Evaluation of Foliar Uptake of Eight Fungicides Using a New Measuring Tool, Congo Red Method

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Using a new evaluation method, Congo red method, the foliar uptakes of eight fungicides were measured. Among the fungicides tested, fenarimol, myclobutanil, edifenphos, isoprothiolane, and tricyclazole were found to be penetrable (11.0~42.8%), and were continuously absorbed into the leaf regardless of their formulation types. Procymidone, dimethomorph, and ethaboxam were seldomly absorbed into plant leaf. The uptakes of dimethomorph and ethaboxam were facilitated (18.1~22.7%) in the presence of Koremul-OE-20, as an activator, showing plateau phase at 12 h after application. Foliar uptake of fenarimol and myclobutanil into cucumber plant by spray application was larger than that by dropping application, while observed opposite in the cases of dimethomorph and ethaboxam containing activator. Congo red method in conjunction with spraying application used in the present study produced the clear results on foliar uptake of fungicides and therefore proved to be better than the conventional method in which the dropping application of pesticide has been used.

Key words: *foliar uptake, Congo red, fungicide, cucumber, rice, activator.*

Radiotracer techniques have been the most common choice for measuring foliar uptake of pesticides.[1-6] However, using radioactive compounds requires special care and equipment as well as high preparation cost. Furthermore, since they must be applied as a dissolved solution in general, it is difficult to obtain any practical information about the foliar uptake, particularly in the case of commercial formulations such as wettable powders or suspension concentrates which give suspensions by dilution with water. In these reasons, the radiotracer method is often not suitable for conventional researches and the development of pesticide formulations. Furthermore, it is necessary to find a new evaluation method for measuring foliar uptake of pesticides in case which the technical material or the commercial formulation of pesticide must be used, and which spray application is required.

For measuring the pesticide deposit, dyes[7-12] or organometallic compounds[13,14] are often used as tracers. But, these materials are not suitable for measuring foliar uptake of pesticides, since they also readily penetrate into leaf, or are unwashable by solvents, so that they cannot be recovered thoroughly from the leaf surface by washing.

We had already proposed a new evaluation method for the foliar uptake of pesticide using Congo red as a tracer (Congo red method)[15]. In that study, Congo red was not permeable to leaf under experimental circumstances, and was totally washable by solvents. Foliar uptake of pesticides could then be obtained by comparing the ratio of pesticide to tracer in leaf washings recovered immediately after application, with the ratio of pesticide to tracer in leaf washings at the specified time intervals. *In situ* degradation/dissipation of pesticide on the leaf surface could be compensated by parallel experiment with a control material such as glass plate to which Congo red and pesticide are impermeable.

In this paper, the foliar uptakes of 8 fungicides into cucumber and rice plant were measured by Congo red method to investigate the penetration characteristics of those fungicides to plant leaf.

Materials and Methods

Cucumber and rice plants. Cucumber plants (variety: Paekmi Paektataki, Dongbu Hannong Seeds Co., LTD., Korea) were grown from seed to the one-leaf stage. Each cucumber plant was transplanted to a pot (66 mm i.d. × 66 mm h) filled with commercial fertilized soil, and grown to 45-leaf stage under greenhouse condition (watering by spraying tap water). Only the second leaf of cucumber plant was used for all tests. Rice plants (variety: Dongjinbyo) were grown from seed to the three-leaf stage. Three rice seedlings were transplanted to a pot (66 mm i.d. × 66 mm h) and grown to 4~5-leaf stage of tillers in the greenhouse.

Reagents. Congo red (97%) was purchased from Aldrich Chemical Company, Inc. (USA). Commercial formulations of fungicides and spreader-sticker were purchased as follows:

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Abbreviations: WP, wettable powder; EC, emulsifiable concentrate; HPLC, high performance liquid chromatography.

procymidone WP (50%, Dongbang Agro Co., Korea), fenarimol EC (12.5%, Novartis Agro Korea Ltd.), dimethomorph WP (25%, Dongbang Agro Co., Korea), ethaboxam WP (25%, Misung Agrochemicals Co., Ltd., Korea), myclobutanil WP (6%, Kyungnong Co., Korea), edifenphos EC (30%, Misung Agrochemicals Co., Ltd., Korea), isoprothiolane EC (40%, Hankook Samgong Co., Ltd., Korea), tricyclazole WP (75%, Aventis Crop Science Co., Korea), and Cover liquid formulation (30%, Dongbu Hannong Chemical, Korea). Koremul-OE-20 (polyoxyethylene oleyl ether with *ca.* 20 ethylene oxide units) were obtained from Hannong Chemicals Incorporation, Korea.

