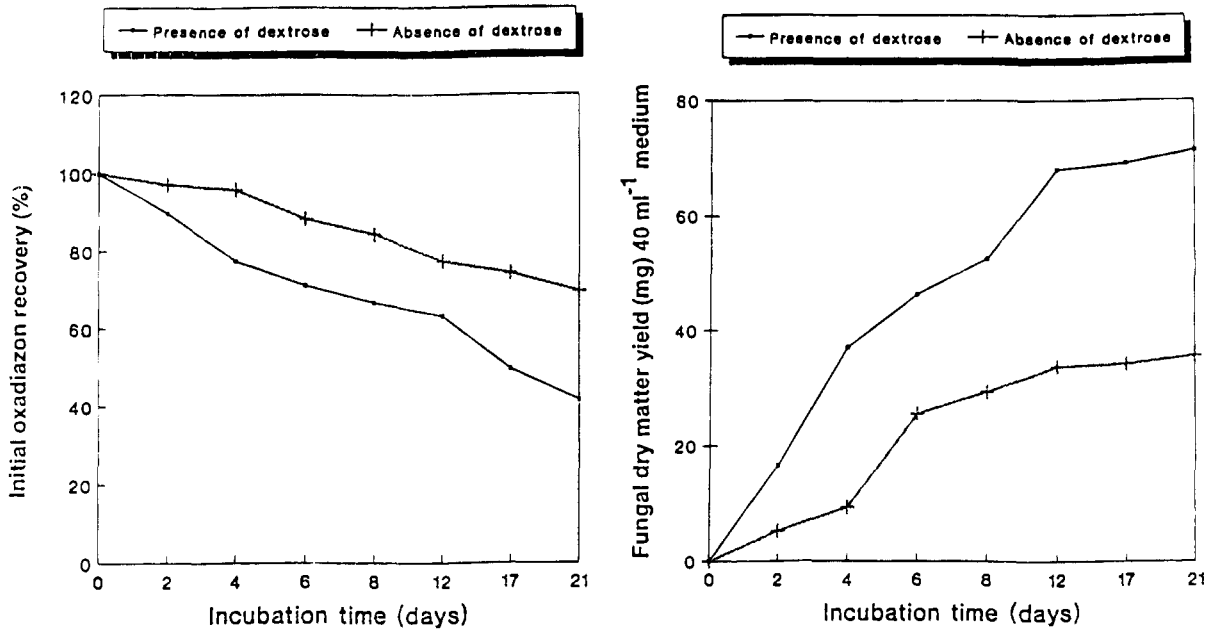


Figure 1. Degradation–utilization pattern of oxadiazon by *F. solani*.

Table 2. Chromatographic Behavior of Oxadiazon and Its Fungal Metabolites

column fraction[a] (250 mL)	product isolated	TLC R_f A[b]	B[b]	C[b]	D[b]	GC–MS R_t (retention time), min
fraction 1	$\mathbf{M_1}$	0.70				9.794
fraction 2	oxadiazon (I)	0.52	0.86		0.75	22.003
	unknown 1	0.31	0.53	0.71	0.62	20.101
fraction 3	$\mathbf{M_2}$	0.23	0.24		0.34	16.321
	$\mathbf{M_3}$	0.17	0.21	0.68	0.25	20.578
fraction 4	unknown 2			0.46	0.19	19.612
	unknown 3			0.42		18.783
	unknown 4			0.39		17.096
fraction 5	unknown 5			0.34		18.667
	unknown 6			0.31		23.007
fraction 6	unknown 7			0.20		15.459
	unknown 8			0.16		17.287

[a] Column fraction: fraction 1 (*n*-hexane); fraction 2 (0.5% acetone in *n*-hexane); fraction 3 (2% acetone in *n*-hexane); fraction 4 (5% acetone in *n*-hexane); fraction 5 (10% acetone in *n*-hexane); fraction 6 (20% acetone in *n*-hexane). [b] TLC solvent system: (A) *n*-hexane–benzene (1:1 v/v); (B) *n*-hexane–acetone (95:5 v/v); (C) *n*-hexane–acetone (8:2 v/v); (D) benzene–ethyl acetate (95:5 v/v).

