

Persistence of oxyfluorfen residues in the soil of paddy field and detection of its residues in crop produce

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Oxyfluorefen is a post-emergence herbicides. It is highly active at low application rates for control of annual and perennial broad-leaf weeds and sedges in rice. Rice is the most important food crop of the world. Currently approximately 75% population depends on rice for their daily sustenance. Almost 90% of the total crop is produced in Asia (McDonanld 1994). In India, rice (*Oryza sativa* L.) is the important staple food crop in terms of production and consumption. The global rice production in Asia needs to be increased by 75% by 2020 to feed the world rice consumers. Weeds caused 16–100% yield loss in rice (Zoschke 1990; Kropff 1993). The conventional method of weed control (hoeing or hand weeding) is laborious, expensive and insufficient. Moreover, weeding during critical growth stages is not possible due to increased cost of human labour and its scarce availability. Thus necessitates the use of herbicides. Hence chemical weed control with effective and highly active herbicides has become useful and convenient method.

Oxyfluorfen is a diphenyl-ether herbicide (Fig 1) used as pre- and post-emergent herbicide to control many annual broadleaf and grassy weeds in a variety of field crops (Thakare *et al.* 2002, Shrefler *et al.* 2004, Sondhia and Dixit 2006, 2007). Oxyfluorfen is a contact herbicide and light is

required for its herbicidal activity (EXTOXNET 2002, Edwards *et al.* 1991, Scrano *et al.* 1999a). The most likely route of oxyfluorfen dissipation is soil binding. Oxyfluorfen shows moderate persistent in most of the soil, with a field half-life of about 30 to 112 days (WSSA 1994, USEPA 1992). The main mechanism of degradation in soils is photodegradation and evaporation in moist soils (WSSA 1994). Oxyfluorfen is very well sorbed to most soils and soil binding is highest in soils with high organic matter and clay content. Once oxyfluorfen is adsorbed to soil particles, it is not readily removed (WSSA 1994) and therefore is unlikely to be mobile in most instances, unless the sorptive capacity of the soil is exceeded.

There is an increasing concern about the persistence of pesticide residues in soils, crop produce and subsequent contamination of groundwater. In particular, their mobility, rate of degradation and solubility govern their potential to contaminate water from different routes. It is essential that contamination of agricultural products and groundwater by pesticides should be prevented to protect public health, fish and other aquatic life (USEPA 1992). Oxyfluorfen is classified as a very highly toxic and persistent herbicide. It can persist in the soil and accumulate in terrestrial plants and certain aquatic environments through runoff (USEPA 1992). Thus pesticide concentration in the hydrosol (sediment) may increase every year after its application.

Although there are several reports on the efficacy of oxyfluorefen against various weeds in rice, but there is very few reports on the methods of analysis and its environmental fate in rice agro-ecosystems. Some authors suggested a potential risk of oxyfluorefen, which are active at low rate of application (The California Department of Pesticide Regulation 1992).

Moreover, herbicide residue estimation in soil and edible plant parts is very essential to determine the duration of herbicide activity in soil and residues in crops produce. Thus, actual field monitoring of oxyfluorfen residues is necessary to evaluate accurately the persistence, bioaccumulation and

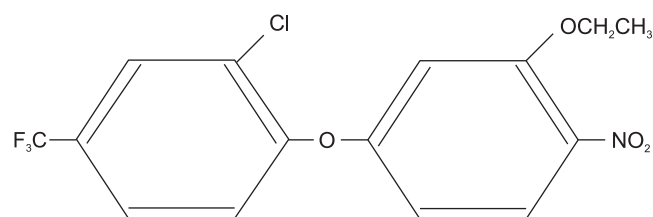


Fig 1 Chemical structure of oxyfluorfen (2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) benzene)

*Short note

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hazard to aquatic organisms. Thus actual field monitoring of oxyfluorfen residues is necessary to evaluate accurately the persistence and bioaccumulation in crop produce. Thus viewing its toxicity and to fill the lack of information on this important aspect, present study was undertaken to evaluate the persistence of oxyfluorfen in soil and bioaccumulation of oxyfluorfen residues in paddy grains and straw.

