Research Article

Residue Kinetics of Ethofumesate in Texturally Diverse Soils of Sugar Beet Crop under Field Conditions

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Received 13 February 2021; Accepted 7 July 2021; Published 20 July 2021

Academic Editor: Franck Rabilloud

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Indiscriminate use of pesticides and growing awareness of environmental and health problems had led to monitoring their residues in soil and crops. Ethofumesate is one of the widely used herbicides for controlling weeds in sugar beet. Dissipation kinetics and terminal residues of ethofumesate were investigated in two diverse soils under subtropical field conditions. Ethofumesate dissipated slowly after application and follows biphasic first-order kinetics in soils. The average half-life for initial and later phases in sandy loam soil, respectively, was 14.54 and 20.42 and 51.83 and 65.21 days, while for silty clay loam, it was 10.09 and 13.00 and 71.42 and 73.10 days, respectively. Recoveries in soil, leaves, and beetroot ranged from 78.15 to 88.05, 77.01 to 88.58, and 76.25 to 84.50%, respectively. The quantitation limit for soil, roots, and leaves was $0.002 \mu\text{g g}^{-1}$. At harvest, no residues were detected in soils, leaves, and sugar beetroots. Residues were below the maximum residue limits in sugar beetroots and leaves as set by EU (0.2 ppm). Ethofumesate is safe from weed control and environmental aspects as it does not persist for a long duration in soils and does not appear to pose any adverse effect on human/animal health under subtropical field conditions.

1. Introduction

Beta vulgaris (sugar beet) is an important sugar crop, containing 14–20% sucrose which is used for making sugar and ethanol, a source of biofuel. Owing to the higher potential of sugar beet as a raw material for the production of sugar and ethanol, tropical sugar beet hybrids were introduced recently. Weeds are a threat to the cultivation and harvest operations of sugar beet and are a major limitation for profitable sugar production. Competition from uncontrolled annual weeds can reduce crop yields by 26–100% [1, 2].

Ethofumesate [(±)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate], a benzofuran group of selective systemic herbicide, is widely applied as pre- and early postemergence herbicide for effectively controlling a wide range of grasses and broad-leaved weeds for up to 10 weeks in sugar beet, other beet crops, turf, ryegrass, pasture grasses, onions, oilseed poppies, strawberries,

sunflowers, Phaseolus beans, tobacco, and other crops at $0.3\text{--}2.0 \text{ kg ha}^{-1}$ and is available as EC, SC, SE, and OD formulations or may be coformulated with phenmedipham, bromoxynil, ioxynil, desmedipham, or metamitron. It acts by inhibiting the growth of meristems, retards cell division, and limits cuticle formation (lipid synthesis) [3, 4]. Ethofumesate is a potential groundwater contaminant [5]. Ethofumesate causes toxicity in chronic animal feeding studies and is also reported to cause some developmental toxicity in the offspring of pregnant rabbits [6]. Foliar injury and growth inhibition caused by ethofumesate vapor were observed on wild higher plant species, showing inhibition potential of ethofumesate on nontarget organisms [7, 8]. Ethofumesate is considered to be “slightly toxic” to freshwater fish [9].

Ethofumesate is considered a persistent herbicide, stable to hydrolysis and anaerobic soil metabolism. Under low moisture aerobic conditions in sandy loam soil, the half-life was 253 days whereas, under moist conditions in silt loam

and sandy loam soils, the half-life was 83 and 122 days, respectively [9]. Scanty information [10] is available on the persistence study of ethofumesate in soils and crops under tropical conditions, although some work on persistence from other regions is reported [11–16].

Excessive and repeated use of agrochemicals leads to accumulation of residues in the soil, crop production, and groundwater causing phytotoxicity, susceptible intercrops or succeeding crops or nontarget organisms, and ultimately health hazards [17–20]. Herbicides are found as bound residues which make them not only unavailable to the targets but also pollute the soil ecosystem in a number of ways. There is a need to monitor herbicides residue in various commodities to assess buildup, biomagnifications, and bioaccumulation and adverse effects if any. The aim of this study was to study the dissipation kinetic of ethofumesate herbicide under subtropical climatic field conditions and monitor persistence in the environment, residues in edible commodities, and groundwater contamination.

