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Persistence behaviour of a mixed formulation (florasulam 10% + halauxifen methyl 10.4% WG) in wheat

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ABSTRACT

The persistence behaviour of Florasulam and halauxifen-methyl were investigated at BCKV Experimental Farm, Mohanpur, West Bengal during two consecutive season of 2012-13 and 2013-14. Extraction of the herbicides was performed with acetonitrile followed by d-SEP clean up with PSA, GCB (for plant sample) and florisil. The residue of florasulam and halauxifen-methyl were quantified by Liquid Chromatography-mass Spectrometry using multiple reactions monitoring (MRM). The half-life of florasulam was found in the range of 1.1-1.4 days and 2.1-2.5 days for wheat plant and soil respectively. The half-life of haluxifen methyl was in the range of 1.0-1.2 days for wheat plant and 1.8-2.1 days for soil. No residue of florasulam and haluxifen-methyl was detected in harvest samples of wheat grain, straw and soil. It seems from this study that, application of the mixed formulation will not pose any residual toxicity problem when applied at recommended dose.

***Keywords*:** Florasulam, halauxifen-methyl, LC-MS/MS, persistence, soil, wheat

Wheat is an important crop worldwide and in India, its production increased from a mere 11.0 million tons during 1960-61 to 93.9 million tons during 2011-12 (Chhokar *et al.*, 2012). More than eight-fold increase in wheat production was mainly due to the adoption of short stature high yielding varieties, increased fertilizers use, irrigation and herbicides. Weeds are regarded as most disdain to crop production. It has been estimated that crop losses due to weed competition are greater than those resulting from combined effect of insect pests and diseases (Abbas *et al*., 2009)

Florasulam [2', 6', 8- trifluoro-5-methoxy- [1,2,4]-triazolo [1,5-C] pyrimidine-2-sulfonanilide] is a selective triazolopyrimidine sulfonanilide post-emergence herbicide. The mode of action for florasulam is through inhibition of the plant enzyme acetolactate synthase (ALS). It is taken up by plant root and shoots and translocated in both xylem and phloem (Tomlin, 2006). Halauxifen methyl (XDE – 729 methyl ester) (4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxy-phenyl)-pyridine-2-carboxylic acid methyl ester) is a novel arylpicolinate herbicide with an auxinic mode of action. These will provide a new option for the control of key broadleaf weeds including those with resistance to other herbicides.

Some analytical methods regarding the estimation of florasulam are recently reported. A high performance liquid chromatography with UV-detection method was developed Anon., 2007) for the quantification of florasulam in soil or sediment and water. Recently, Li *et al.* (2013) developed an analytical method using acetonitrile extraction and quantification by LC-MS/MS for the determination of florasulam in wheat and soil.

Halauxifen-methyl is a newly developed chemical herbicide. This innovative, low-use rate herbicide represents a novel area of chemistry (http://news.agropages.com/News/NewsDetail).

There are meager information on the dissipation and dynamics of halauxifen-methyl on wheat crop in India. Here, authors aimed to establish a method for the quantification of the herbicides using QuEChERS approach and to study the degradation of the same in wheat matrix and soil in West Bengal agro-climatic condition. This work would not only help to establish the MRL of florasulam and halauxifen-methyl in wheat but also to provide guidance on proper use of florasulam and halauxifen-methyl in wheat crop.

MATERIALS AND METHODS

Field experiment was conducted on wheat (Variety: PBW-343) at BCKV Experimental Farm (N $22^0$56' E $88^0$31'). The formulation florasulam 10 % + halauxifen-methyl 10.4% WG was obtained from Dow Agro-Sciences, India. The experiment was designed according to Randomized Block Design (RBD). Each experimental plot was 20 m^2 with 12

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rows per plot. The formulation was applied at doses of 12.76 g a.i. ha^{-1} (T_1: recommended dose) and 25.52 g a.i. ha^{-1} (T_2: double the recommended dose) in 500 L water ha^{-1}. Each treatment was replicated thrice has three replication along with an untreated control (T_3).

Samples of wheat plant (0.5 kg) and 2 kg of surface soil (0–15 cm depth) were collected randomly from each plot at regular time intervals on 0 (1 h after spraying), 1, 3, 5, 7, 10 and 15 days. Wheat straw (0.5 kg), grain (0.5 kg) and soil (2 kg) samples were also taken at the time of harvest. Samples were brought in the laboratory in plastic bags (< 40 µ) and stored at -22 °C. Wheat includes three matrixes: plant, grain and straw. Each matrix was grounded into small pieces or powder with polytron homogenizer (Model: Polytron, PT-MR-3100 Kinemetica AG, Lucerne, Switzerland). Soil samples were passed through 100 mm sieve. The entire processed sample was kept at -22 °C prior to final analysis.