Foliar uptake of fungicides into cucumber plant. Spray solutions of procymidone WP (500 μg/m*l*), fenarimol EC (31.25 μg/m*l*), ethaboxam WP (62.5 μg/m*l*), dimethomorph WP (250 μg/m*l*), and myclobutanil WP (39 μg/m*l*) were prepared by diluting commercial formulations with distilled water. In each spray solution, the concentration of Congo red was adjusted to 400, 50, 50, 200, and 25 μg/m*l*, respectively. In addition, spray solutions of ethaboxam and dimethomorph containing Koremul-OE-20 (1,000 μg/m*l*) were also prepared from WP formulations respectively.

Each dilute solutions was sprayed onto 18 cucumber plants and 18 glass plates (100 mm × 100 mm × 1 mm) in a spray booth (model SB-6, 8001VS hydraulic nozzle, RD Sprayers Inc., USA) operating at 250 kPa to give a volume rate equivalent to 80 *l*/ha under dim fluorescent-light. Immediately after spraying, three cucumber leaves were cut and put into test tubes (32 mm i.d. × 200 mm L) containing aqueous acetonitrile solution respectively. Acetonitrile/water (20 m*l*, 30/70, v/v) was used to wash the leaf deposit of fenarimol, dimethomorph, and ethaboxam, while acetonitrile/water (40/60, v/v) was used for procymidone and myclobutanil. Test tubes were then shaken for two minutes at 60 rpm. Immediately after spraying, three glass plates were also put into petri dishes (150 mm i.d. × 20 mm h) following adding aqueous acetonitrile solution (13 m*l*). Petri dishes were gently shaked for 1 minute. An aliquot of washings were transferred to the capped test tubes. The remaining cucumber plants and glass plates were stored in the dark room (temperature: 20~25°C, relative humidity: 60~90%), and washed at 1, 3, 6, 12, and 24 h after spraying.

Congo red and fungicides in washings were simultaneously analyzed by high-performance liquid chromatography (HPLC) with visible and ultraviolet absorption detection. Operating parameters were shown in Table 1.

Foliar uptake of each fungicide into cucumber plants was calculated from the difference between ratio of active ingredient/Congo red in washings obtained immediately after application, and the ratio observed at time t using equation 1.

$$\text{Uptake}(\%) = \{[({}^{t}A_{gp}/{}^{t}A_{gc})/({}^{0}A_{gp}/{}^{0}A_{gc})]-[({}^{t}A_{pp}/{}^{t}A_{pc})/({}^{0}A_{pp}/{}^{0}A_{pc})]\}100 \quad (1)$$

${}^{t}A_{gp}$: Peak area of pesticide in washing of glass plate at time t
${}^{t}A_{gc}$: Peak area of Congo red in washing of glass plate at time t
${}^{0}A_{gp}$: Peak area of pesticide in washing of plate immediately after application
${}^{0}A_{gc}$: Peak area of Congo red in washing of plate immediately after application
${}^{t}A_{pp}$: Peak area of pesticide in washing of leaf at time t
${}^{t}A_{pc}$: Peak area of Congo red in washing of leaf at time t
${}^{0}A_{pp}$: Peak area of pesticide in washing of leaf immediately after application
${}^{0}A_{pc}$: Peak area of Congo red in washing of leaf immediately after application

Fungicide solutions of fenarimol, dimethomorph, ethaboxam, and myclobutanil were prepared in the same manner mentioned above without Congo red. These solutions (100 μ*l*/leaf) were applied as droplets (droplet size: about 0.3 μ*l*) using a microsyringe to ten cucumber leaves. Immediately after application, five cucumber leaves were cut and washed, and the remaining cucumber leaves were washed at 24 h after application. Fungicides in washings were measured by HPLC. Foliar uptakes of the fungicides into the cucumber plants were calculated from the area difference of active ingredient in washings obtained immediately and at 24 h after application.

Foliar uptake of fungicides into rice plant. Spray solutions of edifenphos EC (600 μg/m*l*), isoprothiolane EC (800 μg/m*l*), and tricyclazole WP (375 μg/m*l*) were prepared by

Table 1. Operating parameters of HPLC for the analysis of Congo red and fungicides.