2. Materials and Methods

2.1. Instruments and Chemicals. Agilent 1120 Compact LC technologies HPLC, Buchi Rotavapor, and SPE (Solid Phase Extraction) miniplastic florisil columns (6.0×0.75 cm id) packed with 500 mg florisil packing material (Sigma-Aldrich, USA) were used for estimation. Analytical grade ethofumesate (98.98% purity) and formulation (50 SC (Suspension Concentrate)) were obtained from courtesy M/S Punjab Chemicals and Crop Protection Limited, India. All the used chemicals were of analytical or HPLC grade (E. Merck/Hi Media).

2.2. Herbicide Treatments and Sampling. Field trials on sugar beet (var. Shubhra) were conducted during the winter season at G.B. Pant University of Agriculture and Technology, Pantnagar ($29^{\circ} 3'$ North, $79^{\circ} 31'$ East with an altitude of 243.84 MSL.). The experimental field plot size was $6 \text{ m} \times 5 \text{ m}$. Sugar beet was planted with a spacing of 50×20 cm. The experiment was laid out in randomized block design with three replications of each treatments. Ethofumesate 50 SC formulation was sprayed at recommended (2.0 kg ha^{-1}) and double recommended (4.0 kg ha^{-1}) doses using knap sac sprayer as postemergent herbicide at 2 leafed stages of sugar beet. A control plot was also maintained where no herbicide was applied. All other recommended packages of practices other than weed control were adopted to raise the crop of sugar beet in experimental plots.

Representative samples from four randomly selected samples (2.0 kg ; 0 – 15 cm depth) of a block of texturally different soils were collected at different time intervals, i.e., 0 (4 hr.), 1 , 3 , 5 , 7 , 10 , 15 , 30 , 45 , 60 , 90 , 120 , and 150 days after herbicide treatment and finally on the harvesting day (162 days after treatment) with the help of tube auger. The soil was pooled, air dried, and passed through a 2 mm sieve before further processing. Subsamples were drawn randomly using the quartering technique and kept in air-tight bags and stored in deep freeze (-20°C) until extraction. The soil was analyzed for pH, CEC, organic carbon, CaCO_3 percent and

proportion of sand silt, and clay fraction by standard analytical procedures. Sugar beet leaves (0.50 kg) and sugar beet (1.0 kg) samples were collected from all treated and control plots at harvest time. Leaves and sugar beetroots were chopped into small pieces using stainless steel knife and macerated in a motorized mixer blender. Groundwater (5 L , 20 m depth) was collected from the nearest borewell (50 m far) at different days. Samples were immediately sent to the laboratory and stored at -20°C until used. Data were subjected to analysis to determine standard deviation among the replicates. The amount of ethofumesate residues was estimated by HPLC.

2.3. Soil Texture and Weather Conditions. The analysis of soil revealed that the texture of soil was of silty clay loam with high silt percentage (45.20%) and organic content (1.04%) with pH (7.27) while the other soil was sandy loam in nature having sand percentage (71.35%) with low organic content (0.44%) and the pH was 6.94 . The temperature during the experimental period varied from 6.3 to $29.5 \pm 1^{\circ}\text{C}$, rainfall varied from 2.5 to 11.2 mm , and relative humidity varied from 27 to $65 \pm 5\%$. The weather condition was almost similar for both years during the experimental periods.

2.4. Extraction Methodology. Extraction was done as given by Pal (2012) [21] with some modifications briefly, representative soil sample (25 gm) was weighed, and 100 mL of acetone: methanol ($5:1$, v/v) was added and shaken for 45 min on an orbital shaker followed by centrifugation at 5000 rpm for 10 min . The process was repeated three times. The combined extract was filtered and transferred into a separatory funnel, and 50 mL of 5% sodium chloride solution was added and washed with 25 mL n-hexane twice. The hexane washing was discarded, the aqueous phase was again partitioned with 50 mL dichloromethane thrice, and the organic layer was collected and passed through anhydrous sodium sulfate to remove traces of water. The solvent was removed by rotary vacuum evaporation at $35 \pm 1^{\circ}\text{C}$ to dry. The residue was dissolved in 2 mL methanol, and the samples were filtered through a $0.22 \mu\text{m}$ PTFE disc filter prior to HPLC analysis.

