Biological Sciences Section

DEGRADATION OF 14C-LABELLED CYFLUTHRIN IN SOIL AND INCORPORATION OF 14C INTO HUMUS FRACTIONS AS AFFECTED BY WHEAT STRAW AMENDMENT AND MOISTURE CONDITIONS

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An incubation experiment was conducted in the laboratory to study the effect of moisture and wheat straw amendment on the mineralization and transformation of phenyl-14C cyfluthrin (active ingredient of Baythroid, an insecticide manufactured by Bayer AG, Leverkusen, Germany) into different soil fractions. The treated soil was incubated at 40, 60 or 100% water holding capacity (WHC) moisture and with or without wheat straw amendment. After 18 weeks of incubation, 44-58% of the cyfluthrin-14C was lost from the soil as CO_2. Mineralization of cyfluthrin was more rapid in unamended soil and at lower moisture regimes; the maximum being at 40% WHC. In unamended soil, a higher proportion of the applied 14C was found in forms extractable with methanol (an efficient solvent of cyfluthrin). In amended soil, on the other hand, a greater proportion of the added 14C was found in bound forms i.e., fulvic acid, humic acid and humins. Transformation of cyfluthrin-14C into stable humic compounds was more intense at lower moisture regimes. However, in all treatments, a higher proportion of bound residues extractable with alkali was present in fulvic acid fraction.

Key words: 14C, Cyfluthrin, Degradation, Moisture, Pesticides, Wheat straw.

Introduction

Use of agro chemicals has resulted in a tremendous increase in crop yields. In Pakistan, a greater portion of the pesticides used in agriculture consists of insecticides and cotton crop consumes up to 80% of the total insecticides used. A significant proportion of the applied chemical reaches the soil during spray application or alongwith rain and/or dew water. In soil, the pesticides affect the soil microflora and their functions which in turn have bearing on the growth and nutrition of plants. In addition, the pesticide residues may find their way into the sub-surface water and then to animal system. The situation warrants studies on the behaviour of pesticides in soil-plant system.

Availability of radio-tracer techniques has greatly facilitated studies on the fate of pesticides in the environment. In Pakistan, however, very few studies have been undertaken on the degradation, stabilization and plant uptake of pesticides using 14C tracer techniques [1-4].

Several physico-chemical factors affect the fate of pesticides in soil, chemical nature of the pesticide being a major factor in determining its fate [5]. Degradation of pesticides is generally slow in heavy-textured soils because of their adsorption onto the clay particles [6]. Similarly, soils rich in organic matter also give protection to these chemicals through adsorption and/or transformation into complex humic substances [7,8]. It is also true, however, that organic amend-

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ment enhances the microbial activity and may thus accelerate the process of degradation. Higher temperatures also enhance the dissipation of pesticides [9-12].

The objective of the present studies was to find out the effect of moisture and organic matter (wheat straw) amendment on the fate of 14C-labelled cyfluthrin, the active ingredient of Baythroid (a product of Bayer AG) which is one of the commonly used insecticides for the control of insect-pests (e.g., *Heliothus* spp and *Spodoptera* spp) of cotton in Pakistan.

Materials and Methods

Soil. The soil used in this study was collected from experimental fields at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Physico-chemical analyses of the air-dried and sieved (<0.5mm) soil showed 0.8% C, 0.05% N, 60% sand, 26% silt, 14% clay and pH 7.4.

Chemical. Phenyl-14C labelled cyfluthrin (active ingredient of Baythroid), was obtained from the Institute of Radioagronomy Research Centre, Julich, Germany. Specific activity of the chemical was 128 μCi mg^{-1}.

Incubation study. Incubation was carried out in the laboratory to study the rate of mineralization of cyfluthrin-14C into $^{14}CO_2$ as affected by moisture regimes and wheat straw amendment. 100g portions of the soil were placed in 12 glass wash bottles. Soil in half of the bottles was amended with 0.5g of powdered wheat straw. Both the amended and unamended soils were treated with 14C-labelled cyfluthrin in

methanol at a rate of 0.4μg g^{-1} soil (6.41 μCi bottle^{-1}) Moisture level in duplicate bottles was adjusted to 40, 60 or 100% water holding capacity (WHC). The bottles were connected to an aeration system providing CO_2-free and humidified air and incubated for 18 weeks at room temperature (26-28°C). The air emerging from the bottles was passed through 100 ml of 1M NaOH solution to trap CO_2. The alkali solution was replaced at regular intervals with fresh volume and aliquots were analyzed for ^{14}C on a liquid scintillation counter (Packard Tricarb 4530) using instagel scintillation cocktail.

