Effects of Spherosomes on Degradation of Pretilachlor and Esprocarb in Soil

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The fate of pretilachlor and esprocarb in soil with or without spherosomes was investigated for 71 days. The estimated half-lives of pretilachlor in nonsterile soil with and without spherosomes were calculated to be 35.6 and 54.3 days, respectively, while those of esprocarb in nonsterile soil with and without spherosomes were 35.5 and 44.8 days, respectively. The degradation of these pesticides appeared to be essentially due to the biological activity of the soil. Spherosomes should enhance the rate of pretilachlor and esprocarb destruction.

Key words —— pretilachlor, esprocarb, pesticide, spherosome

INTRODUCTION

We have previously reported that rice bran and defatted seed were effective in adsorbing pesticides and organochlorine compounds such as chloroform, dichloromethane and benzene.[1] Furthermore, it was confirmed that the spherosomes isolated from these adsorbents were effective in removing these organic compounds.[2] Analytical and laser microscopic data have confirmed that the removal of organochlorine compounds and benzene is dependent on the uptake of these compounds into intracellular particles called spherosomes.[2] Spherosomes are widely distributed among plants and fungi but have not been observed in animal cells. However, spherosomes occur prominently in seeds.[3] Degradation studies in soil are essential for the evaluation of the persistence of chemical compounds. The fate of a pesticide is affected by many factors, such as microorganisms, soil constituents, and its physiocochemical properties.[4] Our research has focused on the adsorption properties of spherosomes and their nutrient effect. The measurement of pesticide levels in soil with or without spherosomes is important for using spherosomes as adsorbent materials for removal of pesticides. The fates of pretilachlor and esprocarb in soil with or without spherosomes were investigated.

This paper provides the first report on the environmental fate of pretilachlor and esprocarb in soil with or without spherosomes.

MATERIALS AND METHODS

Apparatus —— The assay of pretilachlor or esprocarb was performed on a Shimadzu (Kyoto, Japan) Model GC-14B gas chromatograph equipped with a flame ionization detector and a capillary column (ULBON, Shinwa Chemical Industries, Ltd., Kyoto, Japan, HR-52, 30 m × 0.53 mm). The column was maintained at 250°C, while the injection port and detector were maintained at 280°C.

Materials —— Rice bran was purchased at a local market. The soil for these experiments (obtained from a farm in Shiga Prefecture, Kokashi, Japan) was a loam with 2.7% organic matter and pH of 6.1. Spherosomes were isolated from rice bran by a fractionation method. The composition of spherosomes is shown in Table 1. Moisture content was determined by drying a sample for 6 hr at 110°C. Protein concentration was determined by the method of Kjeldahl.[5] Lipids were extracted by the Bligh and Dyer method.[6] The mass of total lipids was determined by drying an aliquot of chloroform extract in a vacuum oven overnight and weighing the resulting lipid residue. Carbohydrate (glucide) was deter-

Table 1. Composition of Spherosomes

Constituent	Concentration (g/100 g)
Water	9.8
Protein	26.6
Llipid	3.9
Carbohydrate	
Glucide	38.4
Fiber	3.6
Ash	17.4

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mined by the Anthrone method.[7] Dietary fiber was determined by the Association of Official Analytical Chemists (AOAC) method.[8] Pretilachlor and esprocarb of analytical standard purity were purchased from Wako Pure Chemical Industries Ltd. (Amagasaki, Japan).

Soil Sterilization —— Soil to be heat-sterilized was placed in glass jars with loosely fitted lids and taken through three cycles of autoclaving for 15 min at 15 psi and 121°C.

Recovery Test —— To determine the method efficiency for pretilachlor and esprocarb, the soil samples (10 g) were fortified with known amounts of analytical standards dissolved in acetone (0.02 mg/g). The soil was extracted with 60 ml of methanol/water (1 : 4) by shaking for 30 min. The total extract and 50 ml of 5% NaCl solution were placed in a separatory funnel and extracted twice with 25 ml of hexane. The hexane solution was concentrated under reduced pressure at 40°C and dissolved in hexane (2 ml), and the aliquot was analyzed by gas chromatography (GC) to determine the concentrations of pretilachlor and esprocarb. Blank samples were used and no interference was found in the determination of pesticide. Recovery data represent four replications.

