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LOSS OF DIAZINON FROM DACCA PADDY FIELD SOILS

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Abstract. Soils from three different areas near to Dacca decomposed aqueous solution of Diazinon. 100 g each of the mixed soils decomposed about 5 mg Diazinon in 24 hr. The rate of decomposition was not influenced by aeration. Little Diazinon was degraded by heat sterilized soil suggesting the action of living organisms. This was confirmed by growing a suspension of a pure culture of soil microflora which decomposed Diazinon and was also heat labile. Decomposition stopped when about half the Diazinon was decomposed.

Granular Diazinon is in extensive use in East Pakistan for the control of rice pests. However, there are various reports from International Rice Research Institute, Manila that Diazinon is decomposed by soil[1] organisms and that decomposition becomes faster with repeated applications, indicating a diminishing value of Diazinon as an insecticide[2] with its continuous use. If this happened in East Pakistan, it would be a major loss to the country. We, therefore, investigated the stability of Diazinon in aqueous solutions in contact with soil samples from East Pakistan.

On finding that Diazinon was lost from aqueous solution in contact with soil, tests were made to determine whether the causes were chemical, physical or biological. This paper describes these investigations.

Experimental

Materials

Soil samples were taken from rice fields outside the villages of Mirpur, Demra and Joydabpur which are situated near to Dacca. Mirpur soil was used for tests unless the soil is specifically indicated.

Pure Diazinon (98.5%) supplied by M/s Geigy, Switzerland, was used as standard insecticide.

Methods

Assay of Diazinon in Aqueous Solution. For the assay, Diazinon was extracted from aqueous solution into n-hexane. The Diazinon in n-hexane solution was then either assayed semiquantitatively using paper chromatography or by gas chromatography using a Pye Panchromatograph.

Extraction of Diazinon from Water. A 50-ml aliquot of aqueous solution of Diazinon in water was extracted with 10 ml n-hexane by shaking in a separating funnel. The n-hexane layer was separated and the aqueous layer extracted with 2 more portions of 5 ml n-hexane. The n-hexane layers were combined and concentrated to 5 ml before assaying Diazinon by gas chromatography.

A Pye Panchromatograph fitted with a 30 cm × 6 mm dia column packed with 5% SE-30 on Chromosorb 'W' maintained at 160°C, was used in conjunction with electron capture detector at 175°C. High purity nitrogen was used as a carrier gas. Each extract was compared by gas chromatography at least three times with standard amounts of Diazinon.

Ascending paper chromatograms were run using Whatman SG-81, a silica-loaded paper with isooctane as solvent, or Whatman No. 1 paper, impregnated with paraffin (10% in ether), with acetone–water, 3:7 (v/v) as solvent. The chromatogram was sprayed with chromogenic agent, a mixture of 10 ml of 2% $AgNO_3$ in distilled water with 90 ml bromophenol blue (0.4%) in acetone, heated for 20 min at 60–70°C and finally rinsed in 0.01% citric acid. Diazinon was identified and estimated semiquantitatively by comparing the size and intensity of the spots with known amounts of the insecticide (4 μg/μl, 2 μg/μl, 1 μg/μl, 0.5 μg/μl, 0.4 μg/μl 0.2 μg/μl on the same chromatograms).

Preparation of Soil. For tests, soils were moulded into roughly spherical pellets of about 1 cm dia.

Apparatus for Percolating Aqueous Diazinon Solutions through Soil Samples. To examine the aerobic decomposition of Diazinon in the presence of soil, an aqueous Diazinon solution was percolated through soil using an apparatus similar to that described by Lees[3] but the joints made with rubber bungs or tubing. 100 g soil pellets were put on a layer of glass wool in a vertical glass tube 18 in long and 1.5 in dia. Aqueous Diazinon solution was pumped over the soil by an air-lift pump operated by suction; after percolating through the soil it returned to the reservoir from which it was pumped.

Results

Sorption. Soil (10 g) and 25 ml Diazinon solution in distilled water (20 p.p.m.) were stirred together to form a paste. The paste was transferred into a centrifuge tube and then centrifuged for 1 hr at 0°C. The supernatant was decanted off and extracted with three portions of 10 ml n-hexane and assayed by gas chromatography. The strength of the aqueous solution was changed little by treating it with soil. The experiment was repeated three times; each time the result was similar and indicated not more than 1 p.p.m. sorption. The experiment was done only with

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the soil sample collected from Mirpur and as little sorption was observed, it was not repeated with soil samples from Demra and Joydabpur.

Diazinon Metabolism by Soil. Mirpur soil (100 g) was kept in contact with 500 ml of 20 p.p.m. Diazinon in aqueous solution at room temperature (apporx 25°C). The aqueous solution was sampled at intervals and assayed for Diazinon. Diazinon was lost from the aqueous solution whether it was percolated over the soil (aerobic tests) or left to stand undisturbed over the soil in a stoppered-flask (anaerobic tests). Loss of Diazinon was similar in both aerobic and anaerobic tests. Diazinon was only slightly decomposed in the absence of soil (Fig. 1).

