

Residue of bensulfuron methyl in soil and rice following its pre- and post-emergence application

P. Janaki¹, C. Nithya², D. Kalaiyarasi², N. Sakthivel², N.K. Prabhakaram²,
C. Chinnusamy²

¹Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University,
Tamil Nadu, India

²Department of Agronomy, Tamil Nadu Agricultural University, Tamil Nadu, India

ABSTRACT

Bensulfuron methyl (BSM) is applied in rice to control a wide range of weeds due to low application rate and high efficiency. A study was conducted to evaluate residues of BSM in soil and rice plant at different doses as pre- and post-emergence application in transplanted rice. The quick easy cheap effective rugged safe (QuEChERS) method was evaluated for BSM residue extraction from different matrices. The limit of detection and limit of quantification was 0.005 and 0.01 µg/g, respectively in soil and rice plant. The average BSM recovery of 91.1, 82.8, 84.5 and 88.7% was obtained from soil, rice straw, grain and husk, respectively. Though, BSM residue was detected (0.011 to 0.017 µg/g) in soil at high dose, it was below maximum residue limit (0.01 µg/g) in rice grain at both the doses of BSM. Hence, the study revealed that the BSM can be safely applied to rice at recommended doses for weed control.

Keywords: *Oryza sativa* L.; weed management; herbicide; contamination; persistence

Rice (*Oryza sativa* L.) is the vital staple food for about 50% of the world's population that lives in Asia. Hence, a worldwide research is continuing to enhance the production and productivity of the rice crop. A major production constraint in rice cultivation is the lack of timely weed management caused by the acute labour scarcity and high manual weeding cost. Thus, the use of herbicides for weed control is encouraged in rice production. Asia accounts for vast majority of the global rice herbicide market and the share continues to grow, since the multinational agrochemical companies acquire the advantage of a more liberal trading climate in Asia (Naylor 1996). Although the use of herbicides has increased the crop production to cope up with food demand, there may be an unintentional exposure of the ecosystem to herbicides residue. Hence, an inappropriate and indiscriminate use of herbicides in rice cultivation could cause environmental contamination through longer persistence.

During last 20 years, low application rate herbicides viz., sulfonyl urea, sulfonamide, and imidazolinone have been developed and registered

for weed control all over the world. They act by inhibiting the action of acetolactate synthase, a key plant enzyme essential for the synthesis of branched-chain amino acids (Moberg and Cross 1990, Stidham 1991). Among the above classes, sulfonyl ureas are registered largely for chemical weed management in rice either as pre- or post-emergence herbicide for controlling the grasses, broad leaved weeds and sedges (Russel et al. 2002). Bensulfuron methyl (BSM) (methyl 2-[(4,6-dimethoxy-pyrimidin-2-yl) carbamoyl sulfamoyl methyl] benzoate), one of the environmental-friendly low dose herbicide belonging to sulfonyl urea is used highly for weed control in rice. It is marketed as Londax (single herbicide) and Londax Power (combination with pretilachlor) in India.

Because of low application rate, its concentration is expected to be low in water and soils. However, it depends on the herbicide molecule and formulation properties, application rate, and areas or type of soils treated with this herbicide. The solubility of BSM depends on pH of water and has high solubility (880 mg/L) in water at pH 8.0

(25°C) and very low solubility (1.1 mg/L) at pH 5.0 (Roberts et al. 1998, Vencill 2002). It is highly stable under slightly alkaline aqueous solutions (pH 8) and degrades slowly under acidic conditions. It is stable to direct photolysis in sterile buffer solutions and degrades rapidly in natural water under sunshine radiation by cleavage of the sulfonylurea linkage to methyl 2-(sulfomethyl) benzoate and (4,6-dimethoxypyrimidin-2-yl) urea, with a degradation half life (DT_{50}) of 3–4 days (Roberts et al. 1998). Bensulfuron methyl degrades also through chemical hydrolysis and microbial processes in moist soils and is classified as immobile to moderately mobile depending on the soil organic matter and pH (Roberts et al. 1998). The major degradation product under aerobic metabolism is CO_2 ; however, under sterile conditions the non-volatile compounds were also produced, namely sulfonamide and pyrimidine amine [2-amino-4,6-dimethoxypyrimidine]. This showed that the microbial degradation is not obligatory for BSM degradation (CDFA 1989).