Determination of Pesticides in Soil —— A 10 g sample was weighed in a 200 ml Erlenmeyer flask, and 60 ml of methanol/water (1 : 4) was added. The soil was analyzed for pretilachlor and esprocarb using the same procedures as described for the recovery test.

Soil Incubation —— The soil (10 g) was weighed into Pyrex glass flasks (100 ml). To each soil subsample was added an adequate amount of water, determined by weighing, to give a soil moisture content of 17%. The moisture contents was maintained, ranging from 15 to 17%, throughout the experiment. This water content was decided upon as follows. Rice fields are filled with water only 20 days after rice-planting. After that the water content of the rice fields is estimated to be 13–25%. Thus, we chose 17% as the value of water content. Each soil sample was uniformly treated with known amounts of analytical standards dissolved in acetone (pretilachlor, 0.0212 mg/g; esprocarb, 0.123 mg/g). After the soil was mixed thoroughly, spherosomes (0.588 mg/g) were added. Treated soil samples were then placed in an incubator in the dark again for up 71 days at 25°C. The treated soil was sampled for soil extraction of the chemicals at 0, 1, 5, 8, 11, 19, 29, 33, 38, 46, 51, 60, and 71 days after treatment. The conditions for the control experiments were the same as the incubation method mentioned above, but without the treatment of standards and spherosomes.

RESULTS AND DISCUSSION

Recovery Studies

The mean recoveries were 91.9% for pretilachlor and 96.1% for esprocarb. Esprocarb was recovered from soil with a high yield using methanol extraction. The limit of quantification was defined for GC as the sample concentration required to give a signal-to-noise ratio of 6 : 1. It was evaluated at 0.00005 mg/g of soil.

Degradation of Pretilachlor and Esprocarb in Soil

The effect of spherosomes on the degradation of pretilachlor and esprocarb was examined using sterile and nonsterile soils. Figure 1 shows residual pretilachlor (%) calculated by the following equation:

$$\text{Residual pesticide (\%)} = (X/M) \times 100$$

where X is the pesticide remaining in soil after treatment and M is the initial pesticide content.

The estimated half-lives of pretilachlor in nonsterile soil with and without spherosomes were calculated to be 35.6 and 54.3 days, respectively. After 71 days of incubation, only 16 and 27.8% of applied pretilachlor remained in nonsterile soils with and without spherosomes, respectively. A simi-

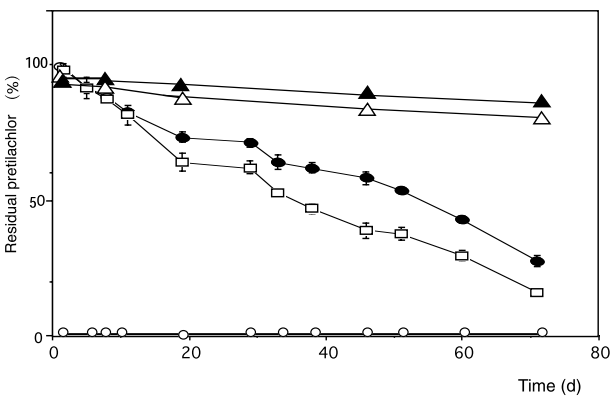

Fig. 1. Degradation of Pretilachlor in Soil

Each value represents the mean ± S.D. of three separate determinations. Pretilachlor, 0.0212 mg/g; spherosomes, 0.588 mg/g. (●) without spherosomes, (□) with spherosomes, (▲) without spherosomes (sterile soil), (△) with spherosomes (sterile soil), (○) control.