Loss increased with time, about 7 p.p.m. was being lost in 8 hr and 12 p.p.m. in 24 hr. When soil was sterilized by autoclaving for 30 min at 15 pounds pressure and 120°C or in the absence of soil, almost no Diazinon was lost from solution whether the test was aerobic or anaerobic. When soil in the percolation apparatus was replaced by short glass rods (1 × 0.5 cm dia) not more than 2 p.p.m. Diazinon disappeared in 24 hr.

This suggests that the cause of decomposition is biological rather than chemical.

A brown material developed on the surface of unsterilized soil samples kept in contact with aqueous Diazinon solution. It did not form on sterile soil. This suggests that the brown material is some organism possibly decomposing Diazinon. A similar brown material has been described by Gunner *et al.*[4]

The brown material grew profusely when streaked onto nutrient agar plates. The growth was increased when Diazinon (1 p.p.m.) was mixed with the nutrient agar, suggesting that Diazinon stimulates the growth of this organism as might be inferred from its action in unsterilized soil exposed to Diazinon.

Culture and Properties of Organism Decomposing Diazinon. The next experiments were done to culture the brown growth in the absence of soil in order to show that it decomposes Diazinon. Some of the brown growth which forms on the surface of the soil in aqueous Diazinon solutions was added to a 5%-peptone solution containing soluble mineral salts and incubated at 37°C for 24 hr when a brown precipitate formed. After thorough mixing, a series of volumes of the brown suspension were added to 100 ml samples of 30 p.p.m. Diazinon solution and each made to 150 ml with water. After 24 hr the Diazinon remaining was assayed. 10 ml suspension decomposed about one third (1.0 mg) of the Diazinon. The loss increased with increasing amounts up to 30 ml biological suspension which decomposed about 55% (Fig. 2) Diazinon present in the solution. Further increase of the amount of biological suspension did not cause more decomposition.

The Diazinon remaining in a mixture of 30 ml biological suspension and 100 ml 30 p.p.m. Diazinon solution, made to 150 ml with distilled water was assayed after 4, 8, 12, 24, 36 and 48 hr. The loss of Diazinon increased with time but after 24 hr when about 56% Diazinon decomposed, the rate of decomposition diminished (Fig. 3). The slowing of decomposition, when almost half the Diazinon decomposed, may be the result of either inhibition of the

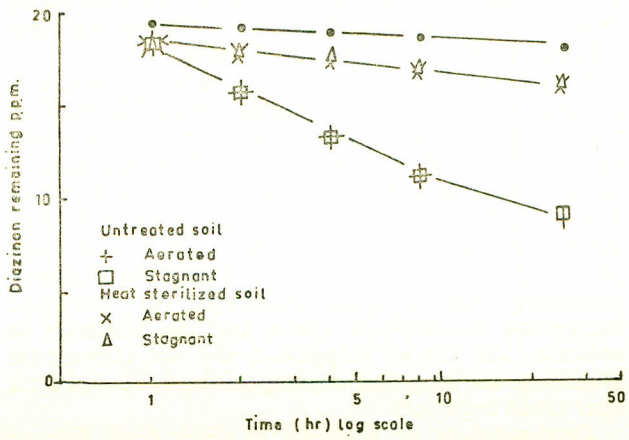

Fig. 1. The effects of heat treatment and aeration on the decomposition of aqueous solutions of Diazinon in contact with soil. (● aqueous Diazinon solution.)

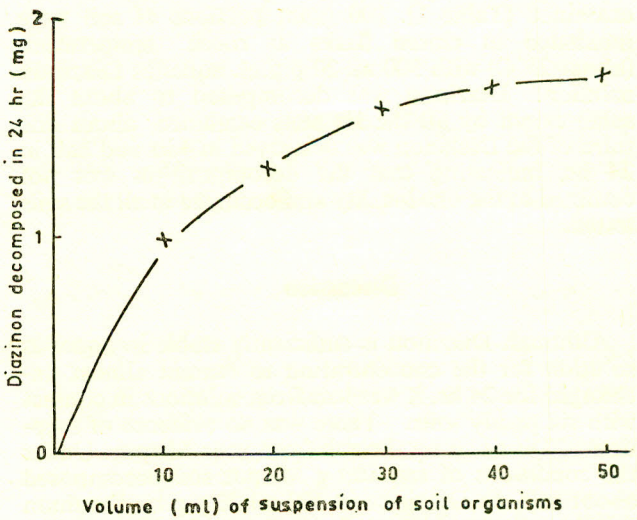

Fig. 2. Decomposition of Diazinon by various quantities of soil organisms cultured in a peptone solution.

