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Movement, Persistence and Uptake by Plants of ^{14}C -labelled Cyfluthrin

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Abstract: A field lysimeter experiment was conducted to study the uptake by plants, dissipation and movement in soil of ^{14}C -cyfluthrin (active ingredient of Baythroid, an insecticide produced by Bayer, Germany). Cotton and wheat plants were grown in succession to study the uptake of ^{14}C . The chemical was worked well into the soil supporting the growth of a healthy cotton plant. The plant harvested at maturity contained 0.376 percent of the applied ^{14}C ; a major portion (ca 65 percent of the total) of the ^{14}C being located in the stem portion. Wheat plants grown after cotton contained 0.11 percent of the initially applied ^{14}C .

Dissipation of ^{14}C from the soil-plant system was fairly rapid and after 9 weeks (during growth of cotton), ca 50 percent of the applied ^{14}C was unaccounted. Subsequent losses were slower and during the remaining study period of 29 weeks, a further decrease of only 10 percent of the applied ^{14}C occurred. Wheat growth and organic amendment caused a decrease in the loss of ^{14}C from the soil-plant system. At all sampling intervals, a greater proportion of ^{14}C was restricted to the top 0-10 cm layer and the amount consistently decreased with depth. In general, >80 percent of the ^{14}C determined in soil at different sampling intervals was present in forms non-extractable with methanol.

Key words: Baythroid, ^{14}C , cotton, cyfluthrin, insecticides, nitrapyrin, soil, wheat

Introduction

In Pakistan, synthetic pyrethroids are used extensively against insect pests of cotton, Baythroid (having cyfluthrin as an active ingredient) being an example of such insecticides. Of the foliarly applied insecticides, a substantial portion may be washed to the soil (McDowell *et al.*, 1987; Willis *et al.*, 1986). According to Maqueda *et al.* (1989) a large proportion of the chemical goes directly to the soil during foliar applications. In soil, a part of the pesticide residues is amenable to uptake by the plants. However, the amount taken up is generally low because of rapid binding onto the soil colloidal complex and restricted mobility (Lee, 1985; Maitlen and Halfhill, 1985; Mikami *et al.*, 1985; Amos and Leahey, 1986). Low permeability of cell membranes to pesticide residues may be another factor inhibiting the uptake by plants. However, the uptake of pesticide residues by plants is facilitated by complexation with humic compounds such as fulvic acid (Schnitzer and Khan, 1978).

A significant proportion of the chemical is adsorbed onto the soil colloidal complex and thus physically protected from further transformations and movement in the soil-plant system. Degradation of pesticides is generally slow in heavy-textured soils because of their adsorption onto the clay particles (Stevenson, 1976). Importance of clays in stabilizing organic compounds has been emphasized by different workers (Cheng *et al.*, 1978; Sorenson, 1975). Similarly, soils rich in organic matter also give protection to these chemicals through adsorption and/or transformation into complex humic substances (Arienzo *et al.*, 1994; Azam *et al.*, 1988; Khan and Hamilton, 1980; Sundaram, 1996, 1997). The fraction of the pesticide in excess of the soil's protecting ability is susceptible to movement and microbial transformations. Root growth and root-driven

microbial activity may also have significant effect on the persistence, movement and transformations of pesticides in soil. However, because of soil's protective role, downward movement of pesticides is generally low (Lee, 1985).

Our objectives were to study the uptake, dissipation from and movement in soil and the distribution in bound and extractable forms of cyfluthrin- ^{14}C applied to soil under field conditions.

Materials and Methods

The experiment conducted on an undisturbed soil column in a cotton field. A squarish soil core measuring 0.6×0.6 m and bearing a 9-week old cotton (*Gossypium hirsutum* L.; var. NIAB-78) plant was dug by cutting the soil from around. A stainless steel rectangular cylinder ($0.6 \times 0.6 \times 1.0$ m) was then pushed onto the core using a polypropylene hammer to avoid excessive disturbance/compaction of the contained soil column. A part of the cylinder (4 in) was kept out of the soil to isolate the lysimeter soil from the rest of the field. This was necessary to prevent mixing of outside soil/water with the lysimeter soil during the experiment. The soil removed from around the newly established core was put back and excessive water applied to get it settled. Within a week, the lysimeter was well established.