amount and, therefore, excluding mass spectra (EI-MS), no further tests were performed with these compounds ($\mathbf{M_1}$ and unknowns 1–8). The electron impact (EI) mass fragmentation patterns of compounds $\mathbf{M_1}$, $\mathbf{M_2}$, and $\mathbf{M_3}$ and oxadiazon are presented in Table 3, and EI-MS data of all other unknown metabolites (unknowns 1–8) of oxadiazon are listed in Table 4. Elution of the column with *n*-hexane (fraction 1) after concentration gave a yellow residue, which on subsequent crystallization yielded $\mathbf{M_1}$ (R_f 0.7, solvent system A). The EI-MS spectrum of compound (Figure 2) $\mathbf{M_1}$ (Table 2) showed the molecular ion *m/z* 204 [$(M)^+$, $C_9H_{10}OCl_2$]. That the molecule possessed an isopropoxy group attached to a benzene moiety was confirmed by the appearance of ionic fragments at *m/z* 162 [$(M - C_3H_7+H^+$] and 133 [$(M - C_3H_7 - CO)^+$]. The presence of two chlorine atoms was evident from the isotopic abundance of *m/z* 206 [$(M + 2)^+$] and 208 [$(M + 4)^+$] along with the formation of ionic fragments at *m/z* 126 [$(162 - Cl - H)^+$] and 98 ($(133 - Cl)^+$]. On the basis of the mass spectral evidence, the structure of metabolite $\mathbf{M_1}$ could be postulated as 2,4-(dichloroisopropoxy)benzene.

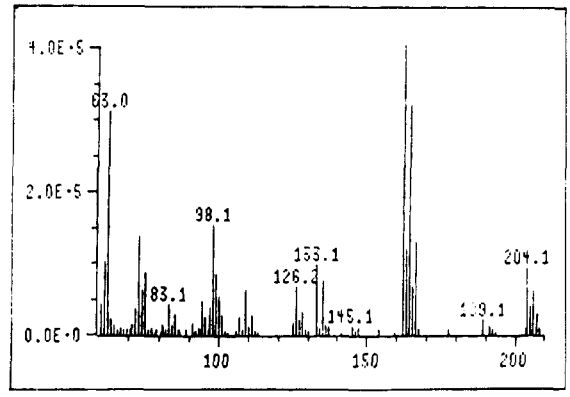


Figure 2. EI-MS of compound $\mathbf{M_1}$.

Again TLC (solvent system B) of concentrated column fraction 2 (0.5% acetone in *n*-hexane) indicated the presence of a mixture of two compounds (R_f 0.86 and 0.53). A white solid of R_f 0.86 (solvent system B) was isolated from fraction 2 by preparative TLC using solvent system A (R_f 0.52), which had identical physical (mmp, CO-TLC) and spectral (CO-IR, mass, and ^{1}H NMR) behavior with that of the parent compound, oxadiazon (**1**).

Column fraction 3 [5% acetone in *n*-hexane (250 mL)] was concentrated and subjected to preparative TLC in solvent system B. The major bands (R_f 0.16–0.33) were scrapped from the plate and extracted with diethyl ether. The latter was then further purified by semi-preparative HPLC using methanol–water (1.1 v/v) as eluting solvent at 0.5 mL min^{-1}. The HPLC eluate of retention time R_t = 28.38 min after concentration afforded a white solid, crystallized from acetone–water as colorless crystals $\mathbf{M_2}$ (mp 118–120 °C). The other eluate (HPLC) of R_t = 22.44 min yielded another white solid, crystallized from *n*-hexane–chloroform (mp 102–104 °C) and designated metabolite $\mathbf{M_3}$.

The ^{1}H NMR chemical shift and coupling information for oxadiazon and its metabolites $\mathbf{M_2}$ and $\mathbf{M_3}$ are enumerated in Figure 3. The presence of an *O*-isopropyl moiety [$-OCH(CH_3)_2$] in all three molecules was evidenced by two methyl (C1′) doublets (δ 1.36–1.42) sharing a 3 Hz coupling with a methine quartet (C1′–

Table 3. Mass [EI-MS] Fragmentation Pattern of Compounds M_1, M_2, and M_3 and Oxadiazon