Since sulfonyl ureas act upon a specific plant enzyme acetolactate synthase that is not found in mammals or other animals, their toxicities to animals is very low (Brown 1990). As they are very active at low concentrations, residual phytotoxicity of sulfonyl ureas to rotation crops such as corn, sunflowers, sugar beets, and dry beans has been already reported in literature (Anderson and Humburg 1987, Curran et al. 1991). The rice plant metabolizes the BSM rapidly with a DT_{50} of 4–6 h. The crop selectivity is due to the slower rate of translocation from roots to shoots and an increased rate of metabolism in rice (Takeda et al. 1986).

The available literature on BSM is limited to bioefficacy, laboratory dissipation studies (Langeland and Lorache 1994) and very few under field conditions (CDFA 1989, EFSA 2008). Similarly, the influence of time of application on the persistence of BSM has not been reported. In view of these facts, the present study was undertaken to study the persistence of BSM in soil and its terminal residues in rice as influenced by the time of application under subtropical arid agro-climatic conditions.

MATERIAL AND METHODS

Experimental details. Field experiments were conducted during the kharif season (June

to September) of 2012 and 2013 at a wet land farm of the Tamil Nadu Agricultural University, Coimbatore with rice (cv. ADT 48) as a test crop. As herbicide bensulfuron methyl (60% dry flowable (DF)) was applied at two different doses (100 and 200 g active ingredient (ai)/ha) as pre- and post-emergence along with control (no herbicide). The experiment was conducted in randomized block design and the treatments were replicated thrice. The pre- and post-emergence application of bensulfuron methyl was done on 3rd and 14th day after transplanting, respectively, using flat fan nozzle and knapsack sprayer with the spray volume of 400 L/ha. All the management practices were followed as prescribed for rice cultivation in the crop production guide. The experimental farm is located at 77°E, 11°N latitude and 426 m a.s.l. Experimental field soil was clay loam in texture and belongs to Typic Chromusterts group and Noyyal soil series. The experimental field soil was medium in organic carbon status (0.60%) with the available nutrient status of low nitrogen (226 kg/ha), medium phosphorus (18.3 kg/ha) and high potassium (458 kg/ha) and has alkaline soil reaction (8.27) and the electrical content of below 0.43 dS/m.

One week after rice harvest, the green gram (*Vigna mungo*) cv. CO 6 was grown in strips without disturbing the layout and observation on germination percent, plant height, number of pods per plant and seed yield at harvest were recorded to assess the carryover effect of BSM applied to the main crop (rice).

Collection of samples. The soil samples were collected for residue analysis at the time of harvest from 0–15 cm depth. Five core soils were randomly taken using soil auger from each treated and untreated plot avoiding outer 20 cm fringes of plot. The core soils were pooled from each plot, air dried, powdered and sieved through 2 mm sieve and stored for analyses. The paddy grain and straw samples were collected from BSM treated and untreated plots at harvest. Grains of rice were removed from husk and crushed using a mechanical blender, while straw was chopped into small pieces using knife.

Meteorological conditions. Weather parameters prevailing during the cropping period were recorded (Figure 1) at weekly intervals. It was observed that the maximum and minimum temperatures ranged from 18.4–33.3°C during kharif 2012 and from 21.6–32.8°C during kharif 2013, respectively. The total rainfall per day recorded ranged from

