

Persistence and bioaccumulation of oxyfluorfen residues in onion

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Abstract A field study was conducted to determine persistence and bioaccumulation of oxyfluorfen residues in onion crop at two growth stages. Oxyfluorfen (23.5% EC) was sprayed at 250 and 500 g ai/ha on the crop (variety, N53). Mature onion and soil samples were collected at harvest. Green onion were collected at 55 days from each treated and control plot and analyzed for oxyfluorfen residues by a validated high-performance liquid chromatography method with an accepted recovery of 78–92% at the minimum detectable concentration of $0.003 \mu\text{g g}^{-1}$. Analysis showed 0.015 and $0.005 \mu\text{g g}^{-1}$ residues of oxyfluorfen at 250 g a.i. ha^{-1} rate in green and mature onion samples, respectively; however, at 500 g a.i. ha^{-1} rates, 0.025 and $0.011 \mu\text{g g}^{-1}$ of oxyfluorfen residues were detected in green and mature onion samples, respectively. Soil samples collected at harvest showed 0.003 and $0.003 \mu\text{g g}^{-1}$ of oxyfluorfen residues at the doses 250 and 500 g a.i. ha^{-1} , respectively. From the study, a pre-harvest interval of 118 days for onion crop after the herbicide application is suggested.

Keywords Oxyfluorfen residues · Soil · Green and mature onion bulbs · HPLC analysis · Persistence · Bioaccumulation

Introduction

Onion (*Allium cepa* L.) is one of important vegetable crop of *Rabi* season, and it forms an indispensable part of many diets, both vegetarian and non-vegetarian, as a flavoring agent. Onion occupies a prominent place among vegetables and is cultivated commercially throughout tropical and subtropical belt of the world. India produce one third of the world's production in onion due to intensive cultivation. Onion is a very slow crop to germinate and grow. Weed infestation in onion is one of the major factors for loss in yield under high rainfall conditions. Yield loss due to weeds infestation in onion is to the tune of 40–80% (Verma and Singh 1997). 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 labor and its scarce availability, thus necessitating the use of herbicides.

Oxyfluorfen is a diphenyl-ether herbicide (Fig. 1) used for broad-spectrum pre- and post-emergent control of annual broadleaf and grassy weeds in a variety of field crops (Thakare et al.

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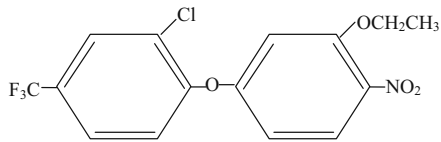


Fig. 1 Chemical structural of oxyfluorfen

2002; Shrefler et al. 2004; Sondhia and Dixit 2006, 2007; Sondhia 2007). Oxyfluorfen is persistent and relatively immobile in soil. Oxyfluorfen can contaminate surface water through spray drift and runoff; however, few researchers suggested that ground water contamination by oxyfluorfen is unlikely because it is relatively immobile in the soil column under laboratory conditions.

Oxyfluorfen shows moderate persistent in most of the soil, with a representative field half-life of about 30 to 40 days (WSSA 1994; USEPA 1992). Oxyfluorfen is not subject to microbial degradation or hydrolysis (Wauchope et al. 1992). The main mechanism of degradation in soils may be photodegradation and evaporation in moist soils (WSSA 1994). In laboratory studies, oxyfluorfen soil half-life was found 6 months, indicating very slow rates of microbial degradation (WSSA 1994; Wauchope et al. 1992). Oxyfluorfen is very well sorbed to most soils (Wauchope et al. 1992). Soil binding is highest in soils with high organic matter and clay content (WSSA 1994; Wauchope et al. 1992). Once oxyfluorfen is adsorbed to soil particles, it is not readily removed (WSSA 1994). It is practically insoluble in water and, therefore, is unlikely to be mobile in most instances, unless the sorptive capacity of the soil is exceeded.

