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Dissipation Kinetics of Ethofumesate in Sugar Beet under Tropical Indian Condition by GC-MS

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The aim of this study was to investigate the dissipation kinetics of ethofumesate belonging to the benzofuranyl alkanesulfonate group in sugar beet plant and field soil as influenced by methods and quantity of application under Indian tropical condition. The quantification of ethofumesate was carried out in ion trap model GC-MS. The method was validated by evaluating the analytical curve, linearity, limits of detection and quantification, precision (repeatability) and accuracy (recovery), after the optimization of extraction parameters for the determination. The method provided limits of quantification of 0.05 mg kg^{-1} for different matrices with the method detection limit of 0.01 mg kg^{-1} for all matrices. The developed method was applied to study the dissipation kinetics and persistence of ethofumesate in sugar beet crop and soil from the field experimentation which received two different doses along with control. It was found that the ethofumesate dissipation followed first order kinetics with a half-life of 7.5 and 5.9 days under single application and 5.3 and 2.6 days under split application in soil and plant respectively. Application of ethofumesate at 0.99 kg a.i. ha^{-1} degraded before the harvest which limited the risk for agricultural environment contamination.

Keywords: Ethofumesate, Dissipation kinetic, Sugar beet, GC-MS analysis.

INTRODUCTION

Ethofumesate (2-ethoxy-3,3-dimethyl-2,3-dihydrobenzofuran-5-yl methanesulphonate) is a herbicide belonging to the benzofuranyl alkanesulfonate group. It is a selective, systemic herbicide and its persistence in soil is longer than the other herbicides used to control weeds in sugar beet crop[1,2]. Degradation of ethofumesate is considered to be microbial and occurs rapidly at 30 °C in dry soil[3]. The DT_{50} of ethofumesate in soil is reported to be ranged from < 35 days in moist soil conditions to > 98 days in cold dry conditions[4]. Haggar and Passman[5] reported that ethofumesate applied in october to a newly planted perennial ryegrass had a half-life of 8 weeks. However, Gardner and Branham[6] found that the half-life of ethofumesate in bare soil was 51 days and 3 days in turf grass. Ethofumesate is metabolized in plants to its 2-hydroxy derivative and to carbon dioxide finally[7] and this rapid metabolism is the basis for its selectivity in sugar beet. Laitinen *et al.*[8] reported that the seasonal variation has high influence on its dissipation. Ethofumesate is moderately mobile in the soil and its mobility and sorption depends on the soil organic carbon[9]. Though few reports on the degradation and adsorption of ethofumesate in soil under laboratory conditions were published, its dynamics under actual field conditions is lacking that too under split application. Hence the present study was undertaken to study the dissipation of ethofumesate in soil and sugar beet plant as influenced by the methods of application under tropical Indian condition.

EXPERIMENTAL

Field experiment was conducted at the experimental farm of the Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu using sugar beet as a test crop during winter, 2008-09 (Var. Cauvery) in a Randomized Block Design with three replications to study the persistence and dissipation of ethofumesate applied in splits. Nine plots each with a size of 30 m^2 were prepared and all sides of the plots were protected with soil boundaries raised to a level of approximately 35 cm height and 25 cm width. Sowing of the crop was taken up as per the standard agricultural practices suggested for sugar beet crop. Two different doses of ethofumesate @ 0.99 and 1.98 kg ai ha^{-1} were sprayed through two methods *viz*., single application on 2-3 leaf stages of weeds and in three equal splits (each time 0.66 kg ai ha^{-1} was applied) during 2, 4-6 and 8-10 leaf stage of weeds. Herbicide spraying was done with knap-sack sprayer using flat fan nozzle with the spray volume of 400 L ha^{-1}. Another three replicates of plot were sprayed with water

alone and maintained as control. Experimental field soil was sandy clay loam (sand 44 %, clay 29.1 % and silt 26.7 %) in texture and the chemical properties were: pH - 8.02, EC - 0.49 dS m^{-1}, organic carbon - 0.48 %, low available nitrogen (152 kg ha^{-1}), medium available phosphorus (18.0 kg ha^{-1}) and high available potassium (432 kg ha^{-1}).

