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Absorption, Movement and Metabolism of ^{14}C-cyfluthrin in Cotton Leaves

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Abstract: A greenhouse experiment was conducted to study the uptake and metabolism in cotton leaves of ^{14}C-labelled cyfluthrin, ($C_{22}H_{18}Cl_2FNO_3$, active ingredient of Baythroid insecticide). The labelled chemical was sprayed onto the selected portions of leaves which were subsequently studied for the movement (using autoradiography) and recovery of ^{14}C (using extraction, purification procedures). Autoradiography showed a fairly rapid movement of ^{14}C in the leaf tissues through vascular tissues. Movement was more efficient when application was made on the mid-rib region. Dissipation of ^{14}C was also fast and even after 2 days, >60 percent of it was unaccounted. Subsequently, however, the losses were slow and amounted to 70.6 percent after 35 days. Thin layer chromatography/co-chromatography of organic extracts followed by linear scanning revealed that >60 percent of the ^{14}C was still present as parent compound. Partial hydrolysis of cyfluthrin was found to be the main process involved in degradation that resulted in two major degradation products or metabolites.

Key words: Baythroid, ^{14}C, Cyfluthrin, cotton, Insecticide

Introduction

Of the different kinds of insecticides used in agriculture, synthetic pyrethroids have become a commercially successful group because of their desirable environmental properties of short-term persistence and comparatively low application rates required for insect control. During spray application, a major portion of the insecticides is intercepted by the plant leaves of which a significant part may enter the leaf tissues through stomata (Preiss *et al.*, 1988). However, compared to other pesticides, relatively less is known about the metabolism and further fate of pyrethroids in the plant tissues. This is particularly true for Pakistan where a very limited work on the fate of pesticides (particularly insecticides) has been reported (Hussain *et al.*, 1984, 1986; Azam *et al.*, 1986, 1988) in spite of their heavy use in agriculture.

From the leaf surfaces, the insecticides are dissipated fairly rapidly, while a portion is incorporated into the plant tissues. Using ^{14}C-Fenpropathrin (a pyrethroid), Mikami *et al.* (1985) reported a quick disappearance of the chemical with 50 percent loss of ^{14}C within the first 11-12 days; after 42 days only about 10 percent of the applied chemical was found on leaves. Most of the applied chemical remained at the site of application (leaf), with little movement in the shoot. Gaughan and Casida (1978) studied the degradation of ^{14}C-labelled Permethrin on cotton and bean plants and observed a loss of ca 30 percent of the ^{14}C within 1 week from cotton. Nash (1983) suggested that volatilization is a major dissipation route for many pesticides. Photodegradation of foliarly applied insecticides may be another significant loss mechanism (Holmstead *et al.*, 1978; Takahashi *et al.,* 1985).

Within the plant tissues, the insecticide residues may be found in extractable and bound forms. Khan *et al.* (1984) characterized the bound residues of Permethrin in radish using tracer techniques and showed that a considerable part of the pesticide residues were bound into plants; net recovery being 67 percent after 21 days of application. Gaughan and Casida (1978) determined a small fraction of Permethrin applied to cotton and bean plants in extractable forms, while a major proportion was found in non-extractable (bound) forms; total recovery being 50 percent.

In Pakistan, Baythroid (with cyfluthrin as the active ingredient) is a commonly used insecticide for cotton which is a major cash crop. Previously, we have reported on the degradation and movement in soil of ^{14}C-cyfluthrin (Lodhi *et al.,* 1996, 2000). Objective of the present investigation was to study the movement, metabolism and dissipation of ^{14}C-labelled Cyfluthrin sprayed onto cotton leaves.

Materials and methods

Cyfluthrin ($C_{22}H_{18}Cl_2FNO_3$) is the common name of the active ingredient of Baythroid with the chemical name, Cyano-(4-fluoro-3-phenoxyphenyl)-methyl-3-(2,2-dichlorethenyl)-2,2-dimethyl-cyclopropanecarboxylate and having molecular weight of 434.0. Structural formulae of the chemical with phenol carbon labelled and the two major metabolites are shown in Fig. 1.

Nine-weeks old cotton plants grown in a filed lysimeter were selected for this experiment. Healthy and well-spread leaves of different plants were selected for application of ^{14}C-cyfluthrin mixed with Baythroid and applied to leaves at 10 µg/leaf delivering 2.5 µCi ^{14}C. The solution was applied using a hypodermal syringe as described by Mikami *et al.* (1985). They used 22 µg/leaf of the chemical as against 10 µg in this study. Before application, however, it was ascertained that no apparent damage is done to the leaves during application. For autoradiography, Cyfluthrin was applied on separate leaves at 4 different positions as shown in Fig. 2. However, for studying the dissipation and movement within the leaf, cyfluthrin was applied only at position 2 in which case 10 leaves were treated.