Herbicide residue estimation in soil and edible plant parts is very essential to determine the duration of herbicide activity in soil and its effect on the crops and to analyze the quality of the food and feed. Onion is an important vegetable crop of the world. It is consumed as green vegetables as well as mature onion bulb. Field studies on persistence and bioaccumulation of oxyfluorfen residues in crop produce is very much limited. Thus, the present study was undertaken to see the extent of persistence and bioaccumulation of oxyfluorfen residues in onion at two different vegetative stages viz. green onion (at 55 days) and mature onion

bulbs (at harvest) from the point of view of consumption of green and mature onion bulbs.

Materials and methods

Experimental

The field experiment was conducted at the Research Farm of National Research Centre for Weed Science, Jabalpur, India during 2006 in *Rabi* season in a randomized block design with three replications. Residue analysis was conducted in the residue laboratory of National Research Centre for Weed Science, Jabalpur. The soil was clay loam in texture (clay, 35.47%; silt, 12.45%; and sand, 52.09%), with nitrogen 300 kg ha⁻¹, phosphorus 40 kg ha⁻¹, and potassium 300 kg ha⁻¹ with organic carbon 0.80%, EC 0.35 mmhos cm⁻¹, and pH 7.1.

Onion was transplanted on 27 December 2006, and oxyfluorfen (23.5% EC) was applied at 250 and 500 g ha⁻¹ in onion crop on 2 January 2007 as early post-emergence herbicide. The soil samples from the oxyfluorfen treated and untreated plots were collected at harvest (118 days after herbicide application) from a depth of 0 to 20 cm after herbicide application and analyzed to see the persistence of oxyfluorfen residues in soil. Five soil cores were randomly taken from each treated and untreated plots using a soil auger up to a depth of 20 cm. The cores were bulked together from each plot, air-dried, powdered, and passed through a 2-mm sieve to achieve uniform mixing. The green onion were sampled at 55 days, and mature onions were collected at 118 days after herbicide application from the oxyfluorfen treated and untreated plots.

Extraction

Soil

Oxyfluorfen residues from soil, green, and mature onion was extracted as described by Sondhia (2007). Ten grams of soil was transferred to 250-ml flasks and extracted with 30 ml of methanol/acetonitrile (2:1) in a mechanical shaker for 2 h, which was filtered. Additional 10 ml

methanol/acetonitrile (2:1) was used as washing solvents. Filtrates were concentrated on a rotary vacuum evaporator to approximately 2 ml and subjected to cleanup.

Extraction of oxyfluorfen from onion

Representative green onion and onion bulbs (25 g) were cut into small pieces, homogenized with acetonitrile (50 ml), filtered, and taken in 250-ml Erlenmeyer flask. Homogenized green onion bulbs and soil samples (25 g) were extracted with 80 ml of methanol/acetonitrile (2:1) for 2 h (repeated twice). They were filtered and added 2 ml H₂SO₄ and shaken carefully. After cooling, contents were taken in 250-ml separatory funnel and partitioned with chloroform (40 ml). Chloroform layer was collected concentrated on a rotary vacuum evaporator at 40°C to approximately 5 ml and subjected to cleanup.

Cleanup

A glass column was packed with 6 g of florisil, and in between, two layers of anhydrous sodium sulfate and activated charcoal (1 g) were added. Concentrated extracts of different soil and onion 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- μ m filter paper.

Validation of the method and detection limit

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 μ g ml⁻¹) were prepared in methanol by diluting the stock solution (1,000 μ g ml⁻¹). Twenty microliter of standard solution was injected in high-performance liquid chromatography (HPLC) and the peak area measured (Table 1). Twenty-five grams green and mature onion samples and 10 g soil samples were placed in an extraction flask. By using a pipette, 1 ml of standard solution of 1 and 0.1 μ g ml⁻¹ of oxyfluorfen was added uniformly on the surface of the

Table 1 Calibration of oxyfluorfen at various concentration levels

| Injected concentration (ppm) | Average area (mabs) |
|------------------------------|-----------------------------------------|
| 4 | 14,454,426 \pm 9,875,860 ^a |
| 1 | 3,890,420 \pm 606,961.8 |
| 0.1 | 1,320,507 \pm 185,144.7 |
| 0.01 | 874,129 \pm 15,164.68 |
| 0.003 | 75,673 \pm 37,031.93 |
| R ² | 0.99 |

Detection limit = 0.003 μ g ml⁻¹; linear equation, $y = 3,460,331.41x + 584,496.10$

^aStandard deviation

matrix and mixed well before adding extraction solvent, further extraction and cleanup process was performed as described in the methodology. Results are presented in Table 2. Quantification of oxyfluorfen residues in soil and onion was accomplished by comparing the peak response for samples with peak area of the standards.