compd	mass fragments, *m/z*	rel abundance, %	probable assignments
M_1	208	02.3	$M^+ + 4$
	206	13.9	$M^+ + 2$
	204	23.1	M^+
	162	100	$M^+ - C_3H_7 + H$
	133	23.3	$M^+ - C_3H_7 - CO$
	126	17.1	$M^+ - C_3H_7 - Cl$
oxadiazon	348	3.6	$M^+ + 4$
	346	23.8	$M^+ + 2$
	344	38.3	M^+
	302	52.5	$M^+ - C_3H_7 + H$
	285	69.2	$M^+ - C_3H_7 - CO_2 + H$
	202	12.6	$M^+ - C_3H_7 - CO_2 - C_4H_9$
	175	100	$M^+ - C_3H_7 - CO_2 - C_4H_9 - HCN + 2H$
M_2	380	2.7	$M^+ + 4$
	378	11.7	$M^+ + 2$
	376	18.4	M^+
	341	76.3	$M^+ - Cl$
	334	7.9	$M^+ - C_3H_7 + H$
	299	100	$M^+ - C_3H_7 - Cl + H$
	250	25.4	$M^+ - C_3H_7 - CO(CH_3)_3 + 2H$
	215	79.8	$M^+ - C_3H_7 - CO(CH_3)_3 - Cl + 2H$
	191	38.6	$M^+ - C_3H_7 - CO(CH_3)_3 - COOCH_3 + 2H$
	176	12.3	$M^+ - C_3H_7 - CO(CH_3)_3 - COOCH_3 - NH + 2H$
	148	7.1	$M^+ - C_3H_7 - CO(CH_3)_3 - COOCH_3 - NH - HCN + H$
	85	50.9	$-COC(CH_3)_3^+$
M_3	322	3.9	$M^+ + 4$
	320	25.5	$M^+ + 2$
	318	38.8	M^+
	276	13.7	$M^+ - C_3H_7 + H$
	241	3.8	$M^+ - C_3H_7 - Cl + H$
	192	36.3	$M^+ - C_3H_7 - COC(CH_3)_3 + 2H$
	176	5.0	$M^+ - C_3H_7 - COC(CH_3)_3 - NH + H$
	149	6.3	$M^+ - C_3H_7 - COC(CH_3)_3 - NH - HCN$
	85	25.0	$-COC(CH_3)_3$

Table 4. Mass Fragmentation Pattern of Unknown Metabolites of Oxadiazon (Unknowns 1–8) and Their Probable Assignments

metabolite	*m/z* [rel abundance, %]	
	M^+	other major ion fragments
unknown 1	298 [26.2]	300 [1.2], 299 [2.7], 255 [$(M - COCH_3)^+$, 20.5], 213 [$(M - COC(CH_3)_3)^+$, 4–8], 199 [17.4], 171 [$(213 - COCH_3 + H)^+$, 10.7], 157 [6.1], 143 [27.2], 129 [$(171 - NH - HCN)^+$, 10.8]
unknown 2	294 [9.5]	263 [$(M - OCH_3)^+$, 5.3], 234 [$(M - COOCH_3 + H)^+$, 1.5], 209 [$(M - COC(CH_3)_3)^+$, 13.7], 180 [18.9], 150 [$(209 - COOCH_3)^+$, 9.5], 136 [$(150 - NH + H)^+$, 11.6], 109 [$(150 - NH - HCN + H)^+$, 32.6], 81 [100]
unknown 3	346 [10.1]	350 [0.9], 348 [6.4], 261 [$(M - COC(CH_3)_3)^+$, 33.7], 245 [$(261 - CH_3)^+$, 22.5], 219 [$(261 - COCH_3 + H)^+$, 11.8], 203 [$(219 - NH_2)^+$, 191 [18.7], 163 [7.9]
unknown 4	270 [9.7]	272 [0.8], 271 [2.8], 242 [6.9], 213 [2.8], 185 [$(M - COC(CH_3)_3)^+$, 6.9], 171 [$(185 - NH + H)^+$, 8.3], 157 [18.1], 143 [$(185 - NH - HCN)^+$, 25], 129 [12.5]
unknown 5	332 [38.5]	336 [2.9], 334 [22.3], 296 [$(M - HCl)^+$, 8.8], 247 [$(M - COC(CH_3)_3)^+$, 47.3], 233 [23.1], 205 [$(247 - COCH_3 + H)^+$, 6.8], 189 [$(205 - NH_2)^+$, 100], 161 [12.1]
unknown 6	340 [45.3]	342 [5.5], 341 [9.4], 284 [100], 241 [25.1], 185 [27.2], 171 [$(M - C_3H_7 - C_4N_9 - CO_2 - HCN + 2H)^+$, 10.9]
unknown 7	256 [11.4]	258 [0.8], 257 [2.5], 199 [$(M - C_4H_9)^+$, 7.1], 185 [6.9], 171 [$(M - COC(CH_3)_3$, 8.6], 157 [$(171 - NH + H)^+$, 12.9], 114 [$(129 - NH)^+$, 17.1], 87 [$(114 - HCN)^+$, 100]
unknown 8	278 [2.7]	223 [$(M - C_4H_9 + H)^+$, 26.9], 147 [$(M - COC(CH_3)_3 - COCH_3 - H)^+$, 100], 121 [45.1], 93 [21.6]