The analytical standard of florasulam and halauxifen-methyl of > 99% purity were obtained from Dow Agro-Sciences, India. All the solvents *viz*. methanol, acetonitrile (HPLC grade) were purchased from J. T. Baker. For cleaning up of the extracted sample, Primary Secondary Amine (PSA: Varian, Harbor City, CA; 40 mm particle size), Florisil (60–100 mesh; Acros, Geel, Belgium) and graphitized carbon black (GCB; United Chemical Technology, Bellefonte, PA: only for plant matrixes) were used. Analytical reagent grade acetic acid was purchased from Merck, India. Magnesium sulphate heptahydrate and soduim chloride was purchased from SRL, India.

The stock solution of florasulam (100 µg ml^{-1}) and halauxifen-methyl (100 µg ml^{-1}) were prepared in methanol and stored in deep freezer. An intermediate standard solution (10 µg ml^{-1}) of the herbicides in mixture was prepared by appropriate dilution with the corresponding solvents. The calibration standards (six calibration points) ranging from 0.01 to 0.5 µg ml^{-1}were prepared by successive dilutions of the intermediate standard.

The calibration curves for florasulam and halauxifen-methyl were obtained by plotting the peak area against the concentration of the corresponding calibration standards. The limit of detection (LOD) of the test compounds was determined by considering a signal-to-noise ratio of 3 with reference to the background noise obtained for the blank sample. The limits of quantification (LOQ) were determined by considering a signal-to-noise ratio of 10.

The homogenized samples, namely, plant (5g), Soil (10g), straw (2g) and grain (5g) were taken in a 50 ml fluorinated ethylene propylene (FEP) centrifuge tube (Nalgene, Rochester, NY) separately. 10 ml milli-Q water was added and samples were acidified with 0.1 ml acetic acid. It was then vortexed for 1 min. for proper incorporation of the acidified water into sample matrix. After 15 min, 10 ml acetonitrile was added and shaken vigorously for 1 min. Then 6g $MgSO_4$ and 1.5 g NaCl was added to it and again vortexed for 2 min followed by 15 minute vertical shaking. Then the sample was centrifuged for 5 min at 5000 rpm. The supernatant (6 ml) was collected to carry out the clean up procedure.

To carry out the clean-up step, PSA, Florisil and GCB (for plant matrix only) were used. 1.5 ml of the extracted aliquot (plant sample) was taken in a pre-weighed 2 ml centrifuge tube with 25 mg PSA, 25 mg florisil and 35 mg GCB. For other matrix (straw, soil and grain) 25 mg PSA and 25 mg florisil was taken in 2 ml centrifuge tube for 1.5 ml aliquot. Afterwards, it was centrifuged at 6000 rpm for 5 minutes. It was then filtered through 0.2µm nylon membrane filter (0.2 µm ultipor N66 nylon 6,6 membrane filter, Pall Corporation) and finally cleaned extract was analyzed by LC-MS/MS.

The recovery experiment was carried out by fortifying fresh untreated plant samples (including straw and grain) and field soil samples in triplicate with the mixture standard at three concentration levels *i.e.* LOQ, LOQ × 5 and LOQ × 50. For both florasulam and halauxifen-methyl these level were 0.01, 0.05 and 0.50 µg/ml.

Matrix matched standards were employed to evaluate the matrix effect. The blank extracts were prepared in a similar fashion as above with untreated control sample of wheat plant and soil. All six calibration standards were prepared in the blank extracts and analyzed.

Quantification of florasulam and halauxifen-methyl residue was done by Liquid chromatography coupled with tandem mass spectrometry. The HPLC separation was performed on a Alliance 2695 separation module liquid chromatograph (Waters, Milford, MA, USA) equipped with a quaternary solvent delivery system by 20 µl via auto sampler on a reversed phase Symmetry C_{18} (5µm; 2.1 × 100 mm) column (Waters, USA) and a Micromass (Manchester, UK) QuattroMicro triple-quadruple spectrometer equipped with an electrospray source (ESI) was used for detection and quantification. Injection volume was 20 µl and the analysis performed with a flow rate of 0.3

ml min^{-1}. The mobile phase was composed of (A) water, 5 mM ammonium acetate and 0.1% acetic acid and (B) methanol, 5 mm ammonium acetate and 0.1% acetic acid. Gradient: 0.0 – 2.0 min – 5.0% B to 95 % B, 2.0-8.0 min – back to the initial condition of 5% B, at 10.0 min, it ends with 5% B.