doi: 10.17221/294/2016-PSE

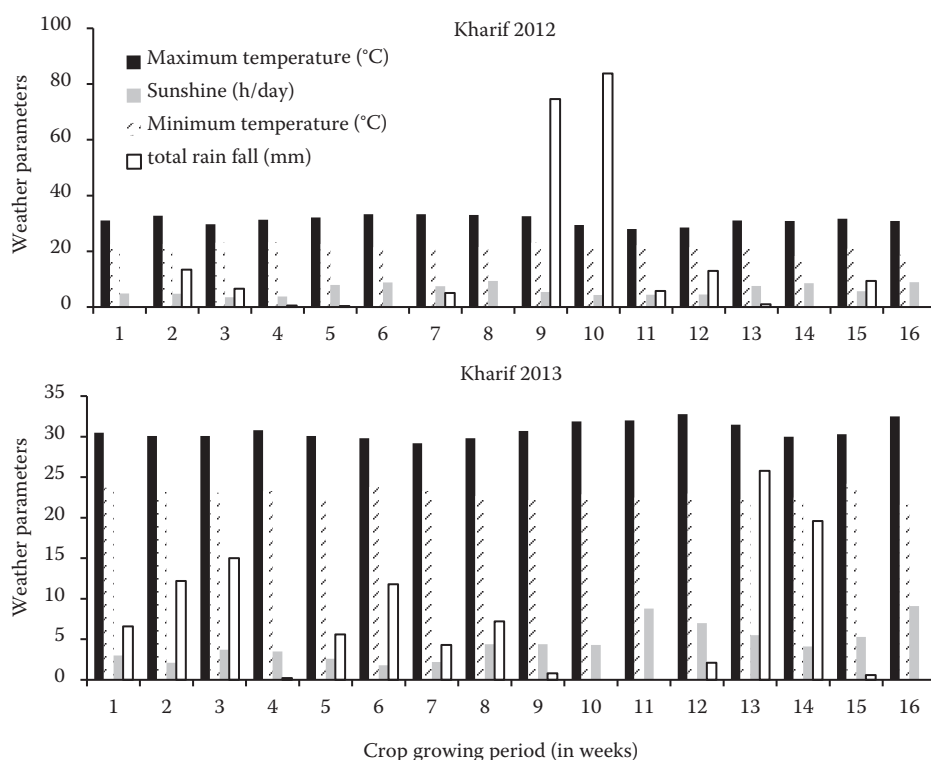


Figure 1. Distribution of weather parameters during the growing period of rice

6.1–165.2 mm during kharif 2012 and 0.6–1.8 mm during kharif 2013.

Determination of bensulfuron methyl residues in soil and plant samples. The BSM residue was extracted by quick easy cheap effective rugged safe (QuEChERS) technique (Janaki et al. 2015) using 1% HOAc in MeCN + dichloromethane and anhydrous $MgSO_4$ and $NaOAc \cdot 3 H_2O$ in vortex mixer. An aliquot was cleaned up using the Bond Elut C18 SPE cartridge with acetonitrile as eluent and then concentrated in rotary vacuum evaporator for LC-DAD analysis.

Validity of the method was tested by conducting the recovery experiments using spiked samples of soil and rice plant parts from the control treatment with the known BSM standards concentration of 0.01, 0.05, 0.1 and 0.5 $\mu g/g$ in three replicates. After spiking, the residue of BSM was extracted and cleaned up as described for samples above. The concentration of BSM was determined by comparing the peak area of the samples and calibration curves of five levels of standards. A quantification limit of 0.01 $\mu g/g$ was used for the calculation. The blank soil and rice plant parts reference was used to establish the limit of quantification. A calibration curve was prepared by plotting concentrations of BSM on x -axis against the average peak area on y -axis (Figure 2).

Instrumentation. The BSM residues were determined using Agilent HPLC (1200 series) with Diode Array Detector (DAD) detector, binary pump and auto sampler having rheodyne injection system. The compound was separated using Agilent Eclipse XDB-C 18, 5 μm , 4.6 \times 150 mm column kept in thermostated oven maintained at 25°C. The instrument was connected to a computer that recorded the response in terms of peak area and height using the EZChrom software (USA). The acetonitrile:water (50:50% v/v) with orthophosphoric acid (pH 3.0) was used as a mobile phase for the separation of BSM with the flow rate of 0.5 mL/min. The injection volume of sample was 20 μL . Detection was performed at 234 nm for all the standards and unknown samples.

