Persistence and Biodegradation of Hinosan in Soil

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Hinosan (O-diethyl S,S-diphenyl phosphorodithiolate) is extensively used in Japan for controlling rice blast caused by Pyricularia oryzae Cav. and has been recommended for rice blast control in India as well. The fate of Hinosan in rice plant has been investigated (ISHIZUKA et al. 1969, TAKASE et al. 1969, UEYAMA et al. 1973); but its behavior in tropical soils is little understood. Recent studies have shown that certain pesticides that persist in nonflooded aerobic soils readily break down in predominantly anaerobic flooded soil system (SETHUNATHAN 1973a). This paper presents data on the relative persistence of Hinosan in flooded and nonflooded soil systems and the role of microorganisms in its degradation.

MATERIALS AND METHODS

Soil Incubation Studies. An alluvial soil (organic matter 1.4%, total nitrogen 0.09%, pH 6.2) from the experimental farm of Central Rice Research Institute was used. Twenty gram portions of the soil contained in 25 x 200 mm test tubes were treated with 2000 ug of technical grade Hinosan (gifted by Nishon Tokushu Noyaku Seizo K.K., 2-Chome, Nihonbashi, Chuoi Ko, Tokyo, Japan) dissolved in 0.1 ml of chloroform. After 24 h for evaporation of chloroform, the soils were flooded with 25 ml of distilled water to provide 3.5 to 4 cm water depth; for nonflooded conditions, the soils were maintained at 50% water holding capacity. In another study to determine the role of biodegradation, 20 g portions of the soils contained in test tubes were autoclaved three times at 15 psi for 30 min at 1 day intervals and 2000 ug of Hinosan in 0.1 ml chloroform was introduced to

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autoclaved and nonautoclaved soils. After 24 h, the soils were flooded with 25 ml of distilled water. For residue analysis, the soils from two replicate tubes were extracted with chloroform-diethyl ether (1:1) and the residues were separated by thin-layer chromatography (t.l.c.) employing hexane-chloroform-methanol (7:2:1) as described for parathion (SETHUNATHAN 1973b).

Bacterial Degradation. An enrichment culture was prepared by 3 or 4 additions of 500 ppm of Hinosan E.C. (Bayer (India) Ltd., Bombay) at 14 day intervals to 20 g soil flooded with 25 ml of distilled water. A loopful of the soil solution (enrichment culture) was transferred to a sterile mineral solution (SETHUNATHAN 1972) supplemented with technical Hinosan as sole carbon source. The mineral solution turned turbid within 72 h and a loopful of this solution was streaked on modified Wakimoto agar medium (SETHUNATHAN 1972). Individual bacterial colonies appearing on the medium were transferred to mineral solution + Hinosan. A bacterium capable of growing in Hinosan medium was thus isolated and purified. To determine the ability of this bacterium to decompose Hinosan, 2000 ug of Hinosan in hexane was introduced to 50 ml sterile Erlenmeyer flasks. After 24 h for evaporation of hexane, 20 ml aliquots of sterile mineral solution, mineral solution + 0.5% glucose and mineral solution + 0.1% yeast extract were added to the flasks under aseptic conditions. The media were inoculated with 0.1 ml of bacterial (7 to 10 days old) suspension in sterile distilled water and incubated at 30°C. Uninoculated media served as controls. After 10 days, the residues from each flask were extracted 3 times with 25 ml portions of chloroform-diethyl ether and then separated by t.l.c.

Estimation of Hinosan. Hinosan from the chromatoplate was decomposed

by digestion with aqua regia and the phosphorus released was determined colorimetrically using sulphomolybdic-aminonaphthol method of FISKE and SUBBAROW (1925). Hinosan from the silica gel areas of the samples in the chromatoplate corresponding to authentic standard were eluted in 5 ml of methanol. After centrifugation at 3500 rpm for 15 min, the supernatant was transferred to 100 ml Erlenmeyer flask and digested with 5 ml of aqua regia. After driving off the excess of HNO_3 vapor with 1 ml of 1:1 HCl, the contents were evaporated to dryness, 10 ml of distilled water added and the contents boiled. After cooling, the contents were transferred to 25 ml volumetric flasks and 1ml of sulphomolybdate solution (0.5%) added. After cooling in ice water bath for 15 min, 1 ml of aminonaphthol reagent was added and the solution was made up to 25 ml. The blue color developed was read at 650 nm. Hinosan was quantitated by multiplying the values of phosphorus with the factor 10.02. Per cent recovery of Hinosan from flooded soils and microbial cultures immediately after application ranged between 50.7 to 64.3.