Parameter	Fungicide							
	Procymidone	Fenarimol	Dimethomorph	Ethaboxam	Myclobutanil	Edifenphos	Isoprothiolane	Tricyclazole
Instruments	Waters 717plus Autosampler, Waters 2487 Detector, WatersPump Model 510							
Column	Nova-Pack® C18 (3.9 mm i.d. × 300 mm L, Waters Co., USA)							
Mobile phase (acetonitrile : water, v/v)	42 : 58	50 : 50	40 : 60	43 : 57	39 : 61	60 : 40	60 : 40	35 : 65
Flow rate (m*l*/min)	2.0	1.5	1.5	1.5	2.5	1.5	1.5	1.5
Wavelength (nm)[a]	229	220	243	310	220	228	282	280
Sample size (μ*l*)	20	20	20	20	20	20	20	20
Retention time (min, Congo red/fungicide)	1.1/21.3	1.2/7.2	1.3/9.9,11.3	1.3/5.5	0.8/13.0	1.0/6.3	1.0/8.6	1.3/3.5

[a]Two wavelengths, 497 nm, and UV, were sequentially used for the detection of Congo red and fungicide.

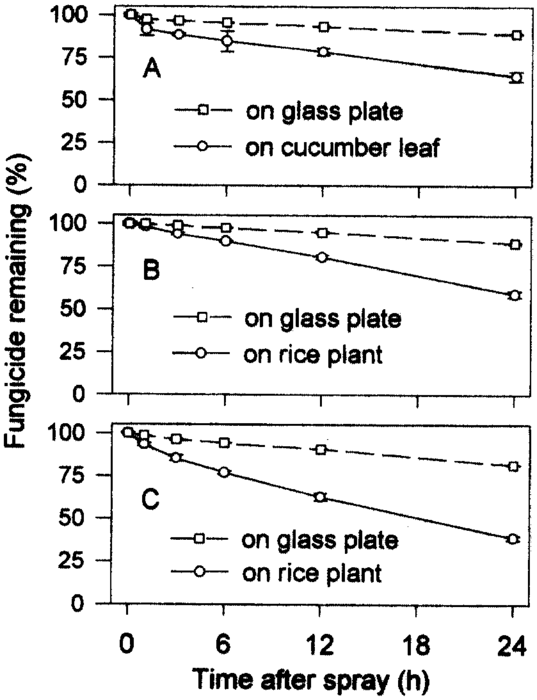

Fig. 1. Fungicide remaining on glass plate and plant surface after spraying. A; 39 μg/m*l* of myclobutanil WP: B; 600 μg/m*l* of edifenphos EC: C; 800 μg/m*l* of isoprothiolane EC.

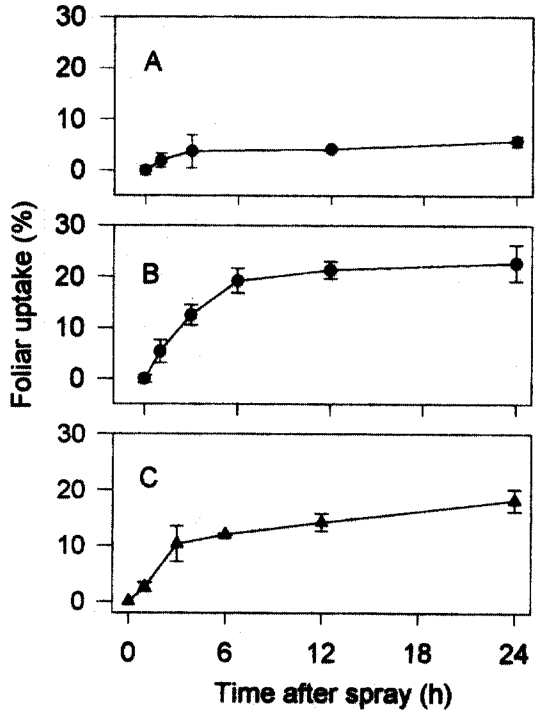

Fig. 2. Foliar uptake of fungicides into cucumber plant after spraying. A; 500 μg/m*l* of procymidone WP: B; 250 μg/m*l* of dimethomorph WP and 1,000 μg/m*l* of Koremul-OE-20: C; 62.5 μg/m*l* of ethaboxam WP and 1,000 μg/m*l* of Koremul-OE-20.

diluting formulations with distilled water. In each spray solution, the concentration of Congo red was adjusted to 600, 600, and 400 μg/m*l*, respectively. Cover liquid formulation (150 μg/m*l*), as a spreader-sticker for tricyclazole, to increase deposit on rice plants, was also added.