A field experiment was conducted at the experimental farm of Directorate of Weed Science Research, Jabalpur, India, in rainy (*khariif*) season of 2006 in a randomized block design with 3 replications. Nine different plots each with a dimension of 8 m × 4 m were prepared. One metre distance was maintained between the plots. All 4 sides of the plots were protected by soil boundaries (bunds) raised to a level of approximately 30 cm height and 30 cm width. Rice seedlings were collected from the nursery and transplanted on 19 July 2006 as per the local agriculture practice at a spacing of 20 cm between the rows and 15 cm between the plants. Four days after transplanting of rice seedlings, 2 different doses of oxyfluorefen (23.5% EC), viz 150 and 300 g/ha were sprayed with Knap-sack sprayer using flat fan nozzle. Another 3 triplicate plots were sprayed with water without any herbicide and maintained as control.

Soil samples were collected at harvest. Three kg of 5-soil cores each were randomly taken from each treated and untreated plots avoiding the outer 20 cm fringes of the plots using a soil auger up to a depth of 20 cm from the surface. Pebbles and other unwanted materials were removed manually. The cores were bulked together from each plot, air-dried, powdered and passed through a 3 mm sieve to achieve uniform mixing. Samples from the control plots were collected before the herbicide treated plots for residue analysis. The soil was clay loam in texture (clay 35.47%, silt 12.55%, and sand 52.08%), N 300 kg/ha, P 62 kg/ha and available P 300 kg/ha with organic carbon 0.81%, EC 0.36 mmhos/cm and pH 7.2. At harvest 500 g of representative rice grains and straw samples were collected from oxyfluorefen treated and untreated plots. Straw samples were cut into small pieces and air-dried. Rice grains and straw samples were then ground on mechanical grinder and used for residue analysis.

Oxyfluorfen residues from soil, paddy grain and straw were extracted and analyzed by HPLC as described by Sondhia (2006). The concentrated extracts of soil samples were filtered through Pall Nylon 0.45 µm filter paper prior to HPLC injection. Rice grains and straw samples were subjected to glass column cleanup. A glass column (10 cm × 2 cm i.d.) was packed with celite (1 g) and activated charcoal (0.25 g) between anhydrous sodium sulfate (2 g) at each end. Concentrated extracts of rice grains and straw samples were poured on the top of the column and eluted with 30 ml of methanol. Elutes were collected and concentrated to approximately 5 ml in a rotary vacuum evaporator and pass

through Pall Nylon 0.45 µm filter paper.

Validation of the method was performed in terms of recovery experiment before analysis of unknown samples. Different known concentrations of oxyfluorfen (4, 1, 0.1, 0.01 and 0.003 µg/ml) were prepared in acetonitrile by diluting the stock solution (1000 µg/ml). 20 µl of standard solution was injected in HPLC and the peak area measured (Table 1). Weighed 25 g of paddy grains and straw samples and 10 g of soil samples into extraction flasks and by using pipette, 1 ml of standard solution of 1.0 and 0.1 µg/ml of oxyfluorfen was added uniformly on the surface of the matrix and mixed well before adding extraction solvent and performed the extraction and cleanup process as described in the methodology. Quantification of oxyfluorfen residues was accomplished by comparing the peak response for samples with peak area of the standards. The recovery and detection limit of oxyfluorfen are shown in Tables 1 and 2. Residue values were not corrected for recovery.

Mean analytical recovery of the oxyfluorfen using the method described is 85–89% (Table 2). Precision standard deviation of replicate analysis is presented in Tables 1 and 2. Oxyfluorfen reference analytical standard were obtained from Dr Ehrenstorfer GmbH, Germany. All the other chemicals and solvents used in the study were analytical grade reagent and were obtained from E-Merck Germany. The HPLC system consisted of a Shimadzu instrument equipped with degasser, LC-10 ATVP pump, SPD-M10 AVP Diode Array Detector (DAD) and Rheodyne injection system was used for analysis. The column was a Phenomenex Luna RP-18 stainless steel column (5 µm particle size, 250 mm×4 mm id).