Soil samples were collected from the treatments during 0, 1, 7, 15, 30, 45, 60 days after the application of herbicides and at the time of sugar beet root lifting. The tubers of sugar beet were sampled for residue analysis on the day of sugar beet lifting. Samples were taken at random (5 soil cores from each plot) in and around the middle of each plot to avoid interference and side effects from the neighboring plots. The soil samples were taken at a soil depth of 0-15 cm, well mixed and stored in polythene bags at -5 °C.

A reference standard of ethofumesate (purity 98.5 %) and the test chemical of formulated ethofumesate were supplied by Punjab Chemicals and Crop Protection Pvt. Ltd., Mumbai, India. All the solvents were analytical grade and purchased locally. Anhydrous sodium sulfate (AR grade) was used as a drying agent for different samples. For GC analysis, HPLC-grade hexane and 0.2 µm filtered milli-Q water were used.

Extraction of ethofumesate residue: Ethofumesate residue was extracted twice from 50 g of sugar beet plant and tuber samples using a mixture of 150 mL of methanol and dichloromethane (1:1 v/v). The samples were mixed and shaken at 200 revolution min^{-1} for 0.5 h on a horizontal shaker and filtered under reduced pressure. The extracts were cleaned by solid phase extraction using preconditioned SPE cartridges with 1 g of florisil[10]. Elution of the samples was carried out using 10 % solution of ethyl acetate in dichloromethane (v/v). A residue of ethofumesate from moistened soil was extracted with acetone. The extract was concentrated and residue was partitioned from aqueous phase with dichloromethane[11]. The dichloromethane extract was concentrated to dryness, re-dissolved in hexane, cleaned-up on silica and analyzed by GC-MS.

Chromatographic conditions: Separation was performed using Chrompack capillary column of 30 m length, 0.32 mm dia. The oven was programmed as follows: Initial: 80 °C hold for 5 min, Ramp of 20 °C/min to 150 °C, hold 5 min, 20 °C/min to 240 °C with final hold at 240 °C for 10 min and the total run time was 38 min. The detection of the herbicide was performed using the Varian gas chromatograph (CP 3800) equipped with ion trap mass spectrometer (model Saturn 2000). The injector and detector were maintained at 260 and 280 °C, respectively. The carrier gas was helium with the constant gas flow rate of 0.5 mL min^{-1} and 1 µL of the sample was injected with the split ratio of 20:1. Approximate retention time of ethofumesate was 18.8 min (Fig. 1a). The identification of the herbicide in the samples was accomplished on the basis of their retention time and by comparison with the NIST library.

Validation of method and detection limit: Validation of the method was performed in terms of recovery studies before the analysis of unknown sample. The recovery of the active substance of ethofumesate was determined by fortification of soil and plant samples at different known concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 µg g^{-1} in three replicates, mixed well, equilibrated for 0.5 h and extracted as described for samples. A linearity check study was carried out with the help of analytical standard following the procedure outlined by Janaki *et al.*[12] for oxyfluorfen.