The beetroot or leave sample (25 gm) was cut and macerated with 100 mL methanol followed by shaking for 45 min , centrifuged, and filtered. The procedure was repeated twice more. The supernatant was pooled and partitioned with 100 mL ($50 + 25 + 25$) dichloromethane. The organic layer was collected, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure at $35 \pm 1^{\circ}\text{C}$. The residue was dissolved in 1 mL methanol and subjected to cleanup. Cleanup was done using a Florisil SPE (solid phase extraction) cartridge, prewashed with 5 mL each of n-hexane. The sample was loaded on a cartridge and eluted with 5 mL dichloromethane: methanol ($2:1$ v/v). The eluted sample was dried under the stream of nitrogen and redissolved in a 1 mL mobile phase. The samples were filtered through a $0.22 \mu\text{m}$ PTFE disc filter prior to HPLC analysis.

Water was collected from the groundwater source at different time intervals. Representative water (100 mL) was

partitioned with 200 mL dichloromethane (100 + 50 + 50). The organic layers were pooled, were dried over anhydrous sodium sulfate, and were evaporated to dryness under reduced pressure at $35 \pm 1^\circ\text{C}$. The residue was dissolved in 1 mL methanol, filtered through 0.22 μm PTFE disc filter, and subjected to HPLC analysis.

2.5. Statistics and Degradation Kinetics. The experiment was laid in randomized block design fashion, and all the treatments were replicated thrice. The experimental data were subjected to statistical analysis to determine standard deviation among the replicates. The amount of ethofumesate residues that remained at different intervals was fitted to first-order exponential decay equation $C_t = C_0 e^{-kt}$ where C_t is the concentration ($\mu\text{g g}^{-1}$) after time t (day), C_0 is the initial concentration ($\mu\text{g g}^{-1}$) at $t = 0$, k is the rate constant (day^{-1}), and t is the time in days. The half-life was computed using the equation, $t_{1/2} = [0.693/k]$, where k is the rate constant in days.

2.6. Chromatographic Conditions. The optimized operating chromatographic parameters were column C-18 [ODS-2, 5 μm , (250 mm $\times$ 4.6 mm)], and the mobile phase was composed of methanol: water (70 : 30, v/v) with an isocratic mode at a flow rate of 1 mL min^{-1} and UV detection at 222 nm.

2.7. Method Validation and Recovery Studies. The method was validated by evaluating analytical curves and linearity, specificity, the limit of detection (LOD), the limit of quantification (LOQ), accuracy (% RSD), and recovery. The linearity of the instrument and the method was evaluated by analytical curves at seven concentration levels (0.001, 0.01, 0.05, 0.5, 1.0, 5.0, and 10.0 $\mu\text{g mL}^{-1}$ ethofumesate) with three replicate injections per concentration. The average detector response in terms of area under the peak was used for the preparation of the calibration curve. The percent relative standard deviation (% RSD) was calculated to check the accuracy of the result. The sensitivity of the method was determined using the ratio between the estimated standard deviation of the linear coefficient and the slope of the analytical curve. The LOD and LOQ for ethofumesate were determined by considering 3 and 10 times ratio of signal to noise, respectively. The obtained values were also checked experimentally.

For recovery studies, processed soil (200 gm), sugar beetroot and leaves (100 gm), and water (250 mL) were taken fortified with ethofumesate to get 0.002, 0.01, 0.1, 1.0, and 2.5 $\mu\text{g mL}^{-1}$ concentrations. The spiked samples were mixed thoroughly. All the experiment was done in triplicate. The methods applied for extraction, cleanup, and analysis were the same as standardized above in Section 2.4.