C2′) appearing in the range between δ 4.43 and 4.58. The two aromatic hydrogens (C3 and C6) of all the compounds (**1**, M_2, and M_3) appeared as two singlets (δ 7.05–7.38 for C_3 and δ 7.42–7.53 for C_6), confirming the presence of two meta oriented aromatic chlorine atoms. However, products M_2 and M_3 showed broad NH resonances (δ 7.93 or 6.32 and 6.47) which are not found in oxadiazon, implying that nitrogens in M_2 and M_3 are not present within the heterocyclic oxadiazoline ring as in oxadiazon. This substantiates that in both M_2 and M_3 the oxadiazoline ring has been cleaved. The three proton multiplets at δ 3.77 along with the 1H multiplet at δ 7.93 (D_2O exchanged) in the molecule M_2 clearly indicated that the product (M_2) contained an *N*-methyl ester functionality. The presence of two para protons (C3 and C6) in both M_2 and M_3 proved beyond doubt that the aromatic part of these two molecules remained unchanged.

The electron impact (EI) mass spectrum of M_2 (Figure 4) indicated the molecular ion at *m/z* 376 (M^+) along with other major peaks at *m/z* 341, 334, 299 (base peak) 250, 215, 191, 176, 148, and 85. The mass fragmentation pattern of the compound (Table 3) showed that the base peak at *m/z* 299 could originate by losing one chlorine atom and $-C_3H_7$ of that isopropoxy side chain from the molecule. The ion fragments at *m/z* 334 and 250 could also be produced due to consecutive loss of 43 mu [$-C_3H_7$ of the isopropoxy side chain] and 84 mu

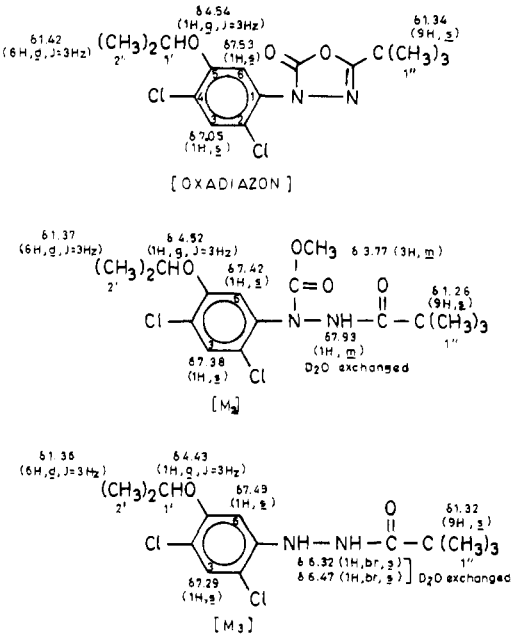


Figure 3. ^{1}H NMR data of compounds $\mathbf{M}_2$ and $\mathbf{M}_3$ and oxadiazon.

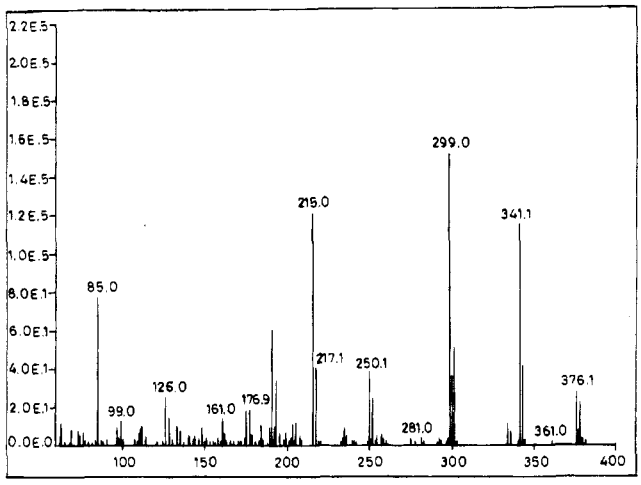


Figure 4. EI-MS of compound $\mathbf{M}_2$.

$[-COC(CH_3)_3]$ from M^+. The ratio of the intensities of the isotopic ions at *m/z* 376 [18.45%, $(M)^+$], 378 [12.33%, $(M + 2)^+$], and 380 [3.36%, $(M + 4)^+$] was 10:7:2, which implied the presence of two chlorine atoms in the molecule.