Accuracy

Mean analytical recovery of the oxyfluorfen using the method described was 78–94% (Table 2). Precision standard deviation of replicate analysis is presented in Tables 1 and 2.

Chemicals

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.

Instrument

The HPLC system consisted of a Shimadzu instrument equipped with degasser, LC-10 ATVP pump, SPD-M10 AVP Diode Array Detector and Rheodyne injection system. The column was a Phenomenex Luna RP-18 stainless steel column (5- μ m particle size, 250 \times 4 mm i.d.). The instrument was connected to a computer detector response having software able to compute detector response in terms of peak area. The injection volume of standards and samples was 20 μ l, and flow was set at 0.8 ml min⁻¹. The analysis was carried out at 205 nm at ambient temperature using

Table 2 Recovery of oxyfluorfen from soil, green and mature onion bulbs

| Matrix | Amount added ($\mu\text{g g}^{-1}$) | Amount recovered ^a ($\mu\text{g g}^{-1}$) | Recovery (%) | Slope | Intercept | Correlation coefficient |
|--------------|---------------------------------------|--------------------------------------------------------|--------------|-------|-----------|-------------------------|
| Soil | 1.0 | 0.960 ± 0.084^b | 96 | 0.971 | -0111 | 1.0 |
| | 0.1 | 0.086 ± 0.007 | 86 | | | |
| Green onion | 1.0 | 0.092 ± 0.049 | 92 | 0.930 | -0.009 | 1.0 |
| | 0.1 | 0.082 ± 0.002 | 82 | | | |
| Mature onion | 1.0 | 0.800 ± 0.028 | 80 | 0.793 | 0.006 | 1.0 |
| | 0.1 | 0.086 ± 0.106 | 86 | | | |

^aAverage of three replications^bStandard deviation

methanol/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.

Results and discussion

Recovery and detection limit

The detection limit of oxyfluorfen is shown in Table 1. The retention time of imazethapyr was found approximately 3.45 min. The instrumental detection limit for oxyfluorfen was estimated as described by Sondhia (2008). The limit of detection was found $0.003 \mu\text{g g}^{-1}$ at signal/noise ratio of 3:1. Detector showed good sensitivity for the oxyfluorfen residues up to $0.0001 \mu\text{g ml}^{-1}$ 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 soil and onion samples showed that recovery percentage for soil varied from 86% to 96%; however, it varied between 82% and 92% in the case of onion bulbs at fortification level of 1.0 and $0.1 \mu\text{g g}^{-1}$ of oxyfluorfen, respectively (Table 2). The limit of detection was $0.003 \mu\text{g g}^{-1}$, and no substrate interferences were observed at this detection limit as evidenced by the control sample analysis. The recoveries of oxyfluorfen from soils at different concentration levels were satisfactory within the range 86–96%, confirming a good repeatability of the method (Table 2).

The equation of analytical calibration graphs, obtained by plotting peak areas in the y-axis

against concentrations of oxyfluorfen in the x-axis within the range of 4 to $0.003 \mu\text{g ml}^{-1}$, was $y = 3,460,331.41x + 584,496.10$, which showed good linearity, and the value of correlation coefficient was 0.99 (Table 1 and Fig. 2).

Residues of oxyfluorfen in soil and green and mature onion

The oxyfluorfen residues in different matrix at different time interval as detected by HPLC are presented in Table 3. The soil samples collected at harvest time from the onion field showed $0.003 \mu\text{g g}^{-1}$ residues of oxyfluorfen at 250 and 500 g ha^{-1} application rates. The concentration of oxyfluorfen in the green onion was found to be 0.015 and $0.025 \mu\text{g g}^{-1}$, where oxyfluorfen was applied at 250 and 500 g ha^{-1} rates, respectively, which was further dissipated to 0.005 and $0.011 \mu\text{g g}^{-1}$ in the mature onion bulbs at 250 and 500 g ha^{-1} doses collected at harvest (Table 1).

