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Factors Affecting Triadimefon Degradation in Soils

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The degradation of triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one] was studied in two soils, mollisol and inseptisol, under varying conditions of moisture and temperature, and the role of cow manure amendment and soil sterilization on fungicide degradation was ascertained. The soil moisture content affected the pathway followed for triadimefon degradation. In nonflooded soils (60% water-holding capacity), triadimefon was reduced to triadimenol, and in flooded soils, it was metabolized to the diol derivative [1-(1*H*-1,2,4-triazol-1-yl)-3,3-dimethylbutan-2-one-1,4-diol]. In nonflooded soils, triadimefon was more persistent in soil having more organic carbon content (mollisol), and the amendment of cow manure (5%) further enhanced its persistence. On the contrary, in flooded soil systems, the higher the soil organic carbon content was, the less persistent was the fungicide, and amendment of cow manure further enhanced its degradation. Triadimefon degradation was faster at 35 °C than at 27 °C. Triadimefon degradation in soils was mediated by the microorganisms, and no triadimefon degradation was observed in sterile soils. Triadimefon (1 mg/kg) did not affect soil phosphatase activity in either of the soils; however, soil dehydrogenase activity was significantly reduced, especially in mollisol soil.

KEYWORDS: Flooded; nonflooded; cow manure; sterilized; sorption; degydrogenase activity; phosphatase activity

INTRODUCTION

Triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one] is a systemic, broad spectrum, eradicant, and protectant fungicide against plant pathogens, especially powdery mildew, loose smut, and rust of cereals and other crops (*1*). It acts by inhibition of ergosterol biosynthesis.

Knowledge of the pathways of degradation and factors influencing the degradation is important in predicting the levels of pesticides likely to remain in soils and allows assessment of the potential risk associated with the exposure. The fate of a soil-applied pesticide is affected by many factors viz. soil constituents, microorganisms, moisture status, sorption, and physicochemical properties of the soils.

Most of the information on persistence of triadimefon is derived from registration data with relatively few studies available in the open literature. In soils, triadimefon is readily converted to triadimenol (*2*−*4*), which is more persistent than triadimefon. Bromilow et al. (*2*) observed a half-life of nearly 1 year for triadimenol in a laboratory incubation study; however, in a field study, no residues of triadimefon and triadimenol were detected even after 12 years of consecutive annual application of triadimefon at 0.25kg/ha. In another study (*3*), the rate of degradation of triadimefon increased 3-fold as the temperature increased from 5 to 18 °C, although the decreasing soil moisture content from 80 to 60% slightly slowed the degradation. Triadimenol (derived from triadimefon) was quite persistent at 10 °C and 80% field capacity with a half-life of more than 2 years. Even under field conditions (*4*), triadimenol, formed from triadimefon applied at 0.5kg/ha, showed a half-life of >400 days. However, no triadimenol was detected 2.5 years after the triadimefon application. Soil fungi can metabolize triadimefon to triadimenol (*5*−*7*) or its isopropyl analogue (*5*). There are no published studies on triadimefon persistence in flooded soils, which are predominantly anaerobic in nature and behave differently to aerobic soils (*8*).

This paper reports the behavior of triadimefon in two soil types. The purpose of this work was to determine the relative importance of temperature, moisture, microorganisms, organic manure, and pesticide sorption on the degradation of triadimefon in soil.

Table 1. Physicochemical Properties of the Soils

soil type	organic carbon (%)	pH	sand (%)	silt (%)	clay (%)
mollisol	1.0	7.0	74	13	9
inceptisol	0.5	8.5	77	9	12

MATERIALS AND METHODS

Soils. Two different agricultural soils were collected from the experimental farms of the Indian Agricultural Research Institute (New Delhi) and G. B. Pant University of Agriculture and Technology (Pantnagar, Uttranchal). The soils were collected from the 0−15 cm soil profile, air-dried, ground to pass through a 2 mm sieve, and stored in plastic bags at room temperature. Physicochemical characteristics of the soils (**Table 1**) were determined by standard methods. The soil pH was determined in a 1:1.25 soil:water suspension using a glass

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electrode (*9*), organic carbon content by the Walkley and Black method (*10*) and soil mechanical fractions by the hygrometer method (*11*).