In another study, 50g portions of soil taken in plastic containers and treated as above were incubated for 18 weeks in sufficient replications to sacrifice duplicate samples at different intervals for the determination of ^{14}C in different fractions. 100g portions of the soil were extracted with 200 ml of methanol (1 hr shaking followed by centrifugation at 5000 rpm for 1 hr and filtration). The filtrate was analyzed for ^{14}C and the residue was fractionated into humic acid, fulvic acid and humins using NaOH-Na$_4$P$_2$O$_7$ as extractant. ^{14}C in different liquid fractions was determined by direct counting using instagel for humic and fulvic acid fractions and PPO-POPOP in toluene for methanol extract. ^{14}C in humin fraction was taken as a difference between applied ^{14}C and that determined in different fractions including $^{14}CO_2$.

Results and Discussion

The data on cumulative losses of $^{14}CO_2$ are presented in Fig.1. After 18 weeks of incubation, maximum $^{14}CO_2$ was evolved at 40 and minimum at 100% WHC in both amended and unamended soils. At 40% WHC, mineralization of cyfluthrin was relatively slow during early period of incubation in both amended and unamended soils and >50% of the total losses occurred during the first 4 weeks. At higher mois-
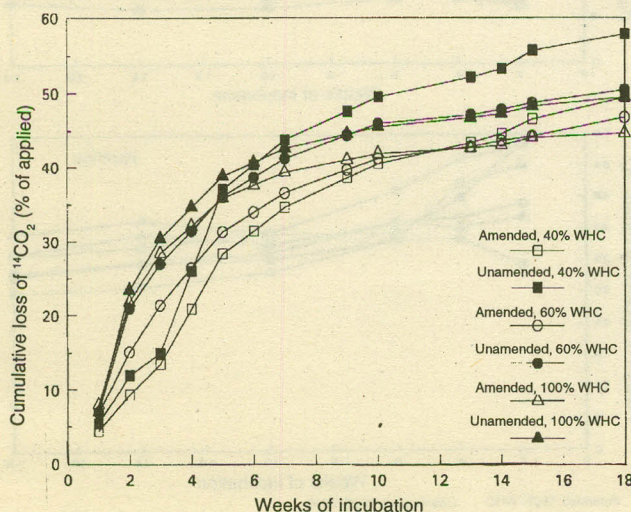
ture (60 and 100% WHC), the rate of mineralization was faster and >50% of the $^{14}CO_2$ losses were recorded during the first 3 weeks of incubation. After 18 weeks of incubation, however, more ^{14}C was lost as CO_2 at 40% WHC. Higher losses of ^{14}C occurred in unamended as compared to amended soil at all moisture levels and after 18 weeks of incubation, the extent of mineralization was 57.8 and 49.5% of the applied ^{14}C in unamended and amended soils, respectively; average values for the 3 moisture regimes were used.

Fractionation of soil showed a consistent decrease in methanol-extractable ^{14}C in all treatments as incubation progressed (Fig. 2). At all incubation intervals, more ^{14}C was extractable in unamended soil at the three moisture regimes. After 18 weeks of incubation, 6.1% of the applied ^{14}C (average for three moisture regimes) was extractable as compared to 3.5% in amended soil. Minimum methanol-extractable ^{14}C at different sampling times was observed at 40% WHC; the differences in extractability were generally less prominent at the two higher moisture regimes in both amended and unamended soils.

At all moisture regimes, a higher percentage of applied ^{14}C was transformed into alkali extractable humic compounds in amended than in unamended soil (Fig.3). More ^{14}C was extractable with alkali at 60% WHC than at the other two moisture regimes. Of the total alkali-extractable ^{14}C, a greater proportion was recovered in the form of fulvic acid at all moisture regimes both in amended and unamended soils. The difference was more obvious in amended soil where fulvic acid contained twice as much ^{14}C as that found in humic acid. The proportion of alkali-extractable ^{14}C in humic acid decreased with the increase in moisture content in both amended and unamended soils. Recovery of ^{14}C in humins decreased with the time of incubation in all treatments but remained higher

Fig. 1. Cumulative loss of applied ^{14}C as CO_2.