Whole leaves as well as leaves along with small portion of the stem were detached at different intervals after spray application of ^{14}C-cyfluthrin using a hypodermal syringe and subjected to autoradiography. The samples were briefly rinsed with a solution of methanol-water (1:2 ratio) to remove superficial residues, wrapped in a thin layer of polyethylene, and transferred to cassettes containing Kodak Diagnostic Film (X-OMAT AR, 13 x 18 cm). The cassettes were left at -20° C for 3 days. The film was removed and transferred to developer mixture in the absence of light and left to develop for 7-9 min without agitation followed by thorough rinsing with distilled water and then transferred to fixer solution. After keeping in fixer solution for about 3 min with intermittent shaking, the films were washed with water and allowed to dry.

Duplicate leaves were harvested after 2, 7, 14, 29 and 35 days of cyfluthrin application, rinsed in dilute methanol (methanol:water, 1:2) to remove surface residues, freeze-dried, finely powdered and subjected to extraction/analysis procedures. Briefly, leaf samples (50 g), water (80 ml) and acetonitrile (170 ml) were taken in a blender and homogenized for 5 min. The slurry was filtered and the residue was dried, combusted and counted for ^{14}C as described by Lodhi *et al.* (1996). The filtrate was shaken with 100 ml hexane in a separating funnel followed by the addition of 100 ml 2 percent NaCl solution. After shaking for 1 min, the mixture was

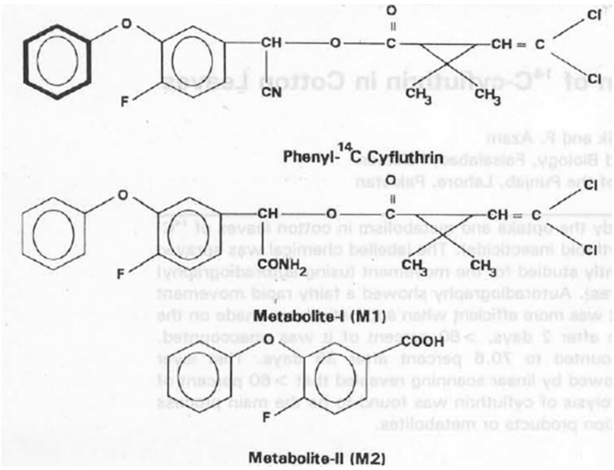

Fig. 1: Structural formulae of cyfluthrin and two metabolites

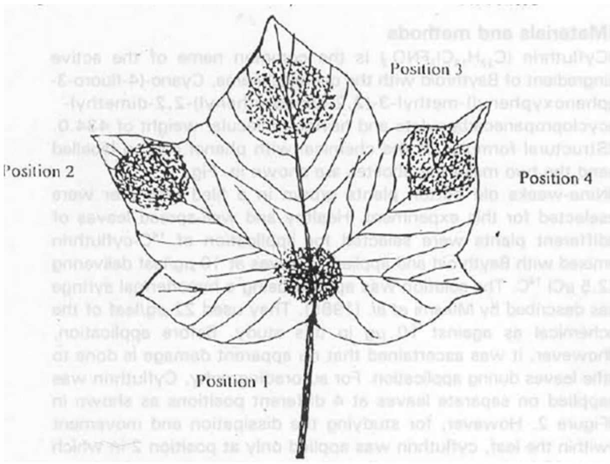

Fig. 2: Cotton leaf showing 4 positions of application of ^{14}C-cyfluthrin

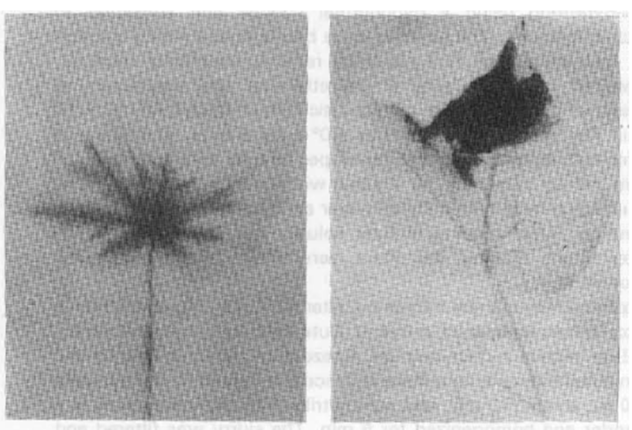

Fig. 3: Autoradiograms of cotton leaves receiving spray application of ^{14}C-cyfluthrin on mid-lab (A) and mid-vein (B) positions

allowed to stand. The organic layer was separated, shaken with 50 ml water for 1 hr, passed through anhydrous sodium sulphate and evaporated to dryness. The residue was redissolved in methanol and an aliquot counted for ^{14}C. The extracts were counted directly, while portions of leaf residues were combusted and then counted as described before. Aqueous portion was counted directly.

