Effect of Spherosomes on Control of *Aphis gossypii* Glover in Cucumbers Using Imidacloprid

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The effectiveness of spherosomes for reducing pesticide accumulation in cucumbers and for the control of *Aphis gossypii* Glover was studied. Spherosomes could efficiently reduce the uptake of imidacloprid in cucumbers. In the case of imidacloprid with spherosomes, the number of *Aphis gossypii* Glover, surprisingly, became zero after 3 and 7 days, and showed almost the same effect as imidacloprid without spherosomes, although the concentration of imidacloprid in cucumbers decreased by about 1/5 in comparision with that without spherosomes. It seems more likely that spherosomes might have a role as adsorbent materials for removal of imidacloprid in soil. The application of spherosomes to farmland is a useful and effective method to keep the concentration of pesticides in crops at a low level, resulting in sustainable agriculture.

Key words —— imidacloprid, *Aphis gossypii* Glover, cucumber, spherosome

INTRODUCTION

Pesticides are considered to be indispensable for the production of an adequate food supply for an increasing world population as well as for the control of insectborne diseases. Pesticide residues in humans are mainly derived from the ingestion of contaminated food. Food is the main source of exposure of the general population to pesticides, and accounts for more than 90% of the total exposure.[1)] Pesticide residues in foods and crops are a direct result of the application of pesticides to crops growing in field, and, to a lesser extent, from pesticide residues remaining in the soil.[2)] Yoshida *et al.*[3)] reported the removal rates of pesticide residues in fruits and vegetables by washing with water. Water washing has been reported to remove malathion residues from vegetables.[4, 5)] Furthermore, there has been much interest in the use of organoclays as adsorbents to remove and remediate pesticides in contaminated agricultural soil.[6–8)] Because of the hydrophilic, negative character of their surfaces, clay minerals, particularly phyllosilicates, have been shown to be very good adsorbents for cationic and highly polar pesticides, but their adsorption capacity for poorly soluble, nonionic organic compounds is usually low.[9–11)] However, few detailed investigations have been made on the removal of pesticide residues in vegetables. We previously reported that rice bran effectively adsorbed organic compounds such as benzene, dichloromethane, carbon tetrachloride, trichloroethylene, and pesticides in environmental water samples[12, 13)] and soil.[14)] Furthermore, it was confirmed that spherosomes isolated from these adsorbents were effective in removing these organic compounds.[15)] Our research has focused on the adsorption properties of spherosomes. In this study, we have elucidated effect of spherosomes on the control of *Aphis gossypii* Glover on cucumber leaf surfaces using imidacloprid.

This paper is the first report on the effect of spherosomes on the control of *Aphis gossypii* Glover in cucumbers using imidacloprid.

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MATERIALS AND METHODS

Apparatus —— Imidacloprid assays were performed using a Shimadzu Model LC-6A (Shimadzu, Kyoto, Japan) and HPLC system with a Wakosil-II 5C18 HG column (4.6 mm × 150 mm, Wako Pure Chemical Industries, Ltd., Osaka, Japan). The column was eluted with a mobile phase containing 25% acetonitrile in water at a flow rate of 1 ml min^{-1}. UV detection (270 nm) was performed with a variable wavelength detector. Under these conditions, the retention time of imidaclopride was 5.4 min.

Rice Bran and Spherosomes —— Rice bran was purchased at a local market. Spherosomes were isolated from rice bran by the fractionation method

Table 1. Composition of Rice Bran and Spherosomes

Constituent	Concentration (g/100 g)	
	Rice bran	Spherosomes
Water	13.5	9.8
Protein	13.2	26.6
Lipid	18.3	3.9
Carbohydrate		
Glucide	38.3	38.4
Fiber	7.8	3.6
Ash	8.9	17.4

developed by our research group.[16] The composition of rice bran and spherosomes is shown in Table 1. The moisture content was determined by drying a sample for 6 hr at 110°C. The protein concentration was determined by the method of Kjeldahl.[17] Lipids were extracted using the Bligh and Dyer method.[18] The total lipid mass was determined by drying an aliquot of chloroform extract in a vacuum oven overnight and weighing the resulting lipid residue. Carbohydrates (glucides) were determined by the Anthrone method.[19] Dietary fiber was determined by the Association of Official Analytical Chemists (AOAC) method.[20] Pesticide standards were purchased for pesticide residue analysis from Wako Pure Chemical Industries Ltd. Sep-Pak Cartridges Plus Silica were purchased from Waters Corporation (Milford, MA, U.S.A.).

Plant and Insect Materials —— Cucumbers (Suzunariyotsuba), seedlings in polypropylene pots (diameter: 15 cm, one plant per pot), were used in this study. All experiments were performed with cucumber plants at the two true-leaf stage. The aphids were reared on cucumber in Otsuka Chemical laboratory for several generations before using in the experiments.

Sample Preparation —— At the two true-leaf stage of cucumber, spherosomes were placed in polypropylene pots (0.5 g/pot), to which imidacloprid (20 mg/pot) was added. Twenty aphids per plant were immediately applied to the cucumber leaves in order to test viability of aphids on the day of the experiment. Aphids were counted at 3, 7, 14, and 21 days post application. Cultivated cucumbers were collected at 3, 7, 14, and 21 days after application.