3. Results and Discussion

Under optimized HPLC conditions, the retention time of ethofumesate was found to be 5.4 minutes with minimum possibility of coelution of impurities as a result of

coextractants. The calibration curve was linear, and the value of the correlation regression coefficient (R^2) was 0.994 representing the fact that the calibration curves were satisfactory and the concentration of herbicide was dependent on detector response. The percent relative standard deviation (RSD) was calculated to check the accuracy/precision of the result. The RSD of ethofumesate was found to be 1.55% indicating the small spread of results. The average percent recoveries of ethofumesate varied from 78.15 to 88.05, 77.01 to 88.58, 76.25 to 84.50, and 89.13 to 93.75% for soil, leaves, roots, and water, respectively (Table 1). The limit of detection (LOD) was 0.0008 $\mu\text{g mL}^{-1}$, and the limit of quantification (LOQ) for soil, roots, leaves, and water was 0.002 $\mu\text{g g}^{-1}$.

The average percent persistence values in sandy loam soil at different time intervals were calculated considering the amount of herbicides on the 0th day (4 hours after application) as 100%. The persistence of ethofumesate at 2.0 kg ha^{-1} decreased to 94.31% on the 1st day and then degraded rapidly up to 57.81% on the 10th day of application. After the 10th day, the rate of degradation was slow and persistence decreased constantly. Persistence decreased to 36.01 and 29.38% on the 30th day and 60th day of application, respectively. On the 100th day after application, the persistence was only 10.90% and was below the quantifiable limit ($<0.002 \mu\text{g g}^{-1}$) on the 120th day of application. At a double recommended application rate, the persistence decreased in almost the same manner as in recommended dose. Herbicide decreased rapidly up to 66.83% on the 10th day, and further dissipation was slow and almost linear dissipation pattern till the 120th day (14.39%). No detectable residue was observed on the 135th day and 150th day of application (Figure 1).

In silty clay loam soil, the percent dissipation values at different time intervals were calculated considering the amount of herbicide on the 0th day (4 hours after application) as 0%. The ethofumesate dissipation at recommended dose increased from 0 to 11.06% from the 0th day (4 hr after application) to the 1st day and increased rapidly up to 52.65% on the 10th day of application. Thereafter, dissipation became slow and was almost linear. The percent dissipation of compound was 61.94% and 78.31% on the 45th and 90th day of application, respectively. On the 120th day, the residue dissipated was 88.49% and was below the detectable limit ($<0.002 \mu\text{g g}^{-1}$) on the 135th day of application. The soil was treated at 4.0 kg a.i. per ha ethofumesate, and the percent dissipation increased from 0 to 5.39% from the 0th day to the 1st day of application. On the 5th and 10th days, the dissipation was rapid and it was 23.28 and 41.66%. From the 15th day, the dissipation was almost gradual and constant up to the 135th day. The dissipation values on the 60th, 90th, and 120th days were 69.11, 74.50, and 86.51%, respectively, and were below the detectable limit on the 150th day of application (Figure 1).

Dissipation followed the first-order kinetics as highly significant R^2 values indicated that the dissipation conformed to the first-order kinetics for both soils and treatments. Ethofumesate in both soils accounted for a biphasic

TABLE 1: Percent recoveries of ethofumesate herbicide from soil, leaves, and beetroot.

Matrix	Conc. (ppm)	Amount injected (ng)	Amount recovered (ng)	% recovery
Ethofumesate	Soil			
	0.002	0.01	0.007 ± 0.005	78.15
	0.01	0.05	0.040 ± 0.05	80.08
	0.1	0.50	0.416 ± 0.02	83.20
	1.0	5.00	4.281 ± 0.05	85.62
	2.5	12.5	11.006 ± 0.04	88.05
	Sugar beet leaves			
	0.002	0.01	0.007 ± 0.003	77.01
	0.01	0.05	0.040 ± 0.04	81.00
	0.1	0.50	0.421 ± 0.02	84.23
	1.0	5.00	4.312 ± 0.05	86.25
	2.5	12.5	11.072 ± 0.03	88.58
	Sugar beetroots			
	0.002	0.01	0.007 ± 0.005	76.25
	0.01	0.05	0.039 ± 0.02	79.14
	0.1	0.50	0.406 ± 0.02	81.25
	1.0	5.00	4.147 ± 0.03	82.95
	2.5	12.5	10.562 ± 0.02	84.50
	Water			
	0.002	0.01	0.008 ± 0.002	89.13
	0.01	0.05	0.045 ± 0.01	90.50
	0.1	0.50	0.456 ± 0.04	91.30
	1.0	5.00	4.607 ± 0.03	92.15
	2.5	12.5	11.718 ± 0.04	93.75