Appropriate volume (0.2-1.0 ml) of the liquid sample and insta-gel (Packard, USA)-scintillation cocktail (5-10 ml) were taken in a 28 ml glass scintillation vial (Packard, USA), and mixed thoroughly. The vials were subjected to counting for ^{14}C using a liquid scintillation counter (Packard Tricarb 4530). The output obtained in the form of disintegrations per minute (dpm) was used for further data processing as required.

Samples of plant material were combusted in a Packard Oxidizer 306 (Hewlett Packard, USA). Known quantities were taken in combustion cups (Packard, USA) and after adding 1-2 drops of combust-aid, the sample was subjected to combustion. The CO_2 trapped in carbosorb was received in a scintillation vial (28-ml) along with the permafluor scintillation cocktail (Packard, USA). The vials were stopperred and subjected to ^{14}C counting as described above.

Results and Discussion

Autoradiography of leaves showed significant presence of ^{14}C within the leaf at locations fairly away from the site of application suggesting not only the uptake but movement of the insecticide within the leaf tissues (Fig. 3). Although pyrethroids are not plant systemic insecticides they can be taken up by plants through the stomata (Preiss *et al.*, 1988). In the present work, movement of cyfluthrin was quite efficient when applied at position 4 i.e., at the point where main veins diverge to different parts of the leaf. Apparently, the movement was both through the xylem and phloem since ^{14}C activity was determined in all directions from the site of application. Significant movement of ^{14}C occurred down the petiole into the woody branches.

Data presented in Table 1 shows the recovery of applied ^{14}C in leaves and its distribution in extractable and bound forms; loss of ^{14}C (unaccounted) is also tabulated. Loss of cyfluthrin-^{14}C from the plant leaves was fairly rapid as suggested by smaller percentage recovered at different time intervals. Even after 2 days of application, more than 60 percent of the applied ^{14}C was unaccounted. Losses during the subsequent period were slow and after 35 days they increased to only 70.6 percent. Several mechanisms may be involved in the dissipation of foliar insecticide. A part of the applied ^{14}C may be washed to the soil as demonstrated by McDowell *et al.* (1987), Maqueda *et al.* (1989) and Willis *et al.* (1986) using simulated rains. In the present study, however, rain was not a factor although overnight dew and leaf drip might have facilitated the washing of applied insecticide from the leaf surfaces. According to Cole *et al.* (1982) photochemical reactions are the primary mechanisms for degradation and loss of pyrethroids sprayed on plant leaves and involve ester cleavage and conjugation. Gaughan and Casida (1978) attributed degradation to hydrolysis followed by rapid conjugation. Nash (1983) conducted a field trial to study the fate of different pesticides from agroecosystems by spraying on wheat and barley and suggested volatilization as the dominant mechanism of pesticide loss from plant leaves. Fritz *et al.* (1992), on the other hand reported little volatilization of cyfluthrin.

Gaughan and Casida (1978) studied the degradation of Permethrin on cotton and bean plants. They reported a loss of ca 30 percent of the ^{14}C within 1 week from cotton with minor differences in labelling position, loss decreased with time and of the applied ^{14}C only 50 percent was recovered after 14 days.

Table 1: Recovery of applied ^{14}C in extractable (aqueous, organic) and bound forms

Days after application	Recovered in leaves				
	Extractable		Bound	Total	Unaccounted
	Aqueous	Organic			
	Percent of applied ^{14}C				
2	12.6±0.6	8.2±0.2	13.2±0.2	34.0±0.3	64.0±2.8
7	10.3±0.3	7.5±0.3	12.9±0.4	30.7±0.8	69.3±1.5
14	11.6±0.4	7.2±0.3	14.6±0.6	31.4±0.5	68.6±1.8
29	14.7±0.2	5.7±0.1	12.5±0.3	32.9±0.7	67.1±2.1
35	12.7±0.5	6.6±0.2	10.1±0.4	29.4±1.1	70.6±3.3

Table 2: Distribution of ^{14}C in cyfluthrin and metabolites in the leaf tissues