The cow manure was obtained locally. The physicochemical characteristics of manure included a pH of 6.3 and an organic carbon content of 21.3%; the total carbon, nitrogen, and hydrogen contents of manure were determined by elemental analysis and were 25.3, 6.7, and 5.8%, respectively.

Chemicals. An analytical grade sample of triadimefon (99% purity) was supplied by Bayer India. Triadimenol was prepared according to the method reported by Deas et al. (*12*). Solvents were of analytical grade.

Degradation Studies. *Effect of Aerobic and Anaerobic Conditions.* Portions (5 g) of soils in sterilized glass test tubes (200 mm × 25 mm) were supplemented with sterile distilled water to obtain nonflooded (60% water-holding capacity) and flooded (soil:water ratio of 1:1.25, w/v) soil conditions. Prior to the addition of triadimefon, flooded soils were incubated at 28 ± 1 °C to allow the development of reducing conditions. Triadimefon (5 μg) was added to the soils in 0.1 mL of acetone. The tubes were closed with cotton plugs and then incubated at 28 ± 1 °C under light. Moisture was maintained by adding a required amount of water at weekly intervals, and at periodic intervals, duplicate samples were removed for analysis.

Effect of Temperature. Soil samples (5 g), both flooded and nonflooded, were prepared as mentioned in previous section. Soil samples were incubated at 28 ± 1 and 35 ± 1 °C. At regular intervals, duplicate samples were removed for analysis.

Effect of Organic Manure. To quantify the effect of such amendments on the persistence of triadimefon, the following experiment was performed. Soils samples (5 g), flooded and nonflooded, without and with 5% cow manure, were prepared as mentioned in the previous section. The soils were incubated at 28 ± 1 °C, and at regular intervals, duplicate samples were removed.

Effect of Soil Sterilization. To ascertain whether triadimefon degradation in soils is mediated by microorganisms, its degradation was compared in sterilized and nonsterilized soils. Soils (5 g) in sterilized tubes (200 mm × 25 mm) were sterilized at 15 bar pressure for 1 h on each of three consecutive days. Soils were supplemented with sterile distilled water to represent flooded and nonflooded conditions. Similarly maintained nonsterile soils served as a control. Prior to fungicide application, flooded soils were allowed to become reducing. Both sterile and nonsterile soils were treated with triadimefon (5 μg) in 0.1 mL of acetone. Samples were incubated at 28 ± 1 °C and at regular intervals samples, in duplicate, were removed for analysis.

Enzymatic Studies. Pesticides are known to affect the metabolic activity of soils and so may be affecting several enzymes. Therefore, the effect of triadimefon on phosphatase and dehydrogenase activity was studied. Nonflooded soil samples (100 g) in 250 mL Erlenmeyer flasks were treated with triadimefon (1 mg/kg) in 0.1 mL of acetone. Soil samples treated with 0.1 mL of acetone alone served as a control. Phosphatase was measured using *p*-nitrophenylphosphate as the substrate (*13*), and the dehydrogenase activity was measured by the method described by Casida et al. (*14*).

Sorption Studies. Soil samples (5 g, oven-dried basis) in stoppered 50 mL glass tubes were equilibrated with 10 mL of aqueous solution of triadimefon on an end-over-end shaker for 4 h. Sorption was performed at four concentrations varying from 2.5 to 20 mg/L, and each concentration was replicated three times. After 4 h of equilibration, the soil suspension was centrifuged at 6000 rpm for 10 min and the supernatant solution was analyzed for triadimefon. The amount of triadimefon sorbed in soil was calculated by subtracting the amount of triadimefon in the supernatant at equilibrium from its initial concentration in aqueous solution. To get the mass balance of triadimefon, for one set of soil, the amount of triadimefon sorbed by the soil was extracted and quantified. Results indicated that there was no degradation of triadimefon during the equilibration.

Redox Potential (Eh) Measurement. Flooded soils are predominantly anaerobic in nature. Therefore, the Eh of the flooded soils was measured during the course of the study. Soil (40 g), with and without 5% cow manure, was flooded with distilled water (50 mL) in 100 mL beakers, which was covered with plastic sheet containing five pinholes for gaseous exchange. At intervals, the soil Eh was measured using an Elico digital meter fitted with a compound platinum-calomel electrode. The electrode was placed in the reduced zone (about 1−2 cm below the oxidized zone) of the flooded soil.