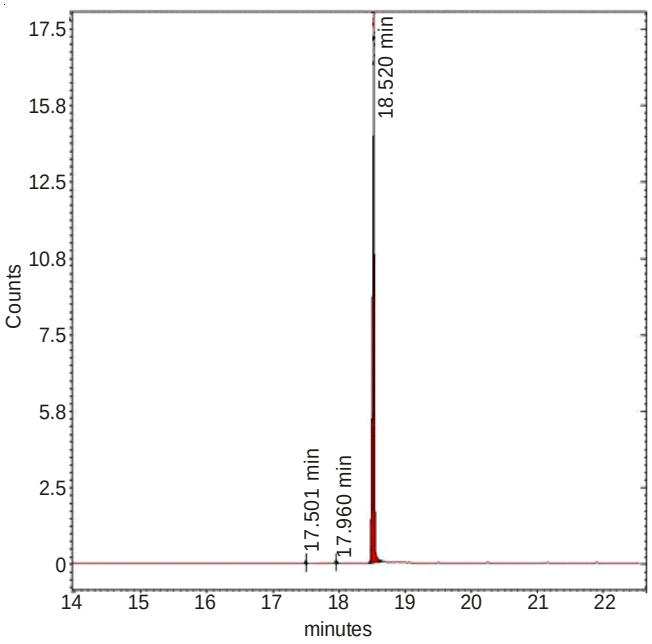

Fig. 1a. Chromatogram of ethofumesate 0.5 ppm standard detected by GC/MS

Data analysis: Rates of dissipation for ethofumesate in soil and plant were calculated using the method described by Timme *et al.*[13]. The dissipation rate constant was calculated by linear regression from the transformed first-order rate equation, $\ln C_i = \ln C_0 - K_t$, where C_i is the ethofumesate concentration as a function of time in days (t), C_0 is the highest ethofumesate concentration and K is the degradation rate constant. The time of dissipation of 50 % (DT_{50}) of the highest concentration was calculated from the equation $DT_{50} = 0{:}693/K$.

RESULTS AND DISCUSSION

Analytical performance, recovery and validation: The standard calibration curve of ethofumesate detected by GC/MS was constructed by plotting the analyte concentration *versus* peak area (Fig. 1b). The calibration curve showed excellent linearity in the concentration ranges of 0.01-1.0 µg mL^{-1} with the regression equation of y = 2E + 07x - 239492 (R^2 = 0.998).

The minimum concentration of herbicide molecule that was detected with acceptable certainty called the limit of detection (LOD) by the instrument was assessed by the repeated injection of the lowest concentration for 7 times. The LOD for ethofumesate was found to be 0.01 µg mL^{-1}.

The method validation was carried out to determine the fortified recoveries, precision and limits of detection of the analytical method. The standard solution of ethofumesate was added to the untreated sugar beet plant and soil at levels of 0.5, 1.0 and 2.0 µg g^{-1}. The fortified samples from all three replications were analyzed. The results suggested that the average recoveries of ethofumesate in plant, root and soil was in the range of 80-89, 83-86 and 90-92 %, respectively (Table-1).

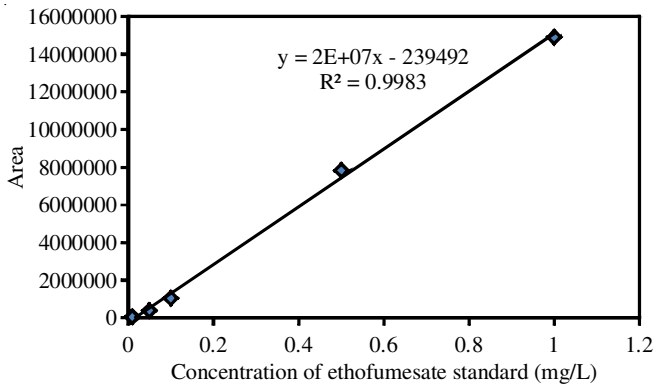

Fig. 1b. Calibration curve of ethofumesate standards detected by GC/MS

TABLE-1
RECOVERY OF ETHOFUMESATE IN SUGAR BEET PLANT, SOIL AND ROOTS

Fortified concentration (µg/g)	Recovery (%)* ± SD		
	Plant	Soil	Roots
0.50	80.36 ± 3.01	91.56 ± 1.84	82.58 ± 2.89
1.00	82.95 ± 1.97	90.70 ± 1.54	85.62 ± 2.07
2.00	88.71 ± 1.21	90.25 ± 1.03	86.33 ± 1.96

*Average of three replications

As the recovery of ethofumesate is more than 80 % from all the substrates, the present method was adopted for the ethofumesate residue extraction and determination in plant and soil samples. The limit of detection (LOD) and limit of quantification (LOQ) of the method followed was 0.001 and 0.005 µg g^{-1} for all the matrices. Mean while plant, root and soil samples from control plots were analyzed and the results showed that the extracts of these matrices did not have any interference with the targeted compound. Precision standard deviation of replicate analysis of different concentration of ethofumesate standard was used to find out LOD and LOQ.