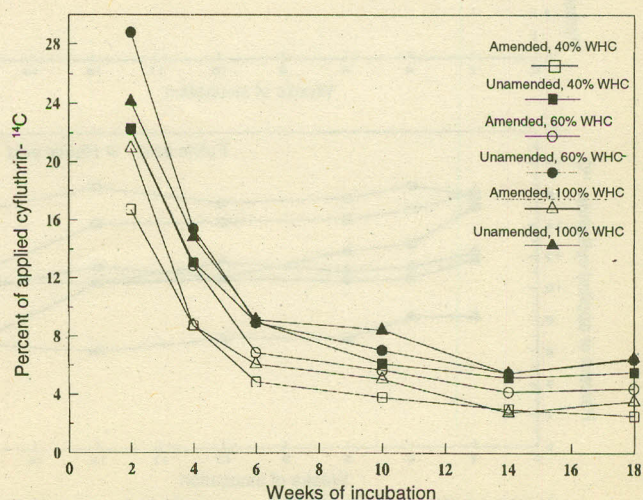Fig. 2. Percent of applied ^{14}C extractable in methanol.

in amended than in unamended soils. In general, a higher proportion of applied ^{14}C was found in humins at 60 and 100% WHC, in both amended and unamended soils.

Microbial degradation of pesticides is an important mechanism of their elimination from the environment [5]. In the present study, a quick and considerable mineralization of phenyl-^{14}C (about 58% of the applied ^{14}C being lost as CO_2) of cyfluthrin was observed. A rapid loss of ^{14}C as CO_2 from ring carbons of 2,4-D and 2,4,5-T that amounted to 70-80% of the applied ^{14}C was observed by other workers also [12]. On the contrary, some other reports showed that phenyl ring oxidation was a minor degradation route. Haider and Azam [13] and Azam *et al.* [14] observed that ring carbons of corn stalks were less susceptible to microbial mineralization. After 12 weeks of incubation, only 16% of the ring-^{14}C was lost as CO_2.

A possible reason for a high mineralization of ^{14}C in the present study may be the low organic matter content of the soil used. This assumption was supported by the findings that more ^{14}C mineralized in unamended soil as compared to amended soil. A higher mineralization of applied organic matter in a soil with low native organic matter content has been reported [15]. In amended soil, the applied ^{14}C could be expected to undergo rapid transformations (chemical, physi-

cal or biological) and thus saved from microbial oxidation. Integration of applied chemicals with native organic matter and its protection from further transformation is reported by several workers [5, 16-18]. It is because of such processes that higher doses of pesticides are generally recommended for organic soils as compared to those for mineral soils [19-20].

An additional factor for higher mineralization of phenyl-^{14}C in our study may be the low clay content of the soil. Clays and organic matter are known to provide protection to the added chemicals and thus decrease their susceptibility to microbial attack [16-18]. The assumption that the applied chemical remained unprotected in unamended soil was indicated by the extractibility of a higher proportion of ^{14}C with methanol.