Table 2. Influence of Soil Conditions on K_{obs} Values for Triadimefon Persistence in Soils

soil conditions	$K_{obs} \times 10^{-3}$ (days^{-1})	$t_{1/2}$	r^2
mollisol—nonflooded	–17.8	16.2	0.989
mollisol—flooded	–39.0	10.2	0.873
mollisol—27 °C	–17.8	16.2	0.989
mollisol—35 °C	–40.9	7.4	0.965
mollisol—nonflooded (no manure)	–17.8	16.2	0.989
mollisol—nonflooded (+ manure)	–15.7	19.2	0.998
mollisol—flooded (no manure)	–39.0	10.2	0.873
mollisol—flooded (+ manure)	–49.6	6.1	0.820
inceptisol—nonflooded	–20.4	14.8	0.968
inceptisol—flooded	–10.7	28.9	0.956
inceptisol—27 °C	–20.4	14.8	0.968
inceptisol—35 °C	–25.5	11.8	0.969
inceptisol—nonflooded (no manure)	–20.4	14.8	0.968
inceptisol—nonflooded (+ manure)	–13.0	23.1	0.995
inceptisol—flooded (no manure)	–10.7	28.9	0.956
inceptisol—flooded (+ organic manure)	–11.4	26.4	0.915

Extraction and Analysis. Triadimefon residues from soils were extracted by equilibrating soils (5 g) with hexane (5 mL) and anhydrous Na_2SO_4 (1 g) for 1 h. After equilibration, the triadimefon residues were quantified using a gas chromatograph (GC) [Hewlett-Packard (Palo Alto, CA)] model 3840 equipped with a ^{63}Ni electron capture detector and fitted with HP-1 column [10 m (l) × 0.50 mm (i.d.) × 2.53 μm film thickness]. The operating parameters were as follows: column, 210 °C; injector, 250 °C; detector, 250 °C; and carrier gas flow, 40 mL/min. Using this extraction procedure, the recovery of triadimefon at fortification levels between 0.1 and 1.0 mg/kg levels was more than 80%. However, for calculation purposes, zero time samples were taken as the 100%.

Triadimefon residues from aqueous samples were extracted by shaking the sample (1 mL) with hexane (2 mL) for 1 min. Anhydrous sodium sulfate (50 mg) was added to each tube to remove any trace of moisture from organic layer. Residues in hexane fraction were estimated by GC as above.

RESULTS AND DISCUSSION

Degradation data from all of the conditions studied fit well to the first-order kinetic equation: $\log(C/C_o) = -K_{obs}t$, where, C_o is the initial concentration of triadimefon (mg/kg), C is its concentration (mg/kg) after time t (days), and K_{obs} is the rate constant of the reaction.

Effect of Soil Type and Aerobic and Anaerobic Conditions. The soil moisture content affected the rate of triadimefon degradation in both soils (**Table 2**). In mollisol, the rate of degradation of triadimefon was higher under flooded than nonflooded conditions with half-lives of 10.2 and 16.2 days, respectively. However, in inseptisol soil triadimefon was degraded at a faster rate under nonflooded than flooded conditions and respective half-lives were 14.7 and 28.9 days. The GC chromatogram of organic solvent extract from nonflooded mollisol soil showed one extra peak (other than the peak for triadimefon) at a retention time of 2.35 min indicating the formation of only one detectable metabolite. GC-MS spectra of this metabolite have a molecular ion peak at *m*/*z* 295, and this matches with the mass spectrum of authentic triadimenol. Thus, in nonflooded soils, triadimefon is metabolized to triadimenol. Earlier studies have also shown that in soils triadimefon is immediately converted to triadimenol (*2*−*4*). The GC chromatogram of organic extract from flooded mollisol soil showed one additional peak (in addition to the peak for