Estimation of both the herbicides was performed in positive mode by a single multiple reaction monitoring (MRM) with mass transition from parent ion 359.87 to daughter ion 128.90 for florasulam. A second mass transition was used 359.87 > 81.60 for confirmation. For halauxifen-methyl, the two ion transitions were 344.82 > 250.10 and 344.82 > 285.00. The ratio of the peak area of these two daughter ions for florasulam and halauxifen-methyl were 0.149 and 0.789 respectively. The corresponding ratio in the positive samples was determined and confirmed in accordance with European Union guidelines (Anon., 2002).

RESULTS AND DISCUSSION

Quantification of florasulam and halauxifen-methyl was done in a single LC-MS/MS method in which both herbicides were eluting in same gradient programming (Fig. 1). The linearity of the calibration curve was established in the range 0.01–0.5 µg ml^{-1} for both the chemicals with a correlation coefficient (R^2) of the calibration curve > 0.99 (Fig. 2, 3). For matrix calibration, the R^2 was found > 0.99 for both compounds. For both the compounds, matrix suppression was prominent in plant and soil. For wheat plant matrix, it was 8 – 10 % and 12 – 15 % for florasulam and halauxifen-methyl respectively. In soil matrix, it falls in the range of 15 – 18 % and 19 – 22 % respectively for florasulam and halauxifen-methyl.

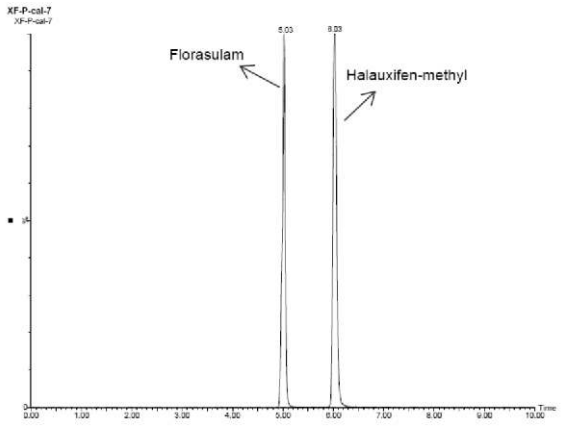

Fig. 1: Chromatogram of analytical standard of florasulam (RT – 5.03 min) and halauxifen-methyl (RT – 6.03 min); Concentration 1.0 µg ml^{-1}

Correlation coefficient: r = 0.999024, r^2 = 0.998049
Calibration curve: 11.0929 * x + 1.89119
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis Trans: None
Correlation coefficient: r = 0.997755, r^2 = 0.995515
Calibration curve: 15.8788 * x + 25.1876
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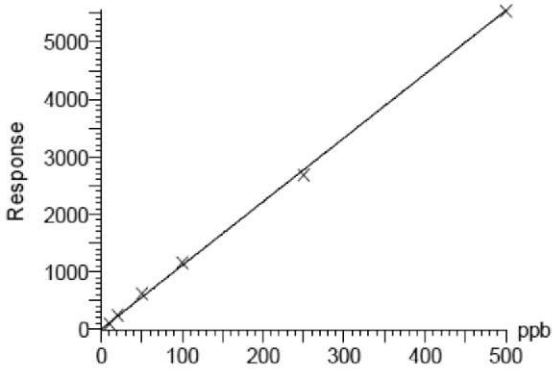


Fig. 2: Calibration curve of florasulam

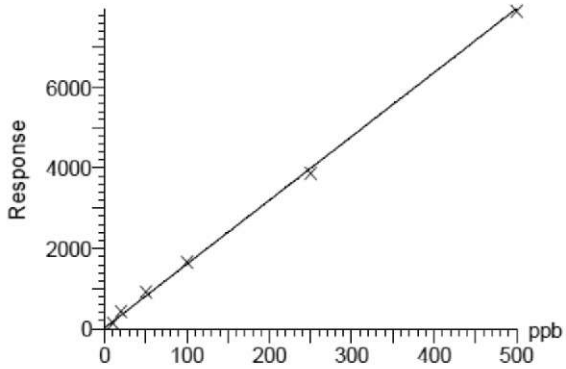


Fig. 3: Calibration curve of halauxifen-methyl

The concentrations of florasulam and halauxifen-methyl in field samples were calculated on the basis of the comparison with the signal in blank samples. For confirmation of residues, the ion ratios pertaining to the two selected mass transitions in actual samples were compared to the ion ratio obtained for calibration standards. Samples showing ion ratios within a range of ± 20% were accepted as confirmatory presence of the said herbicide residues.