These solutions were sprayed onto 18 pots of rice plants and 18 glass plates in spray booth at a rate equivalent to 250 *l*/ha. Acetonitrile/water (15 m*l*, 30/70, v/v) was used to wash the deposit. The foliar uptakes of fungicides into rice plants were measured by the same procedure as described above.

Results and Discussion

Pesticides on leaf surfaces can be dissipated by washing off, volatilization, and degradation including photodegradation and hydrolysis as well as the penetration into leaf. As this study was carried out in the dark, photodecomposition would be negligible. However, volatilization or degradation other than photodecomposition might be also important factors to obscure penetrability of the pesticides. To correct the dissipation by volatilization and/or degradation, glass plates were used under the same conditions because Congo red and fungicides are impermeable to glass plate and they are readily washed by solvent to recover completely. The foliar uptake of pesticides into plants can then be obtained by comparing the ratio of pesticide/Congo red on glass plate to that on the leaf surface.

Fungicides and their concentration employed in this work were selected on the basis of practical use in controlling predominant pathogens occurred in cucumber and rice plant.

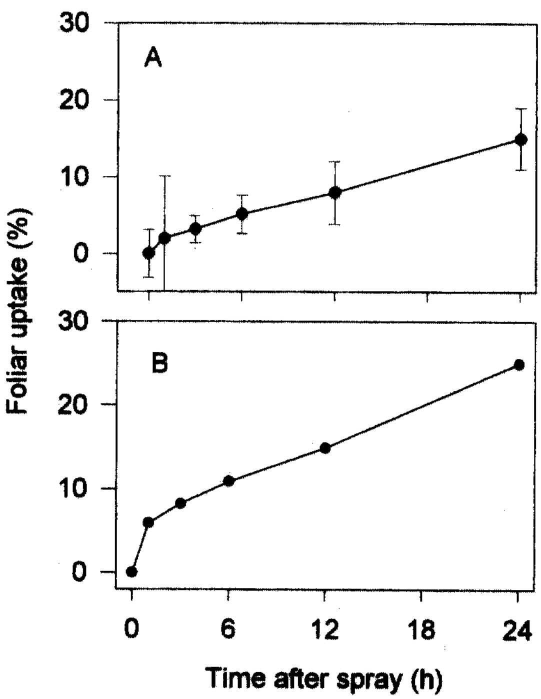

Fig. 3. Foliar uptake of fungicides into cucumber plant after spraying. A; 31.25 μg/m*l* of fenarimol EC: B; 39 μg/m*l* of myclobutanil.

Foliar uptake of fungicides after spraying. No dissipation of procymidone, fenarimol, dimethomorph, ethaboxam

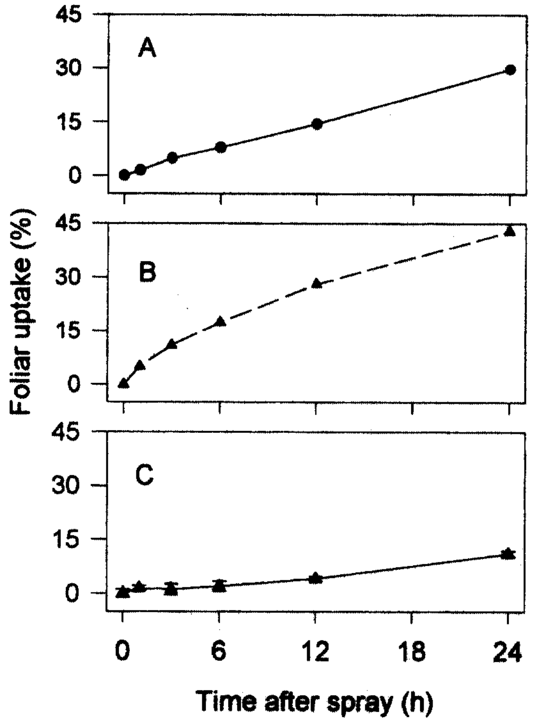

Fig. 4. Foliar uptake of fungicides into rice plant after spraying. A; 600 μg/m*l* of edifenphos EC: B; 800 μg/m*l* of isoprothiolane EC: C; 375 μg/m*l* of tricyclazole WP.