Table 1 Recovery of the oxyfluorfen from soil

Matrix	Fortified concentration (µg/g)	*Concentration found (µg/g)	Per cent Recovery	Standard deviation
Soil	1.0	0.930	93.00	± 0.084
	0.1	0.085	85.00	± 0.002
Paddy straw	1.0	0.858	85.80	±0.042
	0.1	0.084	84.00	±0.001
Paddy grains	1.0	0.910	91.00	±0.159
	0.1	0.084	84.50	±0.002

(*Average of 3 replications)

Table 2 Oxyfluorfen residues in paddy grains and straw at harvest

Treatment (g/ha)	Residues µg/g*		
	Soil	Paddy grains	Straw
Control (water)	<0.003*	<0.003	<0.003
150	0.026±0.003	<0.003	<0.003
300	0.044±0.002	<0.003	<0.003

(*Average of 3 replications)

** Standard deviation

The injection volume of standards and samples were 20 µl and flow was set at 0.8 ml/min. The analysis was carried out at 205 nm at ambient temperature using acetonitrile: water (70 : 30) as mobile phase. Quantification of oxyfluorfen residues was accomplished by comparing the peak area response for samples with peak area of the standards.

The detection limit of oxyfluorfen is shown in Table 1. The retention time of oxyfluorfen was found approximately 3.45 min. The Instrumental detection limit for oxyfluorfen was estimated as described by Sondhia (2008a). The limit of detection was found 0.003 µg/g at signal to noise ratio 3 : 1 and no substrate interferences were observed at this detection limit as evidenced by the control sample analysis. Detector showed good sensitivity for the oxyfluorfen residues up to 0.0001 µg/ml but did not follow the linearity. The soil blanks did not exhibit any peak interfering with the retention time of oxyfluorfen. Therefore, for the soil the extraction did not require clean up.

The recovery experiment conducted with paddy grains, straw and soil samples showed that recovery percentage for soil varied from 85 to 93%, however, it varied between 84–85.8% in the case of paddy straw and 84–91% in case of paddy grains at fortification level of 1.0 and 0.1 µg/g of oxyfluorfen, respectively (Table 2). The recoveries of oxyfluorfen from soils, paddy straw and grains at different concentration levels of 0.1 and 1.0 µg/g were satisfactory being within the range 85–89%, confirmed a good repeatability of the method (Table 2).

The equations of analytical calibration graphs, obtained by plotting peak areas in 'y' axis against concentrations of oxyfluorfen in 'x' axis within the range of 4 to 0.003 µg/ml was, $y = 3460331.41x + 584496.10$, showed good linearity and the value of correlation coefficient was 0.99 (Table 1).

The oxyfluorfen residues in different matrix at different time interval as detected by HPLC are presented in Table 3. Analysis of paddy grain and straw samples collected at harvest showed residues below 0.003 µg/g at the doses 150 and 300 g/ha, respectively. However, in the soil samples, 0.026 and 0.044 µg/g residues were detected at harvest at 150 and 300 g/ha treated plots, respectively. Sondhia (2008a) reported 0.006 and 0.009 µg/g imazosulfuron residues in rice grains where imazosulfuron was applied at 50 and 60 g/ha rates. Imazosulfuron residues in rice straw were not detected at 30 and 40 g/ha treated plots, however, 0.009 and 0.039 µg/g residues were detected where imazosulfuron was applied at 50 and 60 g/ha rates, respectively. Das *et al.* (2003) reported 3% persistence of oxyfluorfen residues after 60 days after application in the rhizosphere soil of rice at 0.12 kg/ha. However, in the present study the residues were below the maximum residues limit (MRL) prescribed for oxyfluorfen in rice in all the substrates. From this study a waiting period of 106 days is suggested for paddy crop after the oxyfluorfen application.