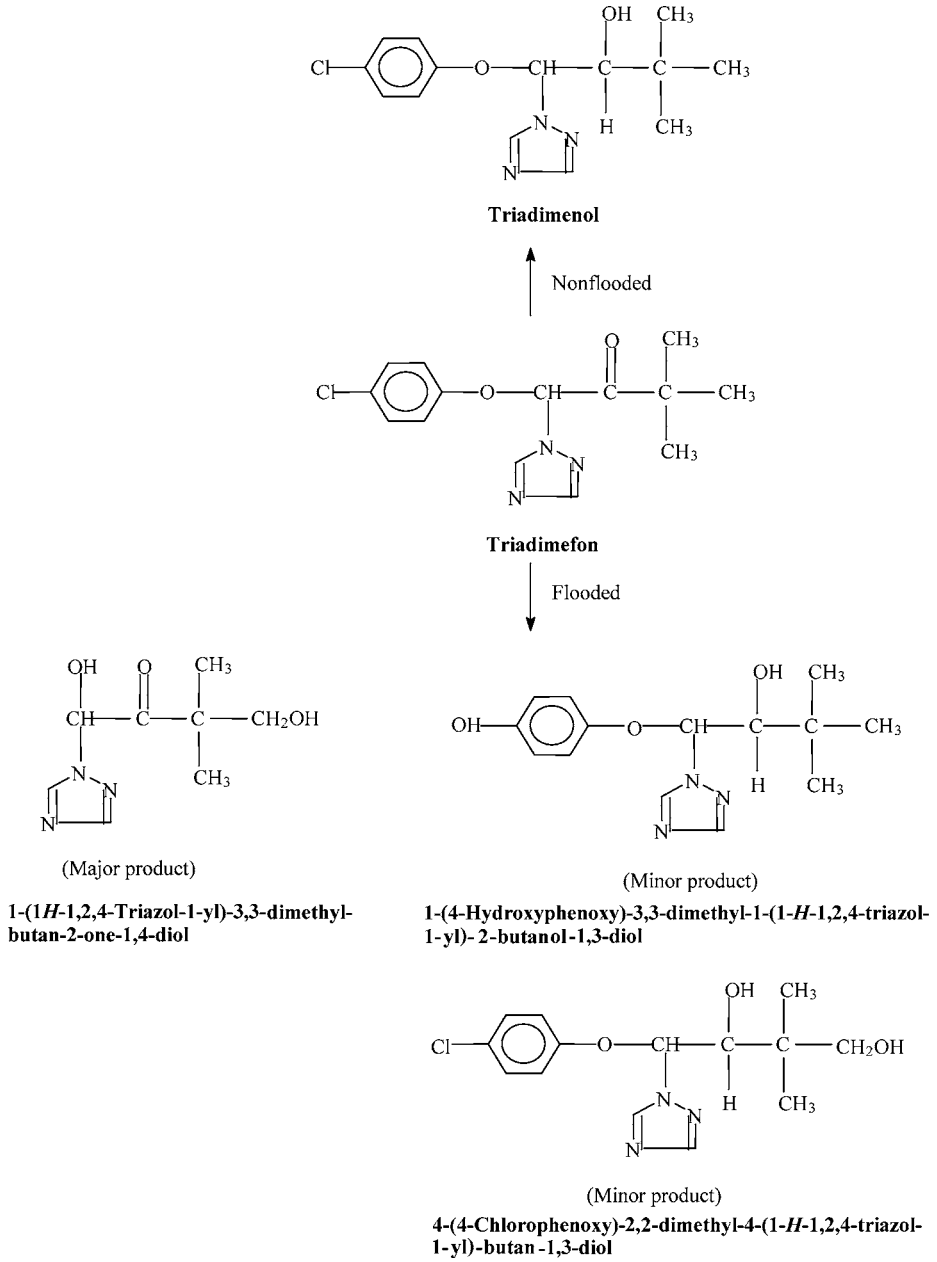

Figure 1. Triadimefon metabolites formed in soils.