Dissipation of ethofumesate in field soil and plant: The data pertaining to the persistence and dissipation of ethofumesate in field soil under different methods of application are presented in Figs. 2 to 3 and Tables 2 to 3. The initial concentration of ethofumesate in sugar beet field soil under single and split application was 0.530 and 0.814 and. 0.253 and 0.401 µg g^{-1}, across two doses respectively. There was a steady decrease in residue content and by 30th day the residues were 0.021 and 0.093 µg g^{-1} from two levels, respectively under single application. Similar results were observed under split application also and the residue on 30th day was 0.024 and 0.059 µg g^{-1} from two levels, respectively. Irrespective of method of application, the ethofumesate residue content went down below detectable limit under low dose; whereas it persists up to 45 and 60 days at high dose under single and split application, respectively. Similar to the soil, there was a steady decrease in residue of ethofumesate in sugar beet plant and by 15th day the residues were 0.017 and 0.081 and 0.009 and 0.031 µg g^{-1} from two levels, respectively under single and split application. Thereafter the residue level decreased below detectable limit.

From the results it is clearly evident that the residues of ethofumesate persist for longer time both in soil and plant under single application than under split application. It is very

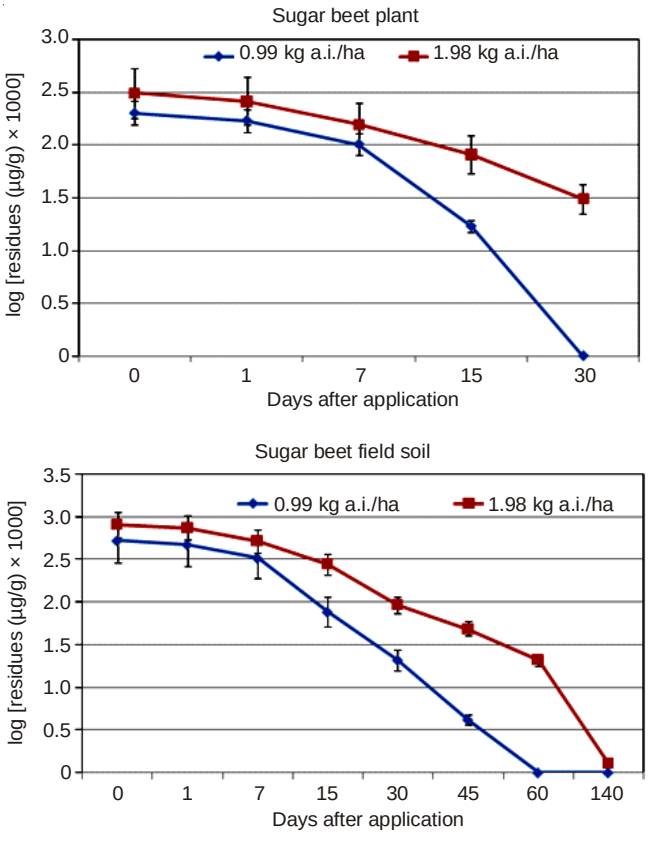

Fig. 2. Persistence and dissipation kinetics of ethofumesate in sugar beet plant and field soil under single time application