Surface 10 cm of the lysimeter soil was then removed and treated with 10.8 mg ^{14}C -labelled cyfluthrin (34130 KBq ^{14}C). Structural formula and position of ^{14}C labelling is shown in Fig. 1. The rate of cyfluthrin application was equivalent to 300 g ha^{-1} or ca $0.2 \mu\text{g g}^{-1}$ soil (considering 52 kg soil in the top 10 cm of the lysimeter). In order to achieve uniform mixing, solution of cyfluthrin in 3 ml methanol was mixed well with enough volume of water (9 litres) to wet the soil to 12 percent WHC. The solution

thus prepared was sprayed onto the soil which was spread in a thin layer. The sprayed soil was thoroughly mixed to ensure good distribution of cyfluthrin and put back into the lysimeter followed by compaction and irrigation.

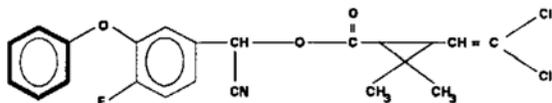


Fig. 1: Structural formula of cyfluthrin (thick line shows the position of ^{14}C labelling)

During the growing period, triplicate soil cores (2 cm diameter) to a depth of 50 cm were obtained after 1, 3, 6, 9 weeks using a stainless steel auger. The soil cores obtained at different intervals were divided into layers measuring 0-10, 10-20, 20-30, 30-40 and 40-50 cm. Required quantities of the soil representative of each depth were freeze-dried and stored for further analysis. Rest of the soil was put back into the holes at respective depth. Cotton plant was harvested at physiological maturity 9 weeks after the application of cyfluthrin. During the growth period (May-November), a total 420 mm rain was received, while 5 irrigations of 74 mm each were given when required. The humidity varied from 40-80 percent; maximum being in the months of July and August and minimum in November. The harvested plant material was partitioned into leaves, stem, seed and root portions and oven-dried at 70°C .

After harvesting cotton, the lysimeter was divided into two by inserting a stainless steel plate in the middle. One half of the lysimeter was sown to wheat (*Triticum aestivum* L., var. Lu-26) and the other half was left fallow. Eight wheat plaits were raised, irrigated as required (5 irrigations of 75 mm each; rainfall amounted to 70 mm during the period December to April; humidity ranged between 30-50 percent) and removed from the soil at maturity. The harvested plants were divided into root, straw and grain portions. All plant components were dried and finely powdered for further analysis. Soil core samples obtained after harvesting wheat as described above were freeze dried and stored for further analyses.

At wheat harvest, both planted and unplanted halves of the lysimeter were further sub-divided into two. One portion from both planted and unplanted halves was amended with 0.5 percent wheat straw, while the other portion was left unamended. For organic amendment, top 10 cm soil of the lysimeter portion to be amended was taken out and the powdered plant material worked well into it. The amended soil was put back into the respective portions. Same procedure was adopted for unamended portion. The soil in the lysimeter was kept moist and core samples taken after 9 weeks (the total time elapsed between initial application

of cyfluthrin and final sampling would be 38 weeks). The soil samples were freeze dried and stored for further analysis.