FIGURE 1: Dissipation of ethofumesate in soils.

degradation pattern at both application rates. It is evident from Table 2, in both soils, the dissipation of ethofumesate during the initial phase was many folds higher than the later phase. The half-life calculated in sandy loam soil and silty clay loam for the initial phase at the recommended rate was 14.54 and 10.09 days, respectively, while, for the later phase, it was 51.84 days and 71.42 days, respectively, whereas, at double recommended rate, the half-life for the first phase,

respectively, was 20.42 and 13.00 days while, for later phase, it was 65.21 and 73.10 days, respectively.

Soil factors like soil texture, temperature, pH, and organic carbon content influence residues, retention, and degradation rates of pesticides [22–25]. Degradation of ethofumesate has been reported in the medium silty loam soil; they found a two-stage degradation process rapid initial phase followed by a slower process [15]. A similar trend in the present study confirms that the degradation of ethofumesate shows a biphasic pattern in both soils. Biphasic dissipation behavior in soils for herbicides has also been reported [26–29]. Ethofumesate is a moderately persistent herbicide with a half-life in bare soil of 51 days compared to 3 days in turfgrass [9, 11]. In the field study, 88–91% of the ethofumesate dissipated after 24 weeks in sandy loam soil, compared to 72–77% in loam soil with a half-life in sandy loam and loamy soils which were 7.7 and 12.6 weeks, respectively [30]. In soils, persistence is also related to soil temperature; DT_{50} of ethofumesate under warm moist conditions was <35 days while, under cold dry conditions, it was >98 days [3]. In sugar beet cultivation, ethofumesate was found to be the most persistent herbicide compared to other herbicides in sandy loam and clay soil types [12]. Schweizer [31] reported the dissipation of ethofumesate in sugar beet soil after 24 weeks of application. The DT_{50} values ranged from 52 to 78 days under field experiments of ethofumesate in sugar beet [32].

In the present investigation, the initial rapid loss might be due to hot subtropical climatic condition which causes higher volatilization/degradation of ethofumesate from the soil surface. Similar studies have been reported [31], where

TABLE 2: Coefficients of determination (R^2), rate of degradation, and half-life for different doses of ethofumesate.

	Sandy loam		Silty clay loam	
	2.0 kg·ha ⁻¹	4.0 kg·ha ⁻¹	2.0 kg·ha ⁻¹	4.0 kg·ha ⁻¹
For the first phase (0–10 days)				
Rate of degradation (λ)	0.0503	0.0360	0.0738	0.0533
Half-life ($t_{1/2}$) days	14.54	20.42	10.09	13.00
Coefficient of regression (R^2)	0.983	0.986	0.985	0.974
For the later phase (10-onward days)				
Rate of degradation (λ)	0.0136	0.0108	0.0103	0.0101
Half-life ($t_{1/2}$)	51.83	65.21	71.42	73.10
Coefficient of regression (R^2)	0.985	0.978	0.988	0.985

ethofumesate persisted about twice as long in soil under cold as that applied under warm weather. Goring et al. [30] also pointed out that factors as chemical structure, temperature, water, and oxygen and various soil properties such as organic matter, pH, and type of microbial population can all affect the rate of degradation of herbicides in soil. Soil type (especially clay and organic matter content) influences speed, run, and final residues of ethofumesate in soils [15].