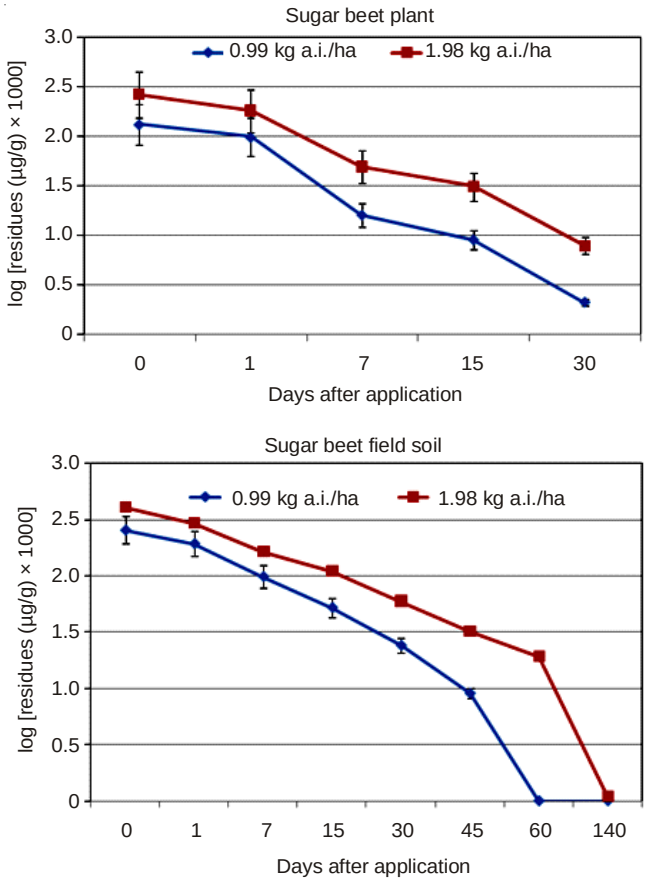

Fig. 3. Persistence and dissipation kinetics of ethofumesate in sugar beet plant and field soil under split application

TABLE-2
REGRESSION RELATION AND HALF LIFE FOR ETHOFUMESATE DEGRADATION IN SUGAR BEET PLANT AND FIELD SOIL UNDER SINGLE TIME APPLICATION

Dose	Regression equation	r^2	Half life (days)
Sugar beet plant			
T_1 = 0.99 kg ai ha^{-1}	y = 2.379-0.077x	0.987	4.20
T_2 = 1.98 kg ai ha^{-1}	y = 2.476-0.033x	0.988	7.70
Field soil			
T_1 = 0.99 kg ai ha^{-1}	y = 2.810-0.058x	0.976	5.39
T_2 = 1.98 kg ai ha^{-1}	y = 2.932-0.033x	0.997	9.67

TABLE-3
REGRESSION RELATION AND HALF LIFE FOR ETHOFUMESATE DEGRADATION IN SUGAR BEET PLANT AND FIELD SOIL UNDER SPLIT APPLICATION

Dose	Regression equation	r^2	Half life (days)
Sugar beet plant			
T_1 = 0.99 kg ai ha^{-1}	y = 2.358-0.071x	0.975	2.30
T_2 = 1.98 kg ai ha^{-1}	y = 2.140-0.141x	0.988	2.90
Field soil			
T_1 = 0.99 kg ai ha^{-1}	y = 2.486-0.032x	0.919	5.11
T_2 = 1.98 kg ai ha^{-1}	y = 2.353-0.084x	0.931	5.38

clear that the dissipation is faster as influenced by the climatic conditions like rain fall and temperature (Table-4). Since the ethofumesate was applied at the vegetative growth stage of the crop, the high rainfall received during that period might have washed out the ethofumesate residues from plant and soil and is responsible for its low persistence time in both plant and soil. Similar results were reported by Janaki and Chinnusamy[14] for the dissipation of metamifop in rice grown soil. Though the ethofumesate is moderately mobile in soil, the optimum soil moisture and temperature could have enhanced the ethofumesate dissipation from soil at a faster rate[7] under both the methods of application. It is possible that photolysis enhanced the observed ethofumesate dissipation in the topsoil[1]. Single application of ethofumesate increased its persistence time both in soil and plant and could be attributed to reduced microbial activity. Low persistence of ethofumesate under split application is attributed to the normal microbial activity which was unaltered by the application of low quantity at a time when compared to single application.