Mineralization of ^{14}C was more at a lower moisture regime and could be attributed to a higher activity of aerobic microbes and/or lower physical protection of the chemical by the soil colloids. At lower moisture regime, the conditions were expected to be more aerobic resulting in a higher oxidation of cyfluthrin-^{14}C into CO_2. At higher moisture, mineralization of cyfluthrin-^{14}C was relatively lower probably due to more intense adsorption of the chemical on the soil colloids resulting in a lower availability of the chemical to microbial
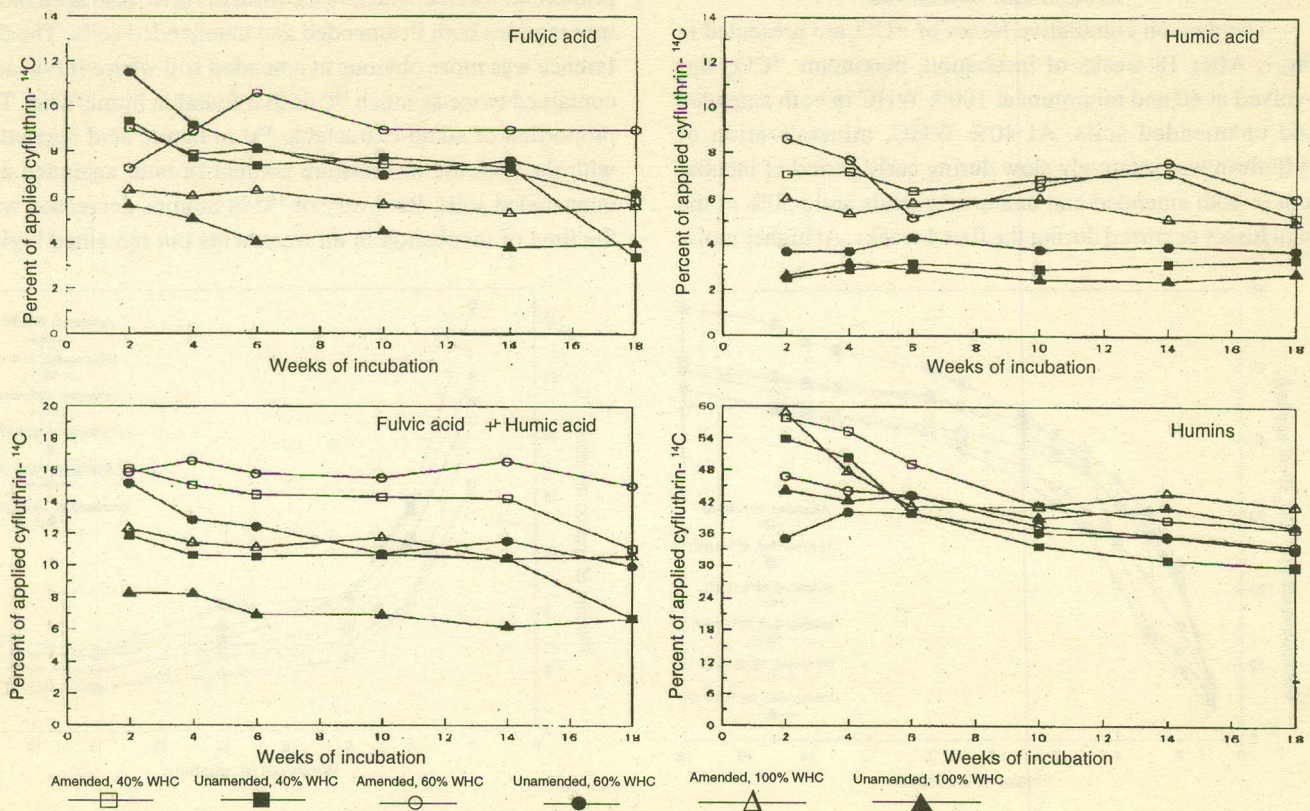

Fig.3. Recovery of applied ^{14}C in soil organic matter fractions.

attack. Further, the availability of O_2 may be limiting the oxidation of cyfluthrin under these conditions. The extractable ^{14}C was, however, lower at lower moisture regimes probably due to its higher mineralization to CO_2 or greater incorporation into microbial biomass. A lower rate of $^{14}CO_2$ losses during early period of incubation at lower moisture regimes might suggest that more ^{14}C was assimilated by the microbes and was released during prolonged incubation following decomposition of the ^{14}C-labelled microbial biomass.

A higher mineralization and extractability of ^{14}C in unamended soil observed in this study could be attributed to its lower transformation into bound forms. The data revealed that in amended soil, significantly higher proportion of added ^{14}C was recovered in humic acid, fulvic acid and humins. A higher stabilization could be either through increased synthetic activities of the microbes or due to a direct incorporation of phenyl-^{14}C into stable humic compounds. Soil microorganisms are known to degrade complex aromatic compounds and use part of the carbon skeleton for the synthesis of humic compounds [21-23]. It is also known that phenolic carbons are incorporated into microbial tissues [24] and the latter may contribute significantly to humus through melanins or via secretion of phenolic compounds which are the building blocks of soil humus [25]. Direct incorporation of ring carbons into humic compounds has also been reported [14].

The results presented here suggest that cyfluthrin is readily degraded particularly in soils having low organic matter content like those found in Pakistan. In addition, a significant part of the applied chemical is rendered inactive due to its incorporation into bound residues. Hence, uptake of cyfluthrin residues by plants (e.g. cotton) and its transfer to the animal food chain may not be significant. Nevertheless, these residues may have some bearing on the soil microbial processes that affect nutrient availability to plants [26].

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