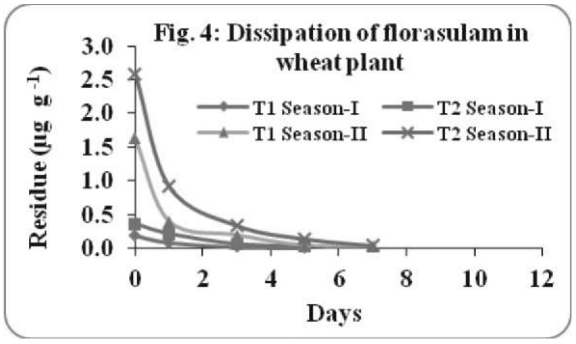
The average recoveries of florasulam in plant and soil samples at LOQ, LOQ × 5 and LOQ × 50 were 92.35 ± 2.19%, 94.41 ± 3.12%, 96.18 ± 1.38% and 88.39 ± 2.09%, 91.82 ± 3.52% and 90.65 ± 4.80% respectively. The average recovery of wheat straw and grain was 84.23 ± 3.09% and 81 ± 4.65% for florasulam. For halauxifen-methyl the recovery was 85 ± 2.54% and 83 ± 3.76% for wheat straw and grain

respectively. This complies with the EU DG SANCO criterion (Anon., 2000), which requires mean recoveries within the range 70–110%.

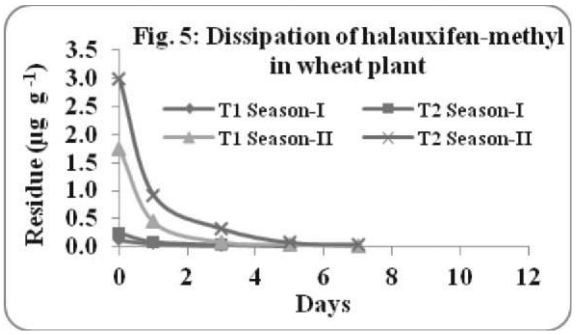
The initial deposits (2 h after spraying) of florasulam in soil were found 0.406 µg g^{-1} (T_1) and 0.879 µg g^{-1} (T_2) in 1st season and 0.277 µg g^{-1} (T_1) and 0.695 µg g^{-1} (T_2) in 2nd season, respectively (Fig. 4). In case of wheat plant, initial residual level for florasulam were found 0.194 µg g^{-1} (T_1) and 0.361 µg g^{-1} (T_2) in season – 1 and 0.277 µg g^{-1} (T_1) and 0.695 µg g^{-1} (T_2) in 2nd season, respectively (Fig. 5). For halauxifen-methyl in soil, the initial deposits (2 h after spraying) were found 0.529 µg g^{-1} (T_1) and 0.821 µg g^{-1} (T_2) in season – 1 and 0.387 µg g^{-1} (T_1) and 0.812 µg g^{-1} (T_2) in season - 2 respectively (Fig – 6). In wheat plant for halauxifen-methyl initially 0.124 µg g^{-1} (T_1) and 0.246 µg g^{-1} (T_2) in season – 1 and 1.748 µg g^{-1} (T_1) and 2.987 µg g^{-1} (T_2) in 2nd season were found, respectively (Fig. 7). The dissipation of both the herbicides follows first order kinetics irrespective of any treatment doses and test matrix. More than 50 % of the initial concentration was dissipated within 5-days after application irrespective of any doses and substrate. The half-life ($T_{1/2}$) of florasulam and halauxifen-methyl was calculated using Hoskins (1961) formula. The half-life of florasulam and halauxifen-methyl in wheat plant were in the range of 1.08 – 1.39 days and 1.01 – 1.18 days. Half-life in field soil falls in the range of 2.05 – 2.45 days and 1.78 – 2.08 days for florasulam and halauxifen-methyl respectively. The statistical analysis is shown in table-1. No residue was detected in untreated control samples irrespective of treatment dose and season. The residues of florasulam and halauxifen-methyl in wheat grain, wheat straw and cropped soil reached below the LOQ value when analyzed at harvest. The degradation of both the herbicides was found faster in plant than soil. Persistence pattern of florasulam observed in this study is somewhat different as reported by Li *et al*. (2013). This is probably due to the different climate, soil type, organic carbon (OC) present in soil and chemical and physical properties of the compound (Smith *et al*., 1977).