For fractionation of ^{14}C present in soil, aliquots (100 g) of soil samples were shaken for 1 h with 200 ml of methanol followed by centrifugation (5000 rpm, 4°C , 1 h). The supernatant (extractable fraction) was filtered and preserved for scintillation counting. The residue (containing bound residues) was dried, sieved and a portion extracted with 0.1 M NaOH + 0.1 M $\text{Na}_2\text{P}_4\text{O}_7$, (1:5, soil:solution ratio, 12 h shaking followed by centrifugation for 1 h at 5000 rpm and 4°C). The residue containing humin fraction was dried, ground, sieved and combusted for the determination of ^{14}C . Supernatant containing humic acid and fulvic acid fractions was filtered and a portion acidified with conc. H_2SO_4 to get the former precipitated. The precipitate was collected by centrifugation (4000 rpm, 4°C , 30 min), dissolved in 0.5 M NaOH and preserved for scintillation counting of ^{14}C . The supernatant containing fulvic acid was counted directly. Aliquots (0.5-1 ml) of the liquid samples were counted on a Packard Tricarb Scintillation Counter (Model 4530) using instagel (Packard, USA) scintillation cocktail.

Triplicate samples of soil and plant material were combusted in a Packard Oxidizer 306 (Hewlett Packard, USA). Weighed quantities of soil were mixed with powdered cellulose in a cellulosic combustion cup (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 stoppered and subjected to ^{14}C counting as described above.

Results

Cotton plant contained 0.376 percent of the applied cyfluthrin- ^{14}C (Table 1). A major portion (0.34 percent) of the total plant ^{14}C , was determined in the root and stalk portions and only a small proportion (0.035 percent) was translocated to the seed portion. Root portion, however, was the most heavily labelled with a specific activity ($\mu\text{Ci } ^{14}\text{C g}^{-1}$ carbon) of 0.071, while the stem, leaves and seeds showed specific activity of 0.0261, 0.0065 and 0.0093, respectively.

Of the residual ^{14}C (i.e., after removing cotton plant), 0.22 percent was found in wheat plants at maturity that was equivalent to only 0.011 percent of the originally applied ^{14}C . Thus the availability to plants of cyfluthrin- ^{14}C decreased substantially with time i.e., 0.376 percent of the applied in cotton Vs 0.011 percent in wheat. A greater proportion of the total plant ^{14}C (82%) in wheat was found in the vegetative parts and only ca 18 percent was translocated to the grain. Root portion was the most heavily labelled, with grain portion showing the lowest specific activity as also observed in cotton. In contrast to cotton, however, ^{14}C was better distributed in the three plant components of wheat.

Table 1: Uptake of ¹⁴C by cotton and wheat and its distribution in different plant components

	Dry matter g	Specific activity	¹⁴ C, % of applied	¹⁴ C, % of residual
Cotton				
Root	25.5	0.0706a*	0.092b	-
Stem	168.4	0.0266b	0.243a	-
Leaves	18.4	0.0065d	0.006d	-
Seed	114.2	0.0093c	0.035c	-
Total	326.6		0.376	-
Wheat				
Root	12.4	0.0040a	0.003b	0.056b
Straw	138.9	0.0008b	0.006a	0.116a
Grain	125.1	0.0003c	0.002c	0.049c
Total	276.4	-	0.011	0.222

*Figures in a column (for either plant type) followed by a similar letter are not significantly different at 5% level of probability according to Duncan's Multiple Range Test

During 9 weeks of cotton growth, 49.6 percent of the applied ¹⁴C was lost from the soil plant system, the values representing a difference of applied and that recovered in soil upto a depth of 50 cm (Table 2). Considering an uptake of 0.38 percent by cotton plant, the remaining 49.2 percent was unaccounted. Of the total losses, over 80 percent occurred during the first 3 weeks after application. Recovery of applied ¹⁴C in both methanol-extractable and non-extractable (bound) forms decreased with the passage of time, essentially due to its loss from the soil plant system. A major portion (generally 80%) of the ¹⁴C found in soil was determined in bound forms, while upto 20 percent was still in extractable forms.