Slower dissipation rate of ethofumesate in the early phase in sandy loam soil could in the present study be attributed to low organic matter and low pH (6.94) whereas a faster dissipation rate in silty clay loam may be attributed to the fact that soil contains higher clay content, high content of organic matter, and relatively higher pH (7.27) which favors the microbial environment and higher microbial population leading to faster degradation. Degradation of ethofumesate in the soil is entirely due to the action of soil microorganisms activity [11, 33], and the activity was largely affected by organic carbon content in the soils [32]. Rahman [34] reported that the dissipation of ethofumesate was faster in warm and wet/moist conditions.

The half-life of ethofumesate in the present results reveals that it was higher at double recommended dose than the recommended dose and was more in silty clay loam compared to sandy loam soil (Table 2). This could be because, at a low application rate, rapid dissipation is due to the effect of many biotic and abiotic factors acting on a less concentrated compound while, at a high rate of application, the dissipation was slow which could be owed to the limited exposure of higher concentrated compound to biotic and abiotic factors. A higher half-life of herbicide/pesticides at a high application rate in soils might also be due to inhibition of soil microbial population, leading to slow degradation of herbicide. The slower dissipation rate of ethofumesate in the later phase of both soils could also be attributed to the soil properties like soil colloids, which strongly bind the herbicide and minimize its losses and also render them chemical less prone to physical and biochemical modes of degradation. McAuliffe and Appleby [35] reported that, upon application of ethofumesate to dry soil, the activity loss is probably due to both strong adsorption and chemical degradation.

At harvest of sugar beet, the active ingredients in leaves and the root were not detected at both application rates for both cropping year and residues were below MRL (Maximum Residue Limits) set by EU (European Union) and

FSSAI. At harvest, the absence of ethofumesate residues may be due to enzymatic action of plant and metabolites formation and/or conjugates with compounds like sugar, amino acid, etc. present in the plant. The EU-MRL and FSSAI (Food Safety and Standards Authority of India) for beetroots and sugar beet are 0.2 $\mu\text{g g}^{-1}$ [36, 37]. Residues of ethofumesate herbicides were detected in roots of sugar beet at the highest applied concentration but did not exceed acceptable values for the EU [13]. In sugar beetroot samples, the residues of active substances were lower ($<0.001 \mu\text{g g}^{-1}$) than those in the soil and were below MRL values [38].

The metabolic pathways of ethofumesate in the soil and plants had been well studied and investigated by other researchers. They found that, in soil, oxidative dealkylation would be expected to initiate ring opening, leading to the carboxylic acid analogous. The major compound found was 2,3-dihydro-3,3-dimethyl-2-oxo-5-benzofuranyl methanesulfonate. In plants, ethofumesate undergoes dealkylation at the ethoxy side-chain and hydroxylation at the 2 positions, yielding ethofumesate-2-hydroxy (NC 8493), ethofumesate lactone (NC 9607), and ethofumesate carboxylic acid (NC 20645) which is conjugated or further degraded to the keto metabolite. The keto metabolite may further undergo ring opening, forming (g-(2-hydroxy-5-methanesulfonyl-oxy-phenyl)-isobutyric acid) which is also conjugated [39, 40].

4. Conclusion

In this study, a simple and reliable residue method for the determination of ethofumesate in sugar beet and soil was used. The validation methods (linearity, LOQ, precision, and RSD) were satisfactory as per international guidelines. Recoveries were in the acceptable range, i.e., from 76 to 93% for the matrices. Dissipation in soil for ethofumesate showed a biphasic degradation pattern. The half-life for the initial phase at the recommended rate in sandy loam soil and silty clay loam was 14.54 and 10.09 days, respectively, while, for the later phase, it was 51.83 days and 71.42 days, respectively. Residues were below MRL in the edible part and were below detection limits in the nonedible part of sugar beet. Ethofumesate optimum usage can be inferred as safe for controlling weeds and consumption of foods for humans and animals and is also less hazardous from an environmental point of view.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

The authors are thankful to PCCPL (M/S Punjab Chemicals and Crop Protection Limited, India) for providing analysis and formulation of ethofumesate for research purposes. The authors are also thankful to G.B. Pant University for providing the necessary facilities for research work.

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