Days after application	Percent of total ^{14}C in organic extracts determined as			
	Cyfluthrin	Metabolite 1	Metabolite 2	Total
2	66.3±2.5	19.7±0.9	9.1±0.6	95.1±6.1
7	71.4±2.2	19.4±0.7	7.2±0.4	98.0±5.3
14	69.1±1.9	21.1±1.1	6.2±0.3	96.4±7.1
29	65.3±2.1	22.5±2.3	8.2±0.4	96.1±4.2
35	60.5±2.3	21.7±0.8	11.8±0.5	94.0±4.1

Mikami *et al*. (1985) found that half of the activity applied to cabbage as Fenpropethrin specifically labelled at cyano, benzyl and cyclopropyl groups was lost within 10-12 days. After 42 days, only 10 percent of the initially applied ^{14}C was detected in leaves without showing movement in the shoot. In the present study, however, autoradiography of the leaves and shoot portions of cotton showed significant movement of cyfluthrin-^{14}C within the plant tissues (Fig. 3). Movement was significantly more efficient when cyfluthrin was applied around the conducting tissues.

A greater proportion of the ^{14}C in leaves was recovered in aqueous and organic extracts and only about one-third was in non-extractable (bound) forms. Although the relative distribution of ^{14}C in different fractions did not show consistency with time, a clear increase in the bound forms was obvious. The increase in bound fraction could be attributed to the hydrolytic activity of the plant esterases towards cyfluthrin leads to the generation of polar metabolites which may be transformed to non-extractable residues. Such metabolites are conjugated with glucose to a great extent and then deposited in the vacuole, which has the effect of reducing the toxicity of metabolite towards the plant because of an inactivation of the functional phenolic group. On the other hand, these sugar conjugates of pesticide metabolites are of considerable concern with respect to the residue problem of xenobiotics since they commonly remain undetected during the routine analysis of pesticides in plant material.

Organic extracts of leaves were subjected to TLC and linear scanning for quantification and identification of ^{14}C labelled fractions. Results presented in Table 2 show that from 64-72 percent of the ^{14}C was still in the form of parent compound, whereas among the two other ^{14}C-labelled compounds, a greater proportion of ^{14}C was attributable to M1: M2 represented a relatively small proportion. However, compared to the results obtained in incubation studies (unpublished data), much higher proportion of the ^{14}C was in M2. There was no consistency in the distribution of ^{14}C in cyfluthrin and metabolites but an apparanet trend was that cyfluthrin ^{14}C decreased, while the metabolites increased. The increase was more obvious for M2. Cole *et al.* (1982) compared the degradation of 4 pyrthroids i.e., Tralomethrin, Tralocythrin, Deltamethrin and Cypermethrin on cotton and bean foliage. Residues on leaves consisted of parent material and various ester photoproducts. Similar observations were reported by Ruzo *et al.* (1982) and Class *et al.* (1990). Additional products included polar conjugates. With the exception of initial debromination and dehydrobromination, the degradation processes and ultimate residues of Tralomethrin and Tralocythrin appeared to be essentially the same as those of others. Mikami *et al.* (1985) suggested that insectcides underwent ester cleavage, hydroxylation at either or both of the dimethyl groups with sebsequent oxidation to carboxylic acid and hydrolysis of the CN group to the $CONH_2$ and COOH groups.

Preiss *et al.* (1988) proposed the degradation route of cyfluthrin in tomatoes and identified 3 main degradation products/metabolites namely; i) 3-phenoxy-4-fluorobenzaldehyde, ii) 3-phenoxy-4-fluorobenzoic acid and iii) 3-phenoxybenzylalcohol. Of these 3-phenoxy-4-fluorobenzoic acid (described as M2 in this study) which results from the oxidation of metabolite (i) of Preiss *et al*. (1988) has been identified in the present studies as well. Metabolite 1, which was identified as the major degradation product in the incubation studies was also present in leaf extracts and might have been produced by partial hydrolysis of cyfluthrin. Zhang and Scott (1994) chemically synthesized metabolites of several 3-phenoxybenzyl pyrethroids excluding cyfluthrin to facilitate identification of different products of degradation/metabolism. According to them there are great similarities between the products of different pyrethroids. For example, products of degradation of Fenvalerate and cyfluthrin were similar (Lee, 1985; Preiss *et al*., 1988). Like-wise the metabolic products of pestcides from different systems (animal, plants, microbes) are also fairly similar (Zhang and Scott, 1994).

In summary, results of this study show that dissipation of cyfluthrin from the leaves is fairly rapid, while a substantial portion enters into the leaves and transported to other plant parts mainly through the vascular tissues. Within the plant leaves, further degradation and metabolism of cyfluthrin is quite fast, although a significant proportion (>60%) of the chemical remains intact even 35 days after application. Partial hydrolysis of cyfluthrin was found to be the main process involved in degradation.

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