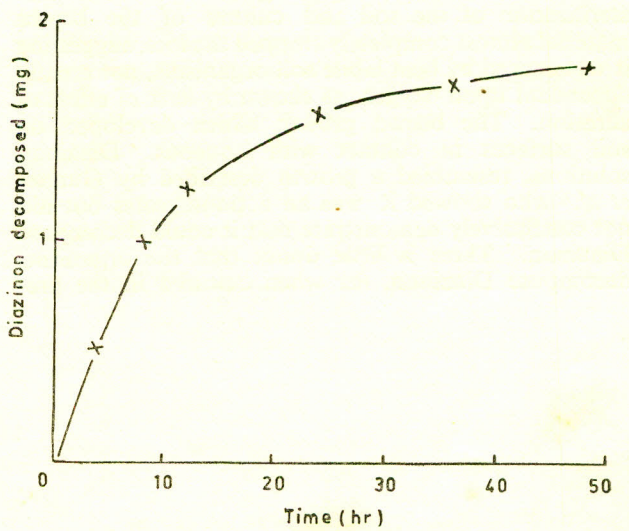

Fig. 3. Decomposition of Diazinon with time by soil organisms cultured in a peptone solution.

reaction by the accumulation of its decomposition products, diminishing 'substrate' (Diazinon) concentration or a combination of both.

Heat Lability of Organism Decomposing Diazinon. A suspension of brown organism cultured in peptone was divided into two parts. One portion was heated to 60°C for 1 hr then untreated and treated suspensions were tested for their ability to decompose Diazinon. 30 ml biological suspension was added to 100 ml 30 p.p.m. aqueous Diazinon solution and made to 150 ml with distilled water. 24 hr later the Diazinon remaining was assayed. Although the untreated suspension decomposed about half the Diazinon in solution, the heated suspension did not decompose Diazinon, showing that decomposition is caused by a heat labile organism.

Decomposition of Diazinon by Soils from Various Places. To determine whether decomposition of Diazinon by soil was peculiar to the sample taken from Mirpur, five other soil samples taken from fields near to the villages of Joydabpur and Demra were examined (Table 1). 100 gram portions of soil were incubated in closed flasks at room temperature (about 25°C) with 500 ml 20 p.p.m. aqueous Diazinon solution. Diazinon was decomposed to about the same extent by all the six soils examined, about one third of the Diazinon was destroyed in 4 hr and half in 24 hr, indicating that the decomposition was not confined to the one locality and occurred in all the soils tested.

TABLE 1. DECOMPOSITION OF DIAZINON IN AQUEOUS SOLUTION STANDING OVER SOILS.*

Soil sample	Diazinon decomposed 4 hr	μg/g soil 24 hr
Mirpur	30	52
	29	52
Joydabpur (a)	31	55
	30	55
(b)	28	53
	28	52
Demra (a)	30	55
	32	54
(b)	31	56
	30	55
(c)	29	54
	29	54

* 500 ml 20 p.p.m. aqueous Diazinon was incubated over 100 g soil pellets at room temperature (about 25°C).

Discussion

Although Diazinon is sufficiently stable in aqueous solution for the concentration to remain almost unchanged for 24 hr, it was lost from solutions in contact with six paddy soils. There was no evidence of sorption of Diazinon on the soil from near Mirpur. Under the conditions of test 100 g Mirpur soil decomposed about half the Diazinon in 500 ml 20 p.p.m. Diazinon solution amounting to about 5 mg Diazinon. The amounts decomposed were similar whether the solutions were aerated or left stagnant. However, heat sterilization of the soil and culture of the brown material almost completely stopped the loss, suggesting it was caused by heat labile soil organisms, not greatly dependent upon oxygen, as shown by lack of effect of aeration. The brown growth which developed on soil surfaces in contact with aqueous Diazinon solutions, resembled a growth described by Gunner *et al.*[4] who showed it was an actinomycetes but did not conclusively demonstrate that it could decompose Diazinon. There is little doubt that the organisms decompose Diazinon, for when cultured in the presence of Diazinon, growth increased both on agar plates and in peptone solution and the suspension grown in peptone decomposed Diazinon, an action stopped by heat. The decomposition seems to be dependent on Diazinon concentration or to be inhibited by the reaction products, because decomposition remains proportional to time and amount of organism used only until about half the Diazinon is degraded. Decomposition of Diazinon by all the six paddy field soils examined indicates a widespread distribution of an organism which limits the residual life of Diazinon and may seriously diminish its value for controlling the pests.

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References

1. F. Pro-Rasmussen, E. Noddegaard and K. Voldum Clausen, J. Sci. Food Agr., **19,** 278 (1968).
2. N. Sethunathan and T. Yoshida, J. Agr. Food Chem., **17,** 1192 (1967).
3. H. Lees, Plant Soil, **1,** 221 (1949).
4. H.B. Gunner, B.M. Zukerman, R.W. Walker, C.W. Miller, K.H. Dendert and R.E. Longley, Plant Soil, **25,** 249 (1966).