The IR spectrum of $\mathbf{M}_2$ also confirmed the existence of >CO in $NHCO(CH_3)_3$ (1682 cm^{-1}) and >CO in $COOCH_3$ (1741 cm^{-1}). On the bais of the above mass spectral as well as IR spectral evidence in combination with ^{1}H NMR data discussed previously, the structure of metabolite $\mathbf{M}_2$ was characterized as 1-(2,4-dichloro-5-isopropoxyphenyl)-1-(methoxycarbonyl)-1,2-trimethylacetyl hydrazine (DIMTH). The IR, mass, and ^{1}H NMR data of compound $\mathbf{M}_2$ were also well in agreement with the reported data for DIMTH (Hirata and Ishizuka, 1975). As methanol, ethyl acetate, and acetone have been used for the extraction and cleanup of metabolite $\mathbf{M}_2$, it would be important to establish that the compound ($\mathbf{M}_2$) was a true metabolite and not an artifact. For this purpose four sets of culture medium were extracted separately with $CHCl_3$, CH_2Cl_2, C_6H_6, and diethyl ether. The GC–MS of each organic extract upon concentration (individually) also showed the existence of $\mathbf{M}_2$. On the other hand, DIMTH ($\mathbf{M}_2$) was also

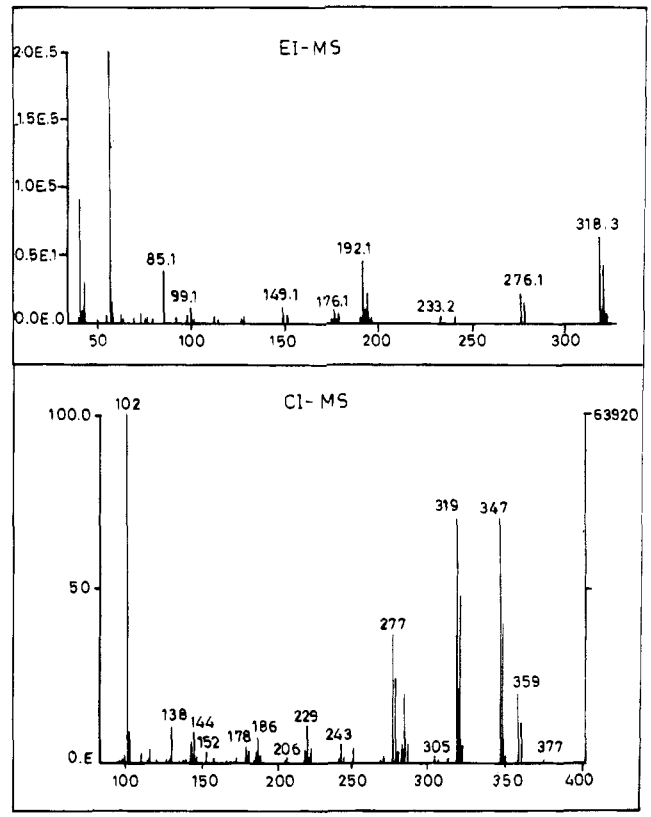


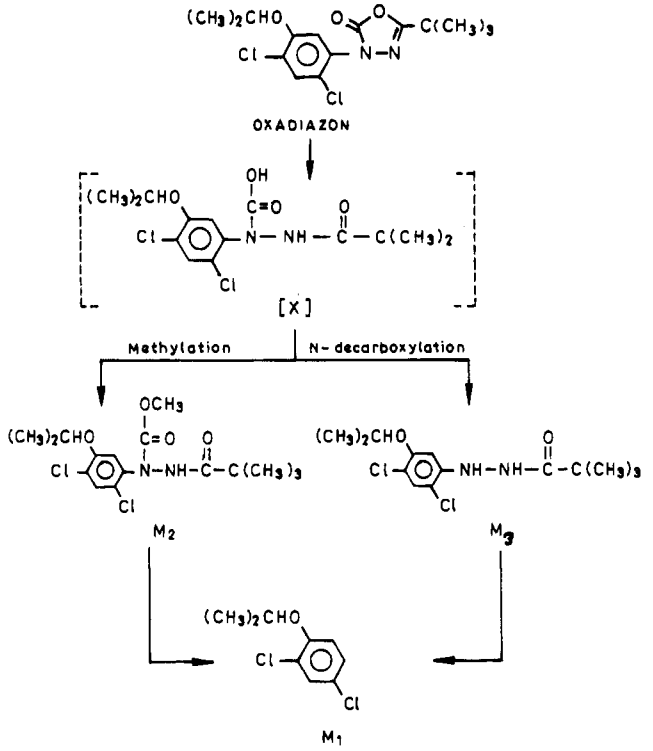
Figure 5. EI-MS and CI-MS of compound $\mathbf{M}_3$.

reported as one of the major metabolites of oxadiazon in rice plant (Hirata and Ishizuka, 1975). It is, therefore, quite reasonable that $\mathbf{M}_2$ was formed during incubation through biological interaction only and not during workup.