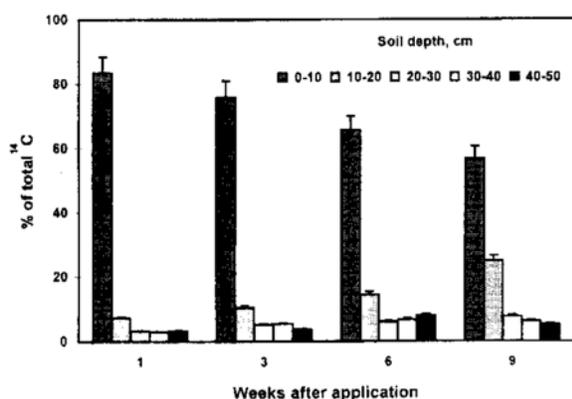


Fig. 2: Percent distribution of ¹⁴C in different soil layers during the growth of cotton

Figure 2 depicts percent distribution of applied ¹⁴C in different soil layers during nine weeks after application. A major portion of the total ¹⁴C was restricted to the top 0-10 cm which was understandable due to high organic matter content of the soil and a rapid adsorption of cyfluthrin on soil colloidal complex. One week after application, 84 percent of the ¹⁴C was determined in the top 0-10 cm and the rest in the remaining 10-50 cm soil layer. With the passage of time, the proportion of ¹⁴C in 0-10 cm soil layer decreased while that in 10-20 cm increased substantially.

Table 2: Distribution (% of applied) of cyfluthrin-¹⁴C in extractable and bound forms and its loss from the soil-plant system during the growth of cotton

Time, Weeks	¹⁴ C in soil, % of applied			¹⁴ C unaccounted % of applied
	Extractable	Bound	Total	
1	14.36a*	63.3a	77.6a	22.33c
3	11.48b	46.3a	57.7b	42.21b
6	10.61b	44.7bc	55.3bc	44.62ab
9	8.44c	50.3c	50.3c	49.61a

*Figures in a column (for either plant type) followed by a similar letter are not significantly different at 5% level of probability according to Duncan's Multiple Range Test

Relatively higher percentages of ¹⁴C were also determined in the deeper soil layers during extended residence time of cyfluthrin in soil. As the major proportion of ¹⁴C was restricted to the top 0-10 cm so was the case with extractable ¹⁴C; generally above 50 percent of the extractable ¹⁴C being determined in this soil layer. In the deeper soil layers less than 1 percent of the applied ¹⁴C was found in extractable forms. At all sampling intervals, the proportion of ¹⁴C in extractable forms generally increased while that in non-extractable forms it decreased with the depth of sampling, minimum being in 0-10 cm layer (data not tabulated). After 1 week, upto 49 percent of the soil ¹⁴C was in extractable forms at a depth of 40-50 cm as compared to 15 percent in the 0-10 cm layer.

Table 3: Per cent Distribution of applied cyfluthrin-¹⁴C in extractable and bound forms and its loss from the soil-plant system as affected by wheat growth and organic amendment

Treatment	¹⁴ C in soil, % of applied			¹⁴ C unaccounted % of applied
	Extractable	Bound	Total	
Fallow	8.11b	34.80b	42.91ab	57.09ab
Planted	9.34a	36.05a	45.39a	54.61b
Fallow-amended	6.66c	34.26b	40.92b	59.08ab
Fallow-unamended	6.87c	31.21c	38.08c	61.92
Planted-amended	6.66c	36.02a	41.53b	58.47ab
Planted-unamended	5.92	34.14b	40.06b	59.94ab

*Figures in a column (for either plant type) followed by a similar letter are not significantly different at 5% level of probability according to Duncan's Multiple Range Test

Wheat growth caused somewhat higher losses of ^{14}C from the soil-plant system i.e., 54.6 percent ^{14}C was lost from planted vs 57.1 percent from unplanted portion of the lysimeter (Table 3). Losses of ^{14}C were reduced substantially during the subsequent period of 9 weeks when both plant and unplanted portions were left with or without organic matter amendment. However, the differences in differently treated soil were not significant. A higher proportion (81-85 percent) of the soil ^{14}C was found in non-extractable forms, while 15-19 percent was extractable with methanol in different treatments. Although small differences in the recovery of applied ^{14}C in extractable and non-extractable forms were caused by wheat growth, percent distribution of the soil ^{14}C was not affected. The differences due to organic amendment were not very clear and non-significant. The amount of total as well as extractable and bound ^{14}C decreased with the depth of sampling as also observed for samples collected after cotton.