RESULTS AND DISCUSSION

Recoveries and detection limit. Equations of analytical calibration graphs, obtained by plotting peak areas on the y axis against concentrations of BSM on the x axis showed good linearity (Figure 2) with the correlation coefficient of 0.979. The retention time of BSM standards and samples was 4.50 ± 0.2 min (Figures 3 and 4) under the given instrumental conditions of HPLC-DAD. The av-

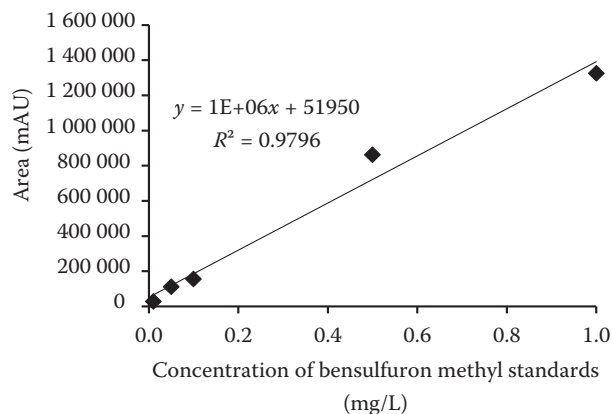


Figure 2. Calibration of bensulfuron methyl standard at concentration levels of 0.01–5.0 $\mu\text{g}/\text{mL}$

erage recovery of BSM obtained from soil, rice straw, grain and husk was 91.1, 82.8, 84.5 and 88.7%, respectively. The results of the study are presented in Table 1. The limit of detection and quantification of BSM in all the matrices was found to be 0.005 and 0.01 $\mu\text{g}/\text{g}$, respectively, with the signal to noise ratio of 3:1. The recovery of BSM from different matrices was found to be acceptable up to fortification level of 0.01 $\mu\text{g}/\text{g}$ (Table 1) and was also found satisfactory at different concentration levels. Niell et al. (2010) also reported the BSM recovery of 91% from rice grain by modified QuEChERS extraction using 1% acetic acid in acetonitrile, MgSO_4 and sodium acetate.

Terminal residues of bensulfuron methyl in rice grain, husk and straw. Irrespective of time (pre- or post-emergence) and dose (100 or 200 g/ha) of application, the BSM residue was below the quantification limit of 0.01 $\mu\text{g}/\text{g}$ in the rice straw, grain and husk at the time of harvest in both years (Kharif 2012, 2013). This could be due to the selectivity of rice crop to the BSM residue by slower rate of translocation from roots to shoots and through an increased rate of metabolism. Takeda et al. (1986) reported that the rice shoots metabolized BSM with a DT_{50} of 4–6 h while sensitive broad-leaved and sedge weeds did not degrade BSM ($\text{DT}_{50} > 50$ h). According to Priester (1985), the metabolism proceeds initially through *o*-demethylation of the pyrimidine ring to yield methyl α -(4-hydroxy-6-methoxypyrimidin-2-ylcarbamoyl sulfamoyl)-*o*-toluate and the hydrolysis of the parent methyl ester to produce bensulfuron (Usui et al. 1993). Similar results of less than 0.03 mg/kg of BSM

residue in rough rice, husk and rice straw were also reported by Wu et al. (2000).

The residue of BSM in rice grain, husk and straw was below the maximum residue limit (MRL) in rice parts set by the EFSA (0.02 mg/kg) for the European union (EFSA 2008), FSSAI (0.01 mg/kg) for India (FSSAI 2015), FSCA (0.02 mg/kg in rice and 0.05 mg/kg in rice bran) for Australia (FSCA 2014) and Japan (0.1 mg/kg) by Clever and Sato (2011). Wei and Chen (1995) also reported a below MRL residue of BSM (0.0116 $\mu\text{g}/\text{g}$) in rice after 98 days of application. The present results suggest that the normal rate (100 g/ha) of BSM application is environmentally safe as its residues were found below the MRL in rice grain and straw sets by different agencies of various countries.