and tricyclazole was observed even on the glass plate. However, a significant dissipation of myclobutanil, edifenphos, and isoprothiolane was observed on the glass plate with recoveries of 89.2±3.6, 88.9±0.6, and 81.7±0.1%, respectively, at 24 h after spraying (Fig. 1). These dissipations from glass plate were supposedly due to the volatilization and hydrolysis of fungicides.[16]

Foliar uptake of procymidone (Fig. 2A), dimethomorph and ethaboxam into cucumber plants was observed as small as 5.7±1.0, 0.5±1.5, and 0.1±1.0%, respectively at 24 h after spraying. Therefore these fungicides were practically not penetrable to leaf surface at the concentration tested. But uptake of dimethomorph and ethaboxam into cucumber leaf was greatly enhanced by addition of Koremul-OE-20 resulting 22.7±3.6 and 18.1±2.0% of uptake rate, respectively (Fig. 2B and 2C).

Fenarimol uptake into the cucumber leaf (Fig. 3A) and tricyclazole uptake into rice leaf (Fig. 4C) were 15.1±4.0 and 11.0±0.9%, respectively, while the foliar uptakes of myclobutanil (Fig. 3B), edifenphos (Fig. 4A), and isoprothiolane (Fig. 4B) reached 25.0±2.7, 29.8±1.9, and 42.8±1.4%, respectively, at 24 h after spraying. The latter three results were the corrected data by subtracting remainings on leaf surface from on glass plate in considering the significant dissipation from the leaf surfaces of plants.

The foliar uptakes of fenarimol, myclobutanil, edifenphos, isoprothiolane, and tricyclazole into cucumber or rice plants were found to increase nearly proportional to the time elapsed. These might be typical patterns observed in the uptake of systemic fungicides as reported.[17] Although an activator surfactant, Koremul-OE-20, facilitated the uptake of dimethomorph and ethaboxam into cucumber leaf remarkably, but its effect rapidly diminished with time.

Table 2. Influence of application method on foliar uptake of fungicides into cucumber plant.

Fungicide	Foliar uptake at 24 h after application (%)	
	Spraying[a]	Droplet[b]
Fenarimol	15.14.0	11.43.9
Dimethomorph[c]	22.73.6	48.84.1
Ethaboxam[c]	22.05.8	18.02.0
Myclobutanil	25.02.7	15.23.1

[a]Mean and error of 3 samples.
[b]Mean and error of 5 samples.
[c]Koremul-OE-20 was added as an activator.

Since the uptake tests were carried out separately, and the application dosages were different from each others, the relationships between uptakes and water solubilities or partition coefficients of fungicides could not be estimated precisely. But, it was noticed, at a glance, that fungicide having water solubility higher than 50 mg/*l*, such as myclobutanil, edifenphos, isoprothiolane, and tricyclazole, was easily absorbed into plant leaf. And the octanol/water partition coefficient ($logP_{ow}$) of fungicides was not closely related with foliar uptake.

Influence of application method to foliar uptake. In the conventional foliar uptake study, the limited amount of radioactive pesticide should often be applied as droplets on leaf surface. However, in the present study, the spray method using track sprayer was used under the controlled condition, because the foliage application of pesticide in field has been made by spraying in general. In this respect, fungicide solutions were applied as droplets to compare with the spraying method used in the present study. The uptake rates of fenarimol, dimethomorph (containing Koremul-OE-20), ethaboxam (containing Koremul-OE-20), and myclobutanil into cucumber leaves at 24 h after dropping application were very different from those obtained by spraying application with Congo red (Table 2). Foliar uptake of fenarimol and myclobutanil (known as 'systemic fungicides') obtained by spray application was larger than that obtained by a dropping application. However, dimethomorph and ethaboxam containing Koremul-OE-20 as an activator showed the reverse responses compared to cases of fenarimol and myclobutanil. This suggests that the concentrated activator with drying of water in deposit further enhanced the foliar uptake of fungicides. This is well-known phenomenon that the foliar uptake of pesticide containing an activator increases with the concentration of the activator.[18-20] These results also indicated that the conventional dropping method used in the radiotracer technique, could not give the practical information for both systemic and non-systemic fungicides, and particularly for activator-containing formulations. Congo red method in conjunction with spraying application used in the present study gave, therefore, more reliable results on foliar uptake of fungicides and proved to be better than the conventional method in which the dropping application of pesticide has been used.

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