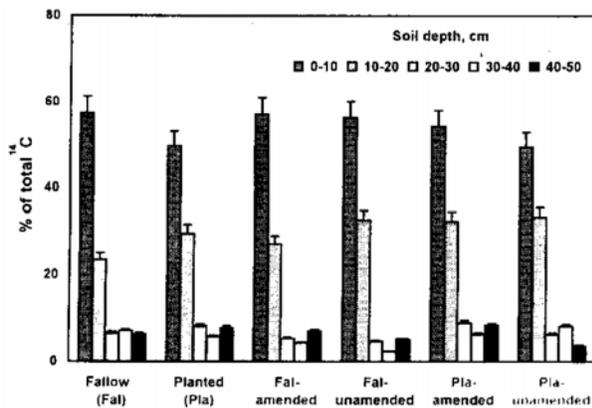


Fig. 3: Percent distribution of ^{14}C in different soil layers as affected by plant growth and organic amendment

Data presented in Fig. 3 shows that a major proportion of the total ^{14}C was restricted to the top 0-10 cm layer as was the case during growth of cotton (Fig. 2). In planted portion, relatively lower proportion of soil ^{14}C was found in the 0-10 cm layer as compared to unplanted (fallow) portion, while it had more ^{14}C in the 10-20 cm layer. This would suggest that plant growth may facilitate the movement of pesticide residues to greater soil depths. Organic amendment lead to a somewhat greater concentration of ^{14}C in the top 0-10 cm layer in both planted and unplanted portions suggesting its protective role. Nevertheless, relatively higher concentrations of ^{14}C were determined in deeper layers of amended as compared to the unamended soil.

Discussion

Results obtained in this study revealed a small uptake of ^{14}C by cotton plants, the seed portion was the least labelled. These observations are in line with many other studies. For example, Mikami *et al.* (1985) found very little ^{14}C in the shoot portion of bean plants grown to maturity in soils treated with ^{14}C fenpropethrin at the rate of 1 ppm. Hascoet and Andre (1978) also found negligible amounts of Decamethrin in cotton seed. Lee (1985) sprayed ^{14}C -labelled Fenvalerate on the soil surface and noted a meager uptake by plants. In the present study, 0.22 percent of the residual ^{14}C after harvesting cotton was found in wheat plants at maturity that was equivalent to only 0.011 percent of the originally applied ^{14}C . Thus the availability to plants of cyfluthrin- ^{14}C decreased substantially with time i.e., 0.376 percent of the applied in cotton vs 0.011 percent in wheat. Similar observations have been reported by Leahey and Carpenter (1980). Amos and Leahey (1986) detected very small quantities of ^{14}C -tefluthrin residues in maize grains which were identified mainly as free and conjugated metabolites of tefluthrin itself.

In view of the very low net ^{14}C content of plants in the present study, no effort was made to identify the ^{14}C -labelled compounds. It can be argued, however, that a greater proportion of the ^{14}C would be expected as cyfluthrin and one of the metabolites i.e., CONH_2 -cyfluthrin. This metabolite is produced by attack on the CN bond (Preiss *et al.*, 1988). Some of our unpublished results suggest that even 5 weeks after spray application to cotton leaves, >60 percent of the extractable ^{14}C was found as cyfluthrin, while 20 percent was determined as CONH_2 -cyfluthrin.