During experimental period an annual rainfall of 799 mm

were received and the minimum and maximum temperature during study period was 12.8 to 33.4°C, relative minimum and maximum humidity was 56–92% was recorded. High humidity during the experimental period enhances the rate of degradation of oxyfluorfen. High rainfall (799 mm) might have washed out the residues through run-off and leaching and thus less residue level of oxyfluorfen was detected in soil (Sondhia 2008c). Oxyfluorfen has also been shown to drift from application sites to nearby areas and has the potential to affect terrestrial plants and aquatic ecological systems at all levels, as it is toxic to plants, invertebrates, and fish (USEPA 1992).

Low concentration of the oxyfluorfen in soil is compensated due to high organic matter content which in turn increased microbial activity, which increased the rate of degradation (Shelton and Parkin 1991, Sondhia 2005, Sondhia *et al.* 2007, Sondhia and Singhai 2008, Sondhia 8b). Besides the organic matter, the clay content can also play an important role in degradation rate of pesticides (Sondhia 2008a, Morrica *et al.* 2001).

Faster dissipation of oxyfluorfen at surface soil may be due to chemical and microbial dissipation process (Sondhia 2005, Sondhia 2008a). As the soil of the experimental field was almost neutral (pH 7.2) having clay and organic matter content 35.47 and 0.81% that may be the reason that less amount of oxyfluorfen detected at harvest in soil in both the application rates. Oxyfluorfen herbicides are generally weakly adsorbed by soil. Thus some researcher suggested less leaching of oxyfluorfen in soil. Moreover, solubility of oxyfluorfen is low in water (0.116 mg litre at 20°C), hence not able to leach from the top-soil to deeper in the soil profile. But through runoffs and leaching this may move to the canal and drain water and thus may not be available in the surface soil (0–20 cm) and hence less amount of oxyfluorfen was detected from the surface soil. The most likely route of dissipation is soil binding but few researcher suggested combination of chemical, biological, physical and environmental factors may operate at different level in influencing the degradation of herbicides (Sondhia 2008a, Sondhia and Singhai 2008).

Since residues were detected even at lower dose in soil but they were found below the maximum residue limit (0.05 mg/kg) (Canada Gazette 2000) thus seeing its persistence in soil a preharvest interval of 106 days for paddy crop after the oxyfluorfen application is suggested. Though residues were found within safe level in soil, paddy grain and straw but continuous use of oxyfluorfen in the same field may be avoided as this may lead to biomagnifications of residues in soil which then bioaccumulate in the crop produce and may also enter to the aquatic system through runoff, drift and leaching and thus affect aquatic life.

SUMMARY

There is increasing concern about persistence of pesticide

residues in soils, agricultural products and subsequent contamination of groundwater. Thus persistence of oxyfluorfen was evaluated under rice cropping system. Oxyfluorfen was sprayed at 150 and 300 g/ha application rates 4 days after transplanting of rice as post-emergence herbicide. Paddy grains, straw and soil samples were collected at harvest and analyzed for oxyfluorfen residues by a validated HPLC method with an accepted recovery of 85–89% at the minimum detectable concentration of 0.003 ppm. Residues of oxyfluorfen in paddy grains and straw were found below 0.003 µg/g in lower and double the recommended dose. In the soil, 0.026 and 0.044 µg/g residues were detected where oxyfluorfen was applied at 150 and 300 g ai/ha rates, respectively. Thus, a safe waiting period of 106 days is suggested for paddy crop after the herbicide application. Though residues were found within safe level in soil, paddy grain and straw but continuous use of oxyfluorfen in the same field may be avoided as this may lead to biomagnifications of residues in soil which then bioaccumulate in the crop produce and may enter to the aquatic system through runoff, drift and leaching and thus affect aquatic life.

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