Chemical ionization mass spectrometry (CI–MS) of compound $\mathbf{M}_3$ with methane as reagent gas (Figure 5) established the molecular ion at *m/z* 218, 318 [70.39%, $(M + H)^+$], 347 [7%, $(M + C_2H_5)^+$], and 359 [1.97%, $(M + C_3H_7)^+$]. On the other hand, the electron impact mass spectrum of the compound (Figure 5) also exhibited the molecular ion at *m/z* 318 [38.8%, $(M)^+$] together with isotopic peaks at *m/z* 320 [$(M + 2)^+$] and 322 [$(M + 4)^+$], confirming the existence of two chlorine atoms. The other major diagnostic fragments at *m/z* 276 [$(M - C_3H_7+H)^+$], 241 [$(276 - Cl)^+$], 192 [$(276 - COC(CH_3)_3 + H)^+$], 176 [$(192 - NH_3)^+$], 149 [$(176 - HCN)^+$], and 85 [$(-COC(CH_3)_3)^+$] were also discernible in the mass fragmentation pattern of metabolite $\mathbf{M}_3$ (Table 3). The prominent ion at *m/z* 57 is a characteristic fragment of aliphatic ketone of the trimethylacetyl side chain and usually accounts for the base peak. Therefore, the ion peaks at *m/z* 192, 85, and 57 arise presumably from the existence of the trimethylacetyl side chain. Deuterium exchange CI-MS with D_2O gave a quasi-molecular ion at *m/z* 222, indicating the presence of two exchangeable hydrogens, which could be further confirmed by the existence of two –NH functionalities attached to the aromatic moiety. From the above mass spectral data coupled with ^{1}H NMR evidence (discussed previously), the structure of $\mathbf{M}_3$ was characterized as 1-trimethylacetyl-2-(2,4-dichloro-5-isopropoxyphenyl) hydrazine.

The electron impact mass spectral data of the other eight unknown metabolites (Table 4) are not sufficient for elucidation of their structures. However, the mass spectra of these metabolites exhibited some important characteristic fragments which would be very helpful for pursuing future research in this direction.

Scheme 1. Plausible Metabolic Pathway of Oxadiazon by *F. solani*



On the basis of the metabolites of oxadiazon (**1**) produced by *F. solani* a plausible pathway involved in oxadiazon transformation is depicted in Scheme 1. It is revealed from the present study that oxadiazoline ring cleavage is the main and first point of oxadiazon metabolism and no dechlorination was observed. Heterocyclic oxadiazoline ring cleavage of oxadiazon yielded an intermediate product (**X**) which underwent N-decarboxylation to form metabolite $\mathbf{M}_3$, while $\mathbf{M}_2$ was formed by methylation. Complete elimination of the oxadiazoline heterocyclic ring from the aromatic moiety of oxadiazon resulted in $\mathbf{M}_1$. The metabolites $\mathbf{M}_1$ and $\mathbf{M}_3$ are now reported for the first time as metabolites of oxadiazon, but no alcoholic, phenolic, and carboxylic acid derivatives of oxadiazon without oxadiazoline ring cleavage could be identified that were previously reported as metabolites of **1** in soil and plants (Ishizuka et al., 1974, 1975; Hirata and Ishizuka, 1975; Ambrosi et al., 1977).

ACKNOWLEDGMENT

We thank Dr. D. Brayford, International Micological Institute, U.K., for kind assistance in identifying and characterizing the isolated soil-borne fungus *F. solani* (Mortius) Sacc. (345650) and express our gratitude to M/s. Rhone-Poulenc (India) Ltd. for supplying technical grade oxadiazon.

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Received for review September 22, 1994. Revised manuscript received May 31, 1995. Accepted June 21, 1995.[⊗] S.K.C. gratefully acknowledges financial support from CSIR, New Delhi (India), as Senior Fellowship for carrying out this work.

JF930486L

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1995.