Losses of ^{14}C were relatively higher from unplanted than planted (with wheat) soil but the difference was not significant ($p=0.05$). This difference could be attributed to the binding/transformation of the ^{14}C -labelled material at the expense of rhizodeposition. Role of organic matter in protecting the xenobiotics from further degradation has already been emphasized. However, there was no significant effect of organic amendment on the loss of ^{14}C whether the soil was previously sown to wheat or left fallow, although slightly more losses occurred from unamended soils. The absence of any effect of organic amendment on the loss of ^{14}C could be due to relatively intractant nature of the cyfluthrin residues after such a long period of presence in soil. In the incubation studies (Lodhi *et al.*, 1996), losses of ^{14}C as CO_2 were relatively more from unamended soil and the difference was attributed to a higher stabilization and reduced degradability of the added cyfluthrin in amended soil.

A major portion of the total ^{14}C applied to the lysimeter soils was restricted to the top 0-10 cm of the soil with higher organic matter content relative to the deeper soil layers and a rapid adsorption of cyfluthrin on soil colloidal complex. One week after application, >80 percent of the ^{14}C was determined in the top 0-10 cm and the rest in the remaining 10-50 cm soil layer. Soil samples collected after cotton also showed higher concentration of ^{14}C in the top 0-10 cm layer. Rapid binding and restricted movement of pesticides

in soils rich in organic matter content has been reported (Arienzo *et al.*, 1994; Mueller and Banks, 1991; Somasundaram *et al.*, 1991; Sundaram, 1996, 1997). Smith *et al.* (1995) studied the effect of moisture, organic matter and redox potential on persistence of cyfluthrin and reported a protective role of organic matter. Lee (1985) also observed little downward movement of Fenvalerate insecticide. Degradation of organic chemicals depends upon their chemistry and to the physical protection resulting from adsorption of substrates to surfaces and from their location within soil aggregates at sites inaccessible to microbes (Paul and Van Veen, 1978; Van Veen *et al.*, 1985). According to Van Veen *et al.* (1985) soils have characteristic capacities to protect or preserve microorganisms. Jenkinson (1977) and Sorenson (1975) have emphasized the importance of clays in stabilizing soil organic matter.

In soil, ¹⁴C in forms unextractable with methanol generally decreased with time and depth of sampling and accounted for >80 percent of the total soil ¹⁴C during the growth of cotton. Similar general trends in the distribution of ¹⁴C in extractable and bound forms were observed for soil samples collected after cotton albeit with some treatment (plant growth and organic amendment) effects which were not very consistent. This would suggest a rapid and persistent binding of the chemical in soil. Turco and Konopka (1990) found that as much as 95 percent of the applied ring-¹⁴C was non-extractable after 28 days. Gerstl and Helling (1985) observed that during 49 days, 54 percent of the ring ¹⁴C remained in soil of which 13 percent was extractable and 87 percent in bound forms. Similar observations were reported by Zhang *et al.* (1984) who studied the persistence, degradation and distribution of ¹⁴C-labelled Deltamethrin insecticide in organic soil under laboratory conditions.

Due to analytical limitations, identity of soil ¹⁴C could not be ascertained. However, a greater proportion of the methanol extractable ¹⁴C would be expected to occur as unaltered cyfluthrin and CONH₂-cyfluthrin. Our unpublished work showed that even after 18 weeks of incubation under laboratory conditions, 67-79 percent of the methanol-extractable residual ¹⁴C was still present as cyfluthrin, while 17-27 percent was determined as CONH₂-cyfluthrin. Using ¹⁴C-labelled pyrethroid, Lee (1985) demonstrated that a major portion (up to 50 percent) of the ¹⁴C recovered in soil after 12 months was in the form of unaltered parent compound. Azam *et al.* (1988) studied the fate of [carbonyl-¹⁴C]methabenzthiazuron in soil and observed more than 90 percent of the residual ¹⁴C as unaltered original compound. Khan *et al.* (1988) demonstrated a long-term persistence of parent compounds and different metabolites in an organic soil. They found 16 percent of the initially applied radioactivity in bound forms after 3 years and identified as original compound as well as metabolites.

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