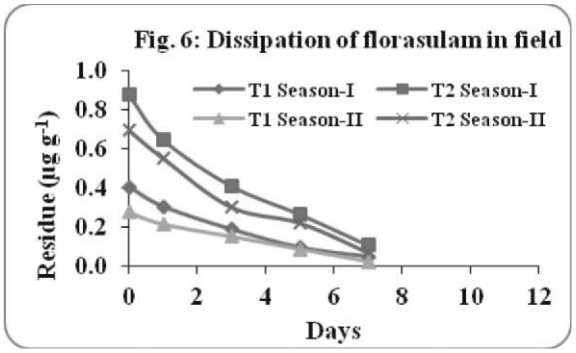
Table 1: Statistical data on the dissipation of florasulam and halauxifen-methyl in wheat plant and soil

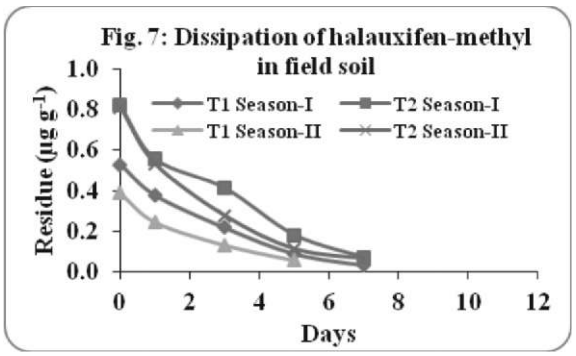
Compound Name	Substrate	Season	Dose	Regression Equation	R^2	Half-life (days)
Florasulam	Soil	Season - I	T_1	y = -0.132x + 2.628	0.99	2.28
			T_2	y = -0.123x + 2.960	0.98	2.45
		Season - II	T_1	y = -0.136x + 2.500	0.94	2.05
			T_2	y = -0.135x + 2.881	0.95	2.23
	Plant	Season - I	T_1	y = -0.240x + 2.222	0.97	1.25
			T_2	y = -0.217x + 2.534	0.98	1.39
		Season - II	T_1	y = -0.280x + 3.075	0.97	1.08
			T_2	y = -0.242x + 3.308	0.99	1.24
Halauxifen-methyl	Soil	Season - I	T_1	y = -0.169x + 2.763	0.98	1.78
			T_2	y = -0.145x + 2.944	0.97	2.08
		Season - II	T_1	y = -0.163x + 2.577	0.99	1.85
			T_2	y = -0.159x + 2.896	0.99	1.89
	Plant	Season - I	T_1	y = -0.297x + 2.081	0.99	1.01
			T_2	y = -0.255x + 2.329	0.99	1.18
		Season - II	T_1	y = -0.267x + 3.021	0.95	1.13
			T_2	y = -0.264x + 3.332	0.97	1.14



Note: T_1: 12.76 g a.i. ha^{-1}; T_2: 25.53 g a.i. ha^{-1}







The present method established for the detection and quantification of florasulam and halauxifen-methyl is cost effective and less time consuming as it comprised of a single method of extraction and single run in LC-MS/MS for both the herbicides. This work would be useful to establish the MRL of florasulam and halauxifen-methyl in wheat which will provide guidance on the proper and safe use of this herbicide in India.

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REFERENCES

Abbas, G., Anjum, A. M., Abbas, Z., Aslam, M. and Akram, A. 2009. Impact of different herbicides on broadleaf weeds and yield of wheat, *Pakistan J. Weed Sci. Res.*, **15**: 1-10

Anonymous 2000. Guidance document on residue analytical methods. European Union Commission Directorate of General Health and Consumer Protection. SANCO/825/00 rev. 6, dated 20 June 2000.

Anonymous 2002. Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, *J. European Communities* L221 (17 August 2002):8–36.

Anonymous 2007. *United States Environmental Protection Agency* (US EPA), Washington DC, Environmental Chemistry Laboratory.

Chhokar, R., Sharma, R. and Sharma, I. 2012. Weed management strategies in wheat – A review, *J. Wheat Res.*, **4**: 1-21

Hoskins, W.M. 1961. Mathematical treatment of the rate of loss of pesticide residue, *FAO Pl. Protection Bull.* **9**:163–68.

Li, Z., Guan, W., Hong, H., Ye, Y. and Ma, Y. (2013), Determination and study on residue and dissipation of florasulam in wheat and soil under field conditions, *Bull. Env. Contam Toxicol*, **90**: 280-84

Smith, A. E. and Walker, A. 1977. A quantitative study of asulam persistence in soil, *Pesticide Sci*, **8**: 449-56.

Tomlin C.D.S. 2006. *The Pesticide Manual*, 14th Edn. BCPC, UK.