Sondhia and Dubey (2006) reported $0.0152 \mu\text{g g}^{-1}$ residues of butachlor in the soil

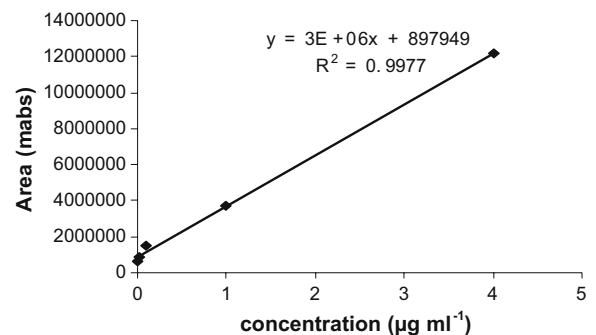
**Fig. 2** Calibration of oxyfluorfen at 4 to $0.003 \mu\text{g ml}^{-1}$ level

Table 3 Residues of oxyfluorfen in soil and green and mature onion bulbs

| Substrate | Oxyfluorfen residues ($\mu\text{g g}^{-1\text{a}}$) | | |
|-------------|-------------------------------------------------------|----------------------------------|-----------------------|
| | Control | 250 g ai/ha | 500 g ai/ha |
| Green onion | <0.003 | 0.015 ($\pm 0.004^{\text{b}}$) | 0.025 (± 0.004) |
| Onion bulbs | <0.003 | 0.005 (± 0.0005) | 0.011 (± 0.002) |
| Soil | <0.003 | 0.003 (± 0.003) | 0.003 (± 0.003) |

^aAverage of three injections

^bStandard deviation

of onion field at the time of harvesting. Lazic (1997) reported that pendimethalin residues decreased during the onion crop vegetative stage, and 50% of the herbicide degraded in an average of 50 days. Pendimethalin residues in young onion were 0.239 ppm and in ripe onion 0.113 ppm (Lazic 1997). Sharma and Mehta (1989) reported 0.103 ppm residues in onion at harvest when the pendimethalin treatment was 2.0 kg ha⁻¹. Tsiropoulos and Miliadis (1998) reported 0.054 ppm residues in onions treated at 2.0 kg ha⁻¹.

Low concentration of the oxyfluorfen in soil is compensated due to high organic matter content, which in turn increased microbial activity and increased the rate of degradation (Jenkinson and Ladd 1981; Shelton and Parkin 1991; Johnson and Sims 1993; Sondhia 2005; Sondhia et al. 2007; Sondhia 2008). Besides the organic matter, clay content can also play an important role in degradation rate of pesticides.

Faster dissipation of oxyfluorfen at surface soil may be due to chemical and microbial dissipation mechanism (Sondhia 2008). The soil pH also plays an important role in the degradation of herbicides (Morrica et al. 2001; Sondhia 2007). As the soil of the experimental field was almost neutral (pH 7.2), this may be reason that oxyfluorfen was not detected at harvest in soil at low application rate. Oxyfluorfen is generally weakly adsorbed by soil, but some researchers suggested high leaching of oxyfluorfen in soil; thus, low amount of oxyfluorfen were detected from the surface soil (Sondhia 2007). The most likely route of dissipation is soil binding, but few researchers suggested that a combination of chemical, biological, physical, and environmental factors may operate at different level in influencing the degradation of herbicides (Sondhia 2008).

Although residues were detected in green and mature onion bulbs, they were found below the maximum residue limit set by the WHO/FAO (0.05 mg/kg). Residues in dry onions bulbs and soil at harvest were also found below the maximum residue limit at higher dose. Based on this study, a pre-harvest interval of 118 days for onion crop after the oxyfluorfen application is suggested. Thus, it may be concluded that early post-emergence application of oxyfluorfen in direct-seeded onion for weed management could be considered as safe from the point of view of consumption of green and mature onion bulbs, as the residues of both the herbicides were below the prescribed maximum residue limits for onion (0.05 mg kg⁻¹). However, continuous use of oxyfluorfen may be avoided, as this may lead to biomagnifications of residues in soil, which can then be bioaccumulated in the crop produce.