Terminal residues of bensulfuron methyl in field soil. The sulfonyl ureas degrade in soil primarily by the microbial or chemical metabolism and conversion through photochemical occurs only in the presence of UV light, which is a minor decomposition mechanism (Singh et al. 2010). The dissipation of sulfonyl ureas in soil is generally influenced by pH and other minor factors are temperature, moisture and organic matter. Though the residue of BSM was not detected in the rice straw, husk and grain at doubled dose of 200 g/ha, its residue was detected in field soil at harvest during kharif 2012 under both the pre- and post-emergence application. It was below

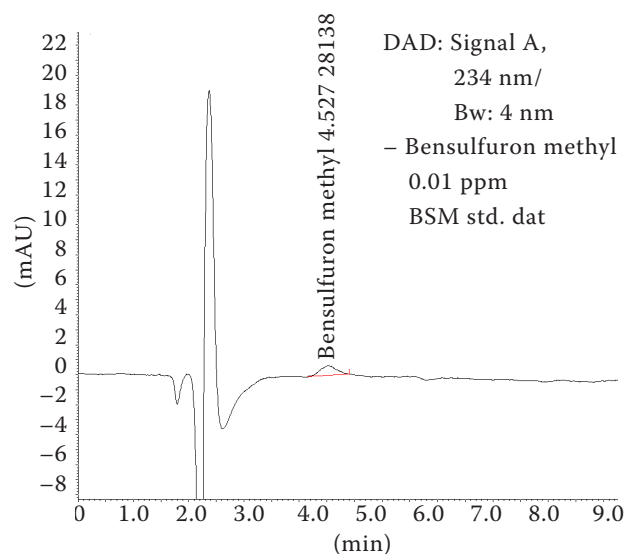


Figure 3. Bensulfuron methyl standard 0.01 $\mu\text{g}/\text{mL}$ detected by high-performance liquid chromatography with diode-array detection (HPLC-DAD)

doi: 10.17221/294/2016-PSE

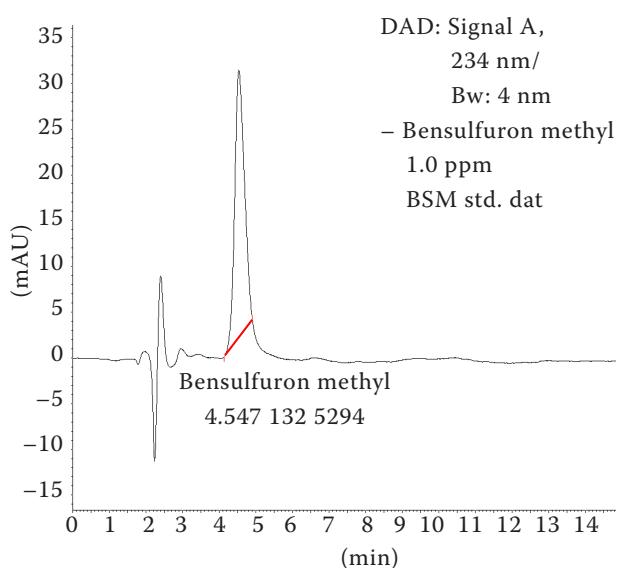


Figure 4. Bensusulfuron methyl standard 1.0 $\mu\text{g}/\text{mL}$ detected by high-performance liquid chromatography with diode-array detection (HPLC-DAD)

the quantification limit of 0.01 $\mu\text{g}/\text{g}$ during kharif 2013 (Table 2 and Figure 5). The quantity of BSM residue detected in soil at post-emergence application is higher (0.017 $\mu\text{g}/\text{g}$) than at pre-emergence (0.011 $\mu\text{g}/\text{g}$) application. The presence of bensusulfuron residues in soil even at the time of harvest suggests that being a weak acid (pK_a of 5.2), it would be relatively stable in the observed high pH of the present experimental field soils (Langeland and Lorache 1994) and hence it persists until harvest. The presence of residue in soil during kharif 2013 below the detectable level might be the results of variation in rainfall and other weather variables (Figure 5). The amount of rainfall received on the first month of bensusulfuron application during kharif 2012 was low when compared to kharif 2013.