Determination of Imidacloprid in Cucumbers —— After cultivating for 3, 7, 14, and 21 days, cucumbers were cut into small pieces and homogenized. Imidacloprid was extracted from the cucumbers (5 g) with 30 ml of acetonitrile for 30 min by shaking, and the extract was concentrated to a volume of 15 ml under reduced pressure. After the addition of 50 ml of sodium chloride (5%) to the solution, imidacloprids was extracted with 30 ml of ethyl acetate twice, and the ethyl acetate layer was evaporated to dryness under reduced pressure. Purification of the imidacloprid extract was carried out by transferring the 2 ml sample to a silica gel column (Waters, silica cartridges, 30 × 12 mm), and eluting with 20 ml of ethyl acetate. The eluate was evaporated to dryness under reduced pressure. The dry residue was dissolved in 10 ml of acetonitrile-water (25 : 75, v/v) and subjected to HPLC to assay the concentration of imidacloprid.

Recovery Test —— To determine the method's efficiency for extracting imidacloprid, imidacloprid was added (5 or 50 μg/g) to the vegetable samples (5 g). The vegetable sample were extracted for 30 min by shaking with 30 ml of acetonitrile. The total extract and 50 ml of 5% NaCl solution were placed in a separatory funnel and extracted with 30 ml of ethyl acetate twice and the ethyl acetate layer was evaporated to dryness under reduced pressure. Purification of the extract for imidacloprid and HPLC analyses was carried out using the same procedure for the determination of pesticides in vegetables. Blank samples were used, and no interference was noted in the determination of imidacloprid. Recovery data represent four replications.

RESULTS AND DISCUSSION

Recovery Studies

The procedure to extract imidacloprid from cucumbers was found be efficient, as indicated by the recoveries from fortified cucumber samples (5 or 50 μg/g), which were 93.7 and 94.1%, respectively. The corresponding standard deviations were 0.45 and 0.51%, respectively.

Imidacloprid Concentrations in Cucumbers with or without Spherosomes

Figure 1 shows imidacloprid concentrations in cucumbers with or without spherosomes measured 3, 7, 14, and 21 days after application. A high concentration of imidacloprid was detected after 3 days in the case without spherosomes, 8.13 μg/g, which was about 7 times higher than that for the case with spherosomes, 1.36 μg/g. After 21 days, the concentration of imidacloprid decreased to 0.29 and 2.17 μg/g with and without spherosomes, respec-

Table 2. Viability of *Aphis gossypii* Glover on Cucumber Leaf Surface

Days after treatment	Number of *Aphis gossypii* Glover/Plant		
	Control	Sample 1	Sample 2
	imidacloprid (−) spherosomes (−)	imidacloprid (+) spherosomes (+)	imidacloprid (+) spherosomes (−)
0	20	20	20
3	73	0	0
7	92	0	0
14	811	5	7
21	1613	10	9

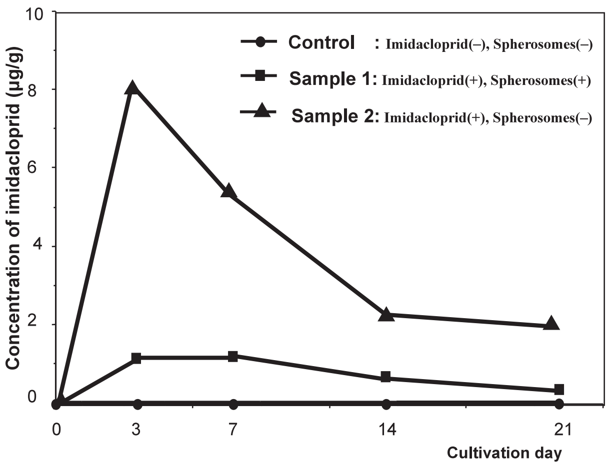

Fig. 1. Concentrations of Imidacloprid in Cucumbers with or without Spherosomes

tively. 2.17 μg/g for the case of without spherosomes exceeds the maximum limits for pesticide residues, which is 1 μg/g. We previously reported the adsorption properties of spherosomes.[12–14] On the basis of the present data, it seems more likely that spherosomes might have a role as adsorbent materials for removal of imidacloprid in soil. Thus, spherosomes could efficiently reduce the uptake of imidacloprid in cucumbers by about 1/5.

Effect of Spherosomes on Effective Control of *Aphis gossypii* Glover in Cucumbers

Table 2 shows the effect of spherosomes on the viability of *Aphis gossypii* Glover on cucumber leaf surfaces. The number of *Aphis gossypii* Glover increased significantly in the case of no usage of imidacloprid and spherosomes (control). In the case of imidacloprid without spherosomes (sample 2), the number of *Aphis gossypii* Glover became zero after 3 and 7 days, decreasing significantly. In the case of imidacloprid with spherosomes (sample 1), the number of *Aphis gossypii* Glover, surprisingly, became zero after 3 and 7 days, and showed almost the same effect as in the case of imidacloprid without spherosomes, although the concentration of imidacloprid in cucumbers decreased by about 1/5 in comparision with imidacloprid without spherosomes. Our study showed that spherosomes could efficiently reduce the uptake of imidacloprid in cucumbers.

The spherosomes that we examined are a residue from rice bran. Rice bran is a waste product generated in the process of making polished rice from brown rice, and is very inexpensive. Utilization of spherosome in the agricultural field is a step toward achiving environmental sustainability, while finding a new value in rice bran. The application of spherosomes to farmland is a useful and effective method to keep the concentration of pesticides in crops at a low level, resulting in sustainable agriculture.

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