triadimefon) with a retention time of 1.23 min, indicating that the triadimefon metabolite, formed in flooded soil, is different from triadimenol, which is formed in nonflooded soils (**Figure 1**). GC-MS analysis of this extract from flooded mollisol soil indicated that the metabolite had a molecular ion peak at *m*/*z* −199 (M^+), a base peak at *m*/*z* −198 ($M^+ - 1$), and fragment ion peaks at *m*/*z* −182 ($M^+ - OH$), 115 [$^+CH_2COC(CH_3)_2CH_2OH$], 103 [$^+CHOHC(CH_3)_2CH_2OH$], 74 [$^+CH(CH_3)_2CH_2OH$], and 68 (1,2,4-triazole). On the basis of the fragmentation pattern, the metabolite was tentatively identified as a diol derivative [1-(1*H*-1,2,4-triazol-1-yl)-3,3-dimethyl-butan-2-one-1,4-diol]. Although the GC chromatogram of the flooded soil extract did not show any other peak, the GC-MS chromatogram (total ion chromatograph) showed two more metabolite peaks at R_t 12.11 and 14.10 min. That at R_t 12.11 min showed a molecular ion peak at *m*/*z* −277 (M^+), a base peak at *m*/*z* −205 [$M^+ - C(CH_3)_3$; −OH], and fragment peaks at *m*/*z* −261 ($M^+ - OH$), 220 (M^+ $C(CH_3)_3$], 189 [$M^+ - CHOH(CH_3)_3$], 175 [$M^+ - OH$; $-CHOH(CH_3)_3$], 68 (1,2,4-triazole), and 57 [$^+C(CH_3)_3$]. This metabolite was tentatively identified as 1-(4-hydroxyphenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol. The mass fragmentation pattern of the metabolite at R_t 14.40 min showed a molecular ion peak at *m*/*z* −311 (M^+), a base peak at *m*/*z* −205 [$M^+ - Cl$; $-^+C(CH_3)_2CH_2OH$], and fragment ion peaks at *m*/*z* −240 ($M^+ - {}^+C(CH_3)_2CH_2OH$], 174 [$M^+ - Cl$; $-^+CHOHC(CH_3)_2CH_2OH$], 128 (*p*-chlorophenol), and 69 (1,2,4-triazole). This metabolite was tentatively identified as [4-(4-chlorophenoxy)-2,2-dimethyl-4-(1H-1,2,4-triazol-1-yl)-1,3-butandiol]. None of the earlier reports showed the formation of 1-(1*H*-1,2,4-triazol-1-yl)-3,3-dimethyl-butan-2-one-1,4-diol during triadimefon metabolism in soil. However, formation of the other two minor products has been shown in photochemical and animal metabolism studies (*15*). This study indicated that degradation of triadimefon in flooded and nonflooded soils followed different pathways. Triadimenol, formed from triadimefon, did not persist in the mollisol soil, and its concentration in soil never exceeds 0.1 mg/kg (10% of the amount of triadimefon applied). None of the triadimefon metabolite formed in mollisol soil (nonflooded and flooded) was recovered from

Table 3. Redox Potential (Eh) of Flooded Soils

incubation (days after flooding)	incubation (days after pesticide application)	Eh (mV)			
		mollisol		inseptisol	
		no manure	+ manure	no manure	+ manure
0		+52 ± 4	+52 ± 4	+120 ± 6	+120 ± 6
10	0	−260 ± 3	−286 ± 1	−158 ± 15	−257 ± 3
15	5	−296 ± 1	−320 ± 2	−240 ± 0	−299 ± 0
20	10	−294 ± 1	−318 ± 2	−239 ± 1	−296 ± 3
30	20	−300 ± 1	−326 ± 1	−245 ± 1	−295 ± 0
40	30	−289 ± 2	−320 ± 1	−240 ± 2	−289 ± 1

inseptisol soil, and a probable explanation for this is that as soon as metabolites are formed, they were further degraded.

Triadimefon was sorbed to a greater extent in mollisol soil as compared to inseptisol soil, with distribution coefficient (K_d) values of 2.48 and 1.39 L/kg, respectively. This result is in agreement with the previous result (*16*) where triadimefon sorption was higher in soils having a higher organic carbon content. Because of the higher triadimefon sorption in mollisol soil, a lesser amount of fungicide was available for degradation in the soil solution (amount actually available for degradation); therefore, the rate of degradation of triademefon was slow in mollisol and the reverse was true for inseptisol soil where a higher amount of triadimefon was available in soil solution for degradation. Similar results were reported by Kim et al. (*17*) for propiconazole where the rate of propiconazole degradation was higher in sandy loam soil than silty clay loam containing more organic matter. More mineralization of propiconazole in sandy loam soil suggested that propiconazole was more accessible to biological forces.

However, sorption results did not explain the degradation of triadimefon under flooded conditions where the fungicide was degraded at a faster rate in mollisol soil than in inseptisol soil. Reduction potential (Eh) measurements indicate that mollisol soil has higher Eh than inseptisol soil (**Table 3**) suggesting a higher anaerobic activity in mollisol soil. A higher degradation rate of triadimefon under flooded conditions can be attributed to anaerobiosis and associated microbial activity.