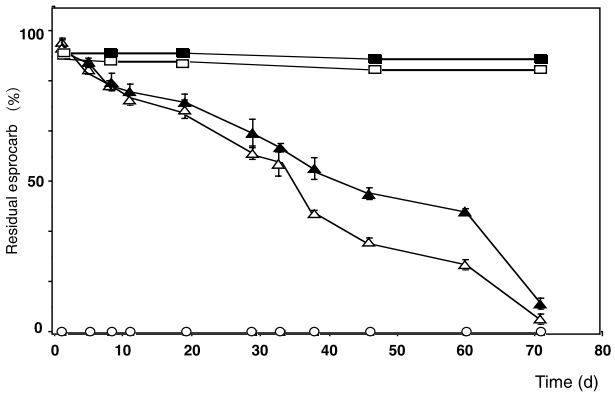

Fig. 2. Degradation of Esprocarb in Soil

Each value represents the mean ± S.D. of three separate determinations. Esprocarb, 0.123 mg/g; spherosomes, 0.588 mg/g. (▲) without spherosomes, (△) with spherosomes, (■) without spherosomes (sterile soil), (□) with spherosomes (sterile soil), (○) control.

lar degradation profile was also observed for esprocarb (Fig. 2). The estimated half-lives of esprocarb in nonsterile soil with and without spherosomes were calculated to be 35.5 and 44.8 days, respectively. After 71 days of incubation, only 4.2 and 7.6% of applied esprocarb remained in nonsterile soils with and without spherosomes, respectively. The percentage of pretilachlor and esprocarb in soil with spherosomes was lower than that in soil without spherosomes. Furthermore, in sterile soil with and without spherosoms, both pretilachlor and esprocarb remained almost unchanged during the incubation period (Figs. 1 and 2). Evidence from the sterile soil experiment indicates that the mechanism of pretilachlor and esprocarb degradation in soils is attributed to microbial degradation. We have previously reported that the efficiency of removal of organochlorine compounds by spherosomes isolated from rice bran was similar to that of rice bran.[2] Our hypothesis is that spherosomes enhance microbial numbers and activity. Environmental factors can greatly influence the degradation rate of chemical compounds in soil, the most important being moisture, pH, organic carbon content, and pesticide formulation. Garcia-Valcarcel and Tadeo[9] reported that degradation rates increased with soil moisture content for hexazinone and simazine, which is in agreement with the results of Walker and Blacklow[10] for atrazine and simazine and those of Bowmer[11] for atrazine; therefore, we maintained constant soil moisture. Pfaender and Alexander[12] reported that the numbers of microorganisms potentially able to cometabolize Dichloro Diphenyl Trichloro ethane (DDT) were high in raw sewage as a result of the addition of glucose and diphenylmethane. The majority of approximately 300 isolates from water and soil could metabolize DDT.[13] Several soil microorganisms isolated from soil contaminated with pesticides have been reported to be active in the degradation of dieldrin.[14] Parr *et al.*[15] reported that the rate of DDT degradation is increased when nutrients are added to soil.

Jacks *et al.*[16] reported that spherosomes isolated from peanuts are composed of 98.1% total lipids and 1.27% protein. Spherosomes isolated from wheat aleurone have also been reported to have a neutral lipid content of 86.9%, 8.5% phospholipids, and 1.8% protein.[17] Adans and Novellie[18] reported that spherosomes from linseed have an average composition of 27% protein, 12% phosphorus, and 8.6% metals. Their protein value is similar to our spherosomes (Table 1). Spherosomes were considerably high in protein and carbohydrate (Table 1). On the basis of the present data, it seems more likely that spherosomes might have a role as a nutrient for growth of microorganisms. Therefore, spherosomes should be active in the growth of microorganisms. We have already reported that a similar result was obtained for the effect of spherosomes on degradation of tetrachloroethylene in soil.[19]

Our study showed that spherosomes should enhance the rate of pretilachlor and esprocarb destruction, suggesting that spherosomes may be applied to soil to reduce pesticides. The spherosomes which we examined were a residue from rice bran. Rice bran is a waste product in the process of making polished rice from brown rice and is very in expensive. From this perspective, the use of spherosomes has merit in bioremediation. We are planning to investigate the effects of spherosomes on the degradation of pretilachlor and esprocarb in soil with high water content in order to clarify the effects within 20 days after rice-planting.

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