The kinetics of ethofumesate dissipation is found to be first order in both the cases. The data was supported by the earlier studies conducted by Tomiln[15] and Kucharski and Sadowski[16]. Half life calculated for plant was 2.6 days for ethofumesate under split application than 5.9 days under single application irrespective of dose of application. Such a low half life is due to the lower initial deposition of ethofumesate after its last application under split application due to the washing out of it from the plant by the rain fall (Table-4). Gardner and Branham[6] has reported such a lower half life of ethofumesate in turf grass. The increase in dose increased the DT_{50} values of ethofumesate under single application and could be established that the fraction of the total herbicide content which was available in the soil solution influence the dissipation of it from soil[17]. Under split application, the DT_{50} of ethofumesate in soil does not varied with the dose of application and established that the application of herbicides in splits does not affect the microbial activity in soil.

Terminal residues in soil and plant: Soil, plant and tubers samples at the time of harvest were analyzed for ethofumesate residue concentration (Table-5). Irrespective of method of application, the dose of application has significant influence on the persistence of ethofumesate in soil and plant. The highest concentration of herbicide active substances was determined in samples from plots where herbicide was applied at double dose. Reduction of herbicide dose caused a decrease of residues at the time of sugar beet lifting in soil and tuber[10]. The presence of metabolites in soil *viz*., oxy ethofumesate and hydroxy ethofumesate was also assessed at the time of harvest of sugar beet. However no residues were detected in soil. This could be ascribed to the high rainfall (Table-4) and intrinsic characteristics of the soil[18] which might have enhanced the ethofumesate dissipation from soil. Influence of soil type especially clay and organic matter content on increasing the dissipation of ethofumesate and its final residue content in soil was reported by Kucharski and Sadowski[16]. Bioassay studies also revealed that the residue of ethofumesate were at non-toxic levels in soil at the time of harvest, though it was above the detectable levels as determined by the GC-MS. However the continuous and inappropriate use of the ethofumesate for the weed control in sugar beet might bioaccumulate in soil and may also cause biomagnification in plants.

TABLE-5
RESIDUE OF ETHOFUMESATE (μg g^{-1}) IN SOIL AND ROOTS OF SUGAR BEET AT HARVEST

Treatments	Single application		Split application	
	Soil	Tuber	Soil	Tuber
T_1 = 0.99 kg ai ha^{-1}	0.0006	BDL	0.0007	BDL
T_2 = 1.98 kg ai ha^{-1}	0.0012	0.0013	0.0013	0.0011

Conclusion

The present method offers good accuracy and precision to determine ethofumesate residues in soil and sugar beet plant parts and this validated method was applied to study the

TABLE-4
WEATHER PARAMETERS PREVAILED DURING CROP GROWTH PERIOD

Parameters	Crop growing period				
	1st fortnight	2nd fortnight	3rd fortnight	4th fortnight	5th fortnight
Average minimum temperature (°C)	22.3	22.7	20.6	22.6	21.4
Average maximum temperature (°C)	31.5	30.8	32.4	31.7	28.9
Total rainfall (mm)	61.1	26.1	0.2	82.4	230.5
Soil temperature minimum (°C) upto 20 cm depth	27.4	25.8	28.1	28.2	24.5
Soil temperature maximum (°C) upto 20 cm depth	33.8	31.7	35.2	34.3	29.5

dissipation behaviour of ethofumesate under two methods of application. Quantity of application and weather variables has influence on the persistence and dissipation of ethofumesate in soil and sugar beet plant. The residues of herbicide dissipated faster in plant than in soil with a half-life of 7.5 and 5.9 days under single application and 5.3 and 2.6 days under split application in soil and plant, respectively. Application of ethofumesate at 0.99 kg a.i. ha^{-1} degraded from the soil and plant before the harvest of sugar beet crop and therefore limited the risk for agricultural environment contamination. However the continuous and inappropriate use of the ethofumesate for the weed control in sugar beet might bioaccumulate in soil and may also cause biomagnification in plants.

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