This might have enhanced BSM sorption to soil and reduced its leaching or runoff. Hence, it was detected above the quantification limit in soil at harvest. The enhanced dissipation of BSM residue during kharif 2013 from soil could be the result of enhanced hydrolysis and microbial degradation by even distribution of rainfall throughout the cropping period (Figure 1). Afyuni et al. (1997) indicated that 1.1% to 2.3% of applied sulfonyl ureas was lost in runoff during a simulated rainfall event 24 h after herbicide application.

Bensusulfuron methyl had medium organic carbon normalized distribution coefficient (K_{oc}) values (205–567), which indicates that it is moderately mobile (EFSA 2008) and more adsorption could be anticipated for clay soils depending on the soil organic matter and pH. The chemical hydrolysis of BSM to ortho carbo methoxy group and further microbial breakdown is the important route of its degradation in soil (Langeland and Lorache 1994). However, in the present study the possibility for chemical hydrolysis is low due to the alkaline pH of the field water (> 8.5) in which it is highly stable. Hence in the present study, the BSM degradation might have been enhanced by the increased sunshine radiation and day temperature independent of pH as suggested by CDEA (1989) through the formation of photo degradation products like beta-lactic acid via the complete breakdown of phenyl ring at low concentrations. Yordy (1987) reported that in moist field soils, BSM has degraded *via* chemical hydrolytic degradation and microbial processes with a DT_{50} of 3–4 weeks by producing the compounds like methyl 2-(aminosulfonyl methyl) benzoate and 4,6-dimethoxy-2-aminopyrimidine and [1*H*-2,3-benzothiazin-4(3*H*)-one 2,2-dioxide] and CO_2 . Wei and Chen (1995) reported the half-life of 28 days for bensusulfuron in soil with the residue

Table 1. Recovery (%) of bensusulfuron methyl from soil, rice grain, husk and straw

Fortified concentration ($\mu\text{g}/\text{g}$)	Soil	Rice grain	Rice husk	Rice straw
0.01	79.0 \pm 2.10	78.0 \pm 1.19	87.0 \pm 2.92	72.0 \pm 2.87
0.05	91.6 \pm 2.36	78.8 \pm 2.45	89.2 \pm 3.01	71.2 \pm 4.17
0.10	97.4 \pm 2.89	86.7 \pm 1.79	87.4 \pm 3.12	90.8 \pm 3.16
0.50	96.2 \pm 3.19	94.4 \pm 2.91	91.0 \pm 3.94	97.2 \pm 4.27
Average recovery (%)	91.1	84.5	88.7	82.8

Mean of three replications; Values \pm are % standard deviation

Table 2. Residue of bensulfuron methyl ($\mu\text{g/g}$) in soil at harvest

Method of application	Kharif 2012				Kharif 2013			
	pre-emergence		post-emergence		pre-emergence		post-emergence	
Dose	100	200	100	200	100	200	100	200
	(g ai/ha)							
Soil	< LOQ	0.011 ± 7.17	< LOQ	0.017 ± 6.89	< LOQ	< LOQ	< LOQ	< LOQ

LOQ – limit of quantification ($0.01 \mu\text{g/g}$); Values \pm are % standard deviation

of $0.0015 \mu\text{g/g}$ in postharvest soil when it was applied at normal rate. Hence, the continuous and indiscriminate use of BSM may be circumvented to overcome the biomagnifications of its residues in soil environment due to its medium to low mobility nature in soil with organic carbon normalized freundlich coefficient (K_{foc}) of 205–561 mL/g (EFSA 2008).

Obrigawitch et al. (1998) found that the presence of 0.1 g/ha active ingredient of sulfonyl ureas is the threshold dose in soil and would be enough to decrease the yields of even the most sensitive non-target plants. Hence, in the present study, sensitive indicator species namely green gram (*Vigna mungo*) was grown as succeeding crop to assess the phytotoxicity of BSM residue in soil. During both years of study, the residual phytotoxicity was not observed on green gram and the parameters

viz., germination percent, plant height, number of pods per plant and seed yield were found to be unaffected by the time and dose of BSM application. The present results suggest that the risk of BSM carryover to succeeding crops planted following pre- or post-emergence application to rice is low in tropical environment at the normal rate as single application. However, the effect of repeated and continuous application needs to be investigated in wetland rice growing environment.