Influence of Temperature. Degradation of triadimefon was faster at a higher incubation temperature (**Table 2**). The half-life values for triadimefon at 28 and 35 °C in mollisol soil were 16.2 and 7.4 days, respectively, with respective values in inseptisol soil being 14.8 and 11.8 days. The faster degradation of triadimefon at higher temperature could be due to a higher microbial activity though; higher volatilization losses at 35 °C cannot be ruled out.

Effect of Soil Sterilization. Comparing degradation in sterilized and nonsterilized soils under both moisture regimes, the degradation of triadimefon in both soils was mediated by microorganisms (**Figure 2**). After 30 days, no degradation of triadimefon was observed in sterilized soils, either flooded or nonflooded. However, over a 30 day period in nonsterile soils, 64−80% of triadimefon was degraded. Earlier reports suggested that soil microorganisms have the ability to degrade triadimefon (*6*, *12*, *18*). Laboratory shake cultures of *Aspergillus niger* converted 5−32% of triadimefon to triadimenol in 2−5 days (*6*), with no further metabolism of triadimenol being detected. However, triadimefon incubated with mycelia mats of *A. niger* was metabolized to its isopropyl derivative [1-(4-chlorophenol)-3-methyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone]. Deas et al. (*12*, *18*) studied triadimefon metabolism using different fungi, viz. *Coriolus versicolor*, *Cladosporium cucumerinum*, *Botrytis cinerea*, and *Fusarium culmorum*, and triadimenol was recovered as the only metabolite.

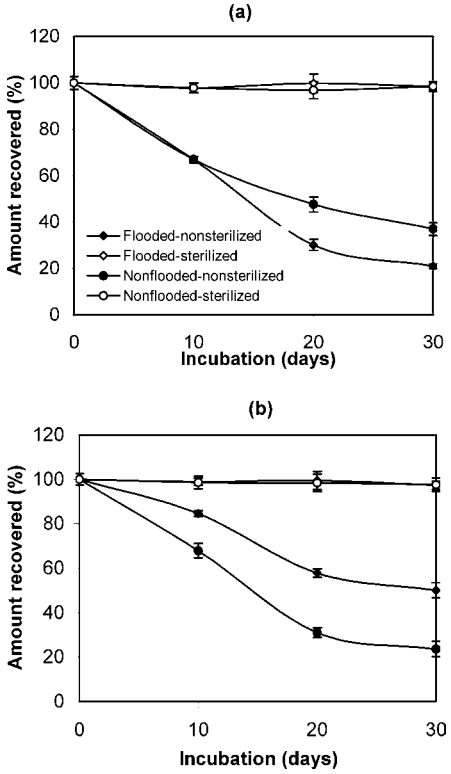

Figure 2. Effect of sterilization on triadimefon degradation in (**a**) mollisol and (**b**) inseptisol soils.

Effect of Organic Manure. Amendment of organic carbon in the form of manure, distillery effluent, or sewage sludge is a recommended agricultural practice to increase soil fertility. Therefore, triadimefon persistence was compared in cow manure amended (5%) and unamended soils. The addition of manure affected the degradation of both triadimefon (**Table 2**) as well as the formation of its metabolites. Application of manure to flooded soils significantly enhanced the formation of the 1,4-diol metabolite. In manure-amended mollisol soil, the 1,4-diol derivative was recovered on the first sampling (day 5), although in manure unamended soil, it was recovered on 30th day of sampling. Reduction potential measurements of manure amended and unamended soils indicated that the application of cow manure to the soils hastened the onset of soil reducing conditions and Eh of manure amended soil dropped faster than that of manure unamended soil (**Table 3**). It is probable that triadimefon degradation in predominantly anaerobic soils (flooded) was mediated by anaerobic microorganisms, and because of faster attainment of reducing conditions following manure application, anaerobic microorganisms proliferated faster and the 1,4-diol derivative was recovered quite early. Similar results were obtained in flooded inseptisol soil. It is interesting to note that I did not recover 1,4-diol derivative from manure unamended inseptisol soil. However, after manure application, 1,4-diol derivative was recovered at day 30. This further confirms that triadimefon degradation in flooded soils was mediated by anaerobic microorganisms.