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References

Jenkinson, D. S., & Ladd, J. N. (1981). Microbial biomass in soil: Measurement and turnover. *Soil Biochemistry*, 5, 415–417.

Johnson, R. M., & Sims, J. T. (1993). Influence of surface and subsoil properties on herbicide sorption by Atlantic and coastal plain soils. *Soil Science*, 155, 339–348.

Lazic, S. (1997). Pendimethalin residues in onion. *Acta Horticulturae*, 462, 571–576.

Morrica, P., Barbato, F., Dello-Iacovo, R., Seccia, S., & Ungaro, F. (2001). Kinetics and mechanism of imazosulfuron hydrolysis. *Journal of Agricultural and Food Chemistry*, 49, 3816–3820. doi:10.1021/jf010088f.

Sharma, R., & Mehta, H. (1989). Studies on pendimethalin and fluchloralin residues in soil and onion. *Indian Journal of Agronomy*, 34, 245–247.

Shelton, D. R., & Parkin, T. B. (1991). Effect of moisture on sorption and biodegradation of carbofuran in soil. *Journal of Agricultural and Food Chemistry*, 39, 2063–2068. doi:10.1021/jf00011a036.

Shrefler, J. W., Taylor, M. J., Roberts, B. W., & Webber, C. L. (2004). Recent experience with fresh bulb-onion production for Oklahoma [abstract]. In *National Allium Research Conference*, p. 85.

Sondhia, S. (2005). Phytotoxicity and persistence of metribuzin residues in black soil. *Toxicological and*

- Environmental Chemistry*, 87, 389–397. doi:10.1080/02772240500165505.
- Sondhia, S. (2007). Evaluation of leaching potential of pendimethalin in clay loam soil. *Pesticide Research Journal*, 19, 119–121.
- Sondhia, S. (2008). Determination of imazosulfuron persistence in rice crop and soil. *Environmental Monitoring and Assessment*, 137, 205–211. doi:10.1007/s10661-007-9741-z.
- Sondhia, S., & Dixit, A. (2006). Oxyfluorfen residues in onion. *Weed Newsletter*, 6, 1–2.
- Sondhia, S., & Dixit, A. (2007). Determination of terminal residues of oxyfluorfen in onion. *Annals of Plant Protection Sciences*, 15, 232–234.
- Sondhia, S., & Dubey, R. P. (2006). Determination of terminal residues of butachlor and pendimethalin in onion. *Pesticide Research Journal*, 18, 85–86.
- Sondhia, S., Singhai, B., & Singh, V. P. (2007). Degradation of sulfosulfuron in sandy clay loam soil and detection of its residues in wheat grains and straw. *Geobios*, 34, 74–76.
- Thakare, P. D., Patil, B. M., Kakade, S. U., & Dangore, S. T. (2002). Studies on chemical weed control in soybean *Glycine max* (L.) Merrill. *Crop Research*, 24, 11–14.
- Tsiropoulos, N., & Miliadis, G. (1998). Field persistence studies on pendimethalin residues in onions and soil after herbicide post emergence application in onion cultivation. *Journal of Agricultural and Food Chemistry*, 46, 291–295. doi:10.1021/jf970712h.
- US Environmental Protection Agency (1992). *Pesticide environmental fate one liner summaries: Oxyfluorfen*. Washington, DC: Environmental Fate and Effects Division.
- Verma, S. K., & Singh, H. T. (1997). Effect of Weed Control measures and fertility on growth and productivity of rainy season onion (*Allium cepa*). *Indian Journal of Agronomy*, 42, 540–543.
- Wauchope, R. D., Buttler, T. M., Hornsby, A. G., Augustijn Beckers, P. W. M., & Burt, J. P. (1992). SCS/ARS/CES Pesticide properties database for environmental decision making. *Reviews of Environmental Contamination and Toxicology*, 123, 1–155.
- Weed Science Society of America. (1994). *Herbicide handbook*, seventh edition. Champaign, IL: Weed Science Society of America.