In conclusion, it can be concluded that the QuEChERS method could be well applied for the BSM extraction from soil and rice parts to the level of below MRL prescribed by different countries. It is observed that the rice grown in soil of neutral to alkaline pH, high sorption capacity and even distribution of high rainfall might lead to less persistence of BSM residues after its pre- or post-emergence single application at normal rate of 100 g/ha . The terminal BSM residue in rice grain, husk and straw was found to be below the maximum residue limit set by the Japan, FSSAI and some European countries when it is applied at the normal rate ranged from $100\text{--}200 \text{ g/ha}$. However, the effect of repeated and continuous application on its dissipation in soil and residues in rice needs to be investigated in wetland rice growing environment as there is a chance for the biomagnifications of herbicide residues in soil and crop.

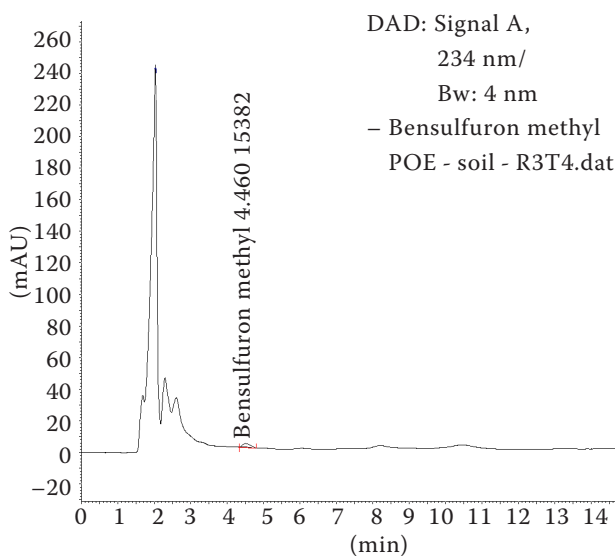


Figure 5. Detection of bensulfuron methyl residue in post-harvest soil during Kharif 2012 by high-performance liquid chromatography with diode-array detection (HPLC-DAD)

Acknowledgements

The authors are grateful to the Directorate of Weed Research, Jabalpur and the Tamil Nadu Agricultural University, India for providing necessary research facilities to carry out the work. The authors declare that they have no conflict of interest regarding this study.