For qualitative analysis of Hinosan and its metabolites, the chromatoplate containing the residues was sprayed with 0.5% palladium chloride solution in 2% HCl.

RESULTS AND DISCUSSION

Hinosan degraded faster under flooded conditions than under nonflooded conditions (Table 1). Under flooded conditions, its concentration declined to 287ug within 7 days and 100 ug in 14 days; the corresponding recoveries of the fungicide from nonflooded soils were 687 and 390 ug. Similar rapid degradation under flooded soil conditions has been noticed also with other organophosphorus insecticides such as diazinon and parathion (SETHUNATHAN 1973a).

TABLE 1

Persistence of Hinosan in an alluvial soil under flooded and non-flooded conditions

Incubation (days)	ug Hinosan recovered/20 g soil	
	Flooded	Nonflooded
0	1033	1046
7	287	687
14	100	390

Heat treatment of the soil prior to fungicide incorporation retarded the degradation of Hinosan. After 7 and 14 days, 740 and 440 ug of the fungicide were recovered from autoclaved soil samples as against the values of 413 and 106 ug from nonautoclaved samples. These figures correspond to recovery of 42% of the initially recovered fungicide from autoclaved soils at the end of 14 days as compared to a recovery of less than 10% from nonautoclaved soils. The reduced and reddish brown oxidized (a few mm thick surface layer) layers which were characteristic of nonsterile soils within few days after flooding (SETHUNATHAN 1973a) were absent in flooded autoclaved soils denoting the suppression of microbial activity following heat treatment. The results suggested the role of microorganisms in the rapid loss of Hinosan from flooded soils.

Thin-layer chromatographic analysis of solvent extracts of soils treated with Hinosan (R_f 0.67) revealed that three compounds (R_f 0.90, 0.81, 0.00) positive to palladium chloride were detected as degradation products in both autoclaved and nonautoclaved soils. Two additional compounds (R_f 0.71, 0.19) were formed in nonautoclaved soils, presumably due to microbiological action. No attempt was

made to identify the metabolites, because authentic compounds were not available.

The bacterium isolated by enrichment culture technique was Gram-negative and small rod-shaped and was identified as a nonflourescent species of Pseudomonas sp. Within 10 days of incubation with this bacterium, Hinosan residues in 25 ml medium declined to 153 ug from an initial recovery of 1167 ug as compared to a recovery of 667 ug from uninoculated media (Table 2). Evidently, Hinosan was utilized as a sole carbon source by the bacterium. Addition of yeast extract further enhanced bacterial decomposition of Hinosan; but sucrose had no effect.

TABLE 2

Degradation of Hinosan in a mineral solution by a bacterium isolated from Hinosan-amended soil under flooded conditions

Treatments	Hinosan recovered[a] after 10 days of incubation (ug/25 ml)
Mineral solution	667
Mineral solution + Inoculum	153
Mineral solution + Sucrose	477
Mineral solution + Sucrose + Inoculum	173
Mineral solution + Yeast extract	607
Mineral solution + Yeast extract + Inoculum	104

a Initial quantity of Hinosan recovered, 1167 ug/25 ml medium.

Qualitative analysis of residues from bacterial cultures showed that three compounds (R_f 0.90, 0.81, 0.00) were formed in both inoculated and uninoculated media. It is not clear from the present

study whether these products were produced in inoculated media by chemical or biological action or a combination of both. Some loss of Hinosan by chemical phenomenon is indicated by a slow decline in its concentration in uninoculated media (Table 2) accompanied by the formation of three compounds. Faster degradation occurred, however, in the presence of the bacterium and two additional metabolites (R_f 0.71, 0.19) were detected. Yeast extract enhanced the formation of these two biological products of Hinosan metabolism.

More information is needed concerning the fate, behavior and pathways of newer pesticides such as Hinosan under conditions of Indian agriculture before their widespread use in pest control is recommended. Viewed from this perspective, the results obtained on the fate of Hinosan in a soil environment are interesting. The data demonstrate that applications of Hinosan in rice blast control may not cause any soil residue hazard in view of its rapid decomposition in soils under flooded conditions.

To sum up, Hinosan decomposed in an alluvial soil more rapidly under flooded conditions than under nonflooded conditions. A bacterium, isolated from flooded soil by enrichment technique, decomposed Hinosan in liquid media. Among five products of Hinosan detected in flooded soils and in bacterial cultures, two products were produced by microbial metabolism.

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