Manure application to nonflooded soils increased the persistence of triadimefon. As triadimefon sorption was more in higher organic carbon content soil, the addition of manure will provide additional sites for the sorption of triadimefon, and because of the decreased availability of triadimefon in soil solution, its degradation was slowed in manure amended soils

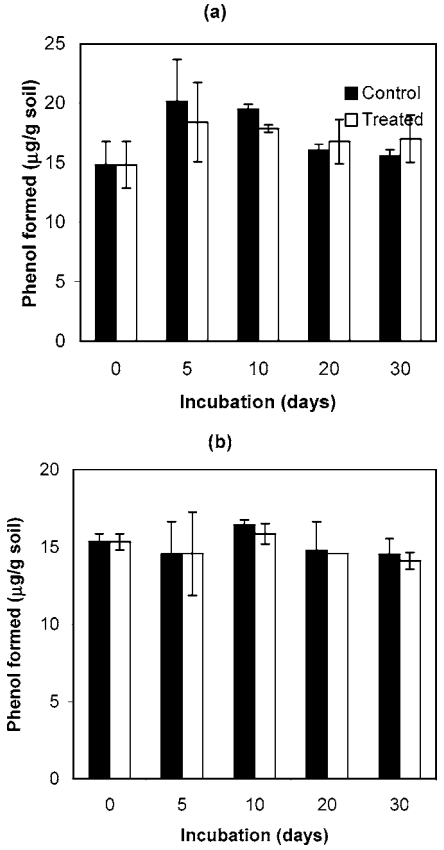

Figure 3. Effect of triadimefon on soil phosphatase activity in (**a**) mollisol and (**b**) inseptisol soils.

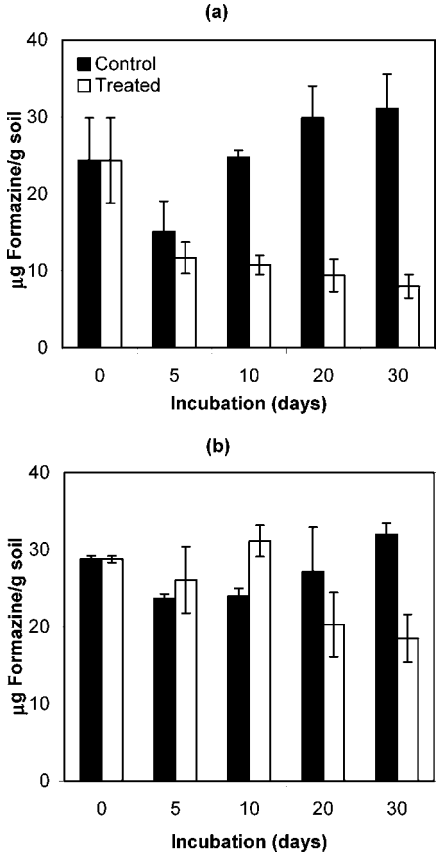

Figure 4. Effect of triadimefon on soil dehydrogenase activity in (**a**) mollisol and (**b**) inseptisol soils.

Effect on Soil Enzymes. Soil enzymes are the indicators of its health. Phosphatase activity plays a crucial role in phosphate mineralization as well as maintaining soil fertility. Similarly, an assay of dehydrogenase activity has been recommended as an index of the general activity of soil microorganisms and can be used as a sensitive marker of soil degradation and soil microbial activity (*14*, *19*, *20*). Triadimefon at 1 mg/kg in both soil types did not affect phosphatase activity in both soil types (**Figures 3** and **4**). However, triadimefon significantly decreased the soil dehydrogenase activity in both of the soils. Initially, dehydrogenase activity decreased even in control soils, indicating that acetone inhibits the enzyme activity. However, after about 10 days, control soils overcame this inhibition and the enzyme activity increased in control soil. However, the activity in triadimefon-treated soils further decreased. After 30 days of incubation, dehydrogenase activity in mollisol and inseptisol soils had decreased by 70 and 50%, respectively, of that in control soils. The inhibitory effect of triadimefon was more pronounced in mollisol soil than inseptisol soil. This may be partially responsible for the slower degradation of triadimefon in nonflooded mollisol soil as compared to inseptisol soil.

The soil moisture content was found to affect the pathway of triadimefon degradation. In nonflooded soils, triadimefon was converted to triadimenol, but in flooded soils, it was converted to 1,4-diol metabolite. The degradation of triadimefon, both in flooded and in nonflooded soils, was essentially due to microbial processes. Soil organic matter significantly affected the persistence of fungicide in amended soils. Triadimefon (1 mg/kg) did not affect the soil phosphatase activity although soil dehydrogenase activity was significantly decreased.

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