doi: 10.17221/294/2016-PSE

REFERENCES

- Afyuni M.M., Wagger M.G., Leidy R.B. (1997): Runoff of two sulfonylurea herbicides in relation to tillage system and rainfall intensity. *Journal of Environmental Quality*, 26: 1318–1326.
- Anderson R.L., Humburg N.E. (1987): Field duration of chlor-sulfuron bioactivity in the central great plains. *Journal of Environmental Quality*, 16: 263–266.
- Brown H.M. (1990): Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. *Pest Management Science*, 29: 263–281.
- CFDA (1989): Bensulfuron Methyl (Londax). California, California Department of Food and Agriculture.
- Clever J., Sato J. (2011): Japan proposes new MRLs for bensulfuron-methyl cyclanilide and Spinosad. Gain Report Number: JA1044. Dt. 11/1/2011. Available at: [www. http://gain.fas.usda.gov/](http://www.gain.fas.usda.gov/)
- Curran W.S., Knake E.L., Liebl R.A. (1991): Corn (*Zea mays*) injury following use of clomazone, chlorimuron, imazaquin, and imazethapyr. *Weed Technology*, 5: 539–544.
- EFSA (2008): Conclusion on pesticide peer review: Conclusion regarding the peer review of the pesticide risk assessment of the active substance bensulfuron. EFSA scientific report 178: 1–102. Available at: <http://www.efsa.europa.eu/en/efsajournal/doc/178r.pdf>
- FSCA (Food Standards Code of Australia) (2014): Available at: <http://www.foodstandards.gov.au/code/code-revision/Documents/Schedules/78%20-%20Schedule%20%20.pdf>
- FSSAI (2015): Available at: http://www.fssai.gov.in/Portals/0/Pdf/Draft_WTO_Notification_Pesticides_23_11_2015.pdf
- Janaki P., Sundaram K.M., Chinnusamy C., Sakthivel N. (2015): Determination of residues of metribuzin in soil and sugarcane by QuEChERS. *Asian Journal of Chemistry*, 27: 3692–3696.
- Langeland K.A., Lorache F.B. (1994): Persistence of bensulfuron methyl and control of hydrilla in shallow ponds. *Journal of Aquatic Plant Management*, 32: 12–14.
- Moberg W.K., Cross B. (1990): Herbicides inhibiting branched-chain amino acid biosynthesis. *Pest Management Science*, 29: 241–246.
- Naylor R. (1996): *Herbicides in Asian Rice: Transitions in Weed Management*. Palo Alto (California): Institute for International Studies, Stanford University and Manila (Philippines): International Rice Research Institute, 270.
- Niell S.L., Pareja L., Geis Asteggiante M.V., Cesio H.H. (2010): Comparison of extraction solvents and conditions for herbicide residues in milled rice with liquid chromatography-diode array detection analysis (LCDAD). *Food Additives and Contaminants: Part A: Chemistry, Analysis, Control, Exposure and amp. Risk Assessment*, 27: 206–211.
- Obrigawitch T.T., Cook G., Wetherington J. (1998): Assessment of effects on non-target plants from sulfonylurea herbicides using field approaches. *Pest Management Science*, 52: 199–217.
- Priester T. (1985): Metabolism of [2-14C-Pyrimidine] DPX-F5384 in Indica rice grown in a rice paddy ecosystem. DuPont Internal Report, AMR 315–385.
- Roberts T.R., Hutson D.H., Lee P.W., Nicholls P.H., Plimmer J.R. (1998): *Metabolic Pathways of Agrochemicals: Part 1: Herbicides and Plant Growth Regulators*. Cambridge, Royal Society of Chemistry, Thomas Graham House, 483–487.
- Russel M.H., Saladini J.L., Lichtner F. (2002): *Sulfonylurea Herbicides*. Cambridge, Royal Society of 3814 Chemistry – Pesticide Outlook, 166–173.
- Singh R.G., Singh S., Singh V., Gupta R.K. (2010): Efficacy of azimsulfuron applied alone and tank mixed with metsulfuron + chlorimuron (almix) in dry direct seeded rice. *Indian Journal of Weed Science*, 42: 168–172.
- Stidham M.A. (1991): Herbicides that inhibit acetohydroxyacid synthase. *Weed Science*, 39: 428–434.
- Takeda S., Erbes D.L., Sweetser P.B., Hay J.V., Yuyama T. (1986): Mode of herbicidal and selective action of DPX-F5384 between rice and weeds. *Weed Research*, 31: 157–163.
- Usui K., Suwanwong S., Ohno A., Ishizka K. (1993): Zasso Kenkyu, 38: 7–11.
- Vencill W.K. (2002): Bensulfuron methyl. In: *Herbicide*. 8th Ed. Lawrence, Weed Science Society of America.
- Wei C., Chen Z.Y. (1995): Residue of ¹⁴C-bensulfuron (Londax) in rice. *Journal of Ecology and Rural Environment*, 11: 17–18.
- Wu L.Q., Hu L.Y., Xu H., Zhu Y. (2000): Residue study of bensulfuron methyl on rice. *Acta Agriculturae Zhejiangensis*, 12: 393–396.
- Yordy J.R. (1987): Aerobic soil metabolism of DPX-F5384. Dupont Internal Report, AMR 782–787.

Received on April 16, 2016
Accepted on August 8, 2016

Corresponding author:

Assist. Prof. Dr. P. Janaki, Ph.D., Tamil Nadu Agricultural University, Department of Agronomy, 641 003 Coimbatore, India; e-mails: janakibalamurugan@rediffmail.com; janaki.p@tnau.ac.in