Journal of Crop and Weed, 14(1): 212-219 (2018)

Bioassay for detecting flucetosulfuron residue in wetland rice soils

S. R. ARYA, E. K. SYRIAC AND [1]S. K. RAJ

Department of Agronomy, College of Agriculture, Vellayani, Thiruvananthapuram – 695 522, India
[1]Coconut Research Station, Balaramapuram, Thiruvananthapuram – 695 501, India

Received : 02-07-2017 ; Revised : 03-03-2018 ; Accepted : 05-03-2018

ABSTRACT

In order to assess the residue of flucetosulfuron in wetland rice soils, bioassay was conducted in two parts viz., identification of suitable indicator plants for flucetosulfuron and assessment of herbicide residue in post experiment soil using selected indicator plant. To identify indicator plant for flucetosulfuron, pot culture experiments were conducted using four test plants viz.,barnyard millet, cucumber, sunflower and maize. Each plant species was allowed to grow in 8 different concentrations of flucetosulfuron viz., 0, 0.01, 0.05, 0.1, 0.5, 1, 10, 50 and 100 µL L^{-1}. The effect of different concentrations of flucetosulfuron on germination percentage, shoot length, root length, shoot fresh and dry weight of each indicator plant species were recorded. Based on statistical analysis, sunflower was selected as the most sensitive indicator plant for assessing the residual effect of flucetosulfuron, since it recorded the highest regression co-efficient for the parameters tested. Among the various parameters compared, shoot length of sunflower was selected as the most suitable parameter to detect the residue of flucetosulfuron in soil. Logarithmic linear regression equation developed for shoot length of sunflower was Y=4.309788-0.64968 ln (X), R^2= 0.946. Field experiments were carried out with 3 different concentrations (20, 25 and 30 g ha^{-1}) of flucetosulfuron at 3 different times of application (2-3, 10-12 and 18-20 days after sowing) for the Kharif and Rabi seasons of 2016-'17. After each filed experiment, bioassay was conducted in post experiment soil and results revealed that there is no toxic residue of flucetosulfuron in the soil of the experimental plots indicating the safety of the chemical.

Keywords: Bioassay, flucetosulfuron, herbicide residue assessment, indicator plants, sunflower, wet seeded rice

Weeds are the most harmful group of pests and one of the major constraints which affect rice productivity (Bhimwal and Pandey, 2014) adversely if not managed during critical period of crop growth. To bring weeds under control without affecting the yield, adoption of weed management practices at critical periods of crop growth is a necessary. Even though hand weeding is the best method, herbicide based weed management is the smartest and viable option due to scarcity and high wages of labour (Anwar *et al*., 2012). Despite some undesirable side effects, no viable alternative is presently available to shift the chemical dependence for weed management in rice (Juraimi *et al*., 2013). Sulfonyl urea group of herbicides are low dose high efficacy herbicides having acetolactase synthase (ALS) inhibition as mode of action in plants, and are safe for mammals. Flucetosulfuron is such a new generation, pyrimidinyl sulfonylurea, broad spectrum herbicide, odourless white solid, soluble in water, acetone, ethyl alcohol, ethyl acetate, n-hexane and methanol.Even though new generation herbicides are required in smaller quantities, their persistence and safety to the succeeding crop in the herbicide applied field must be analysed thoroughly. The phytotoxic activity of the herbicide molecule can be measured by bioassay method which is cost-effective and do not require expensive equipments like High Performance Liquid Chromatograph (HPLC). Bioassays or biological tests applied to the study of herbicides, are based on the response of different species, chosen as controls, to the application of the herbicide under study (Horowitz, 1976).Bioassay is the simplest and direct method of residue assessment. It possesses several advantages over mechanical or chemical methods of residue assessment like determination of both active or biologically active substance and possible degradation products of the herbicide; being based on the observation of the response of the plants to herbicide, it provides more practical information and materials involved and the methodology is simple with high reproducibility (Günther *et al*., 1993).

Bioassays are usually conducted with sensitive plant species, also called as indicator plants or test species. A plant that can be used as an indicator species must be sensitive enough to detect even very small amounts of herbicide in the soil or another substrate. It must also show a gradual increase in susceptibility with increasing herbicide concentrations. The indicator plant should be vigorous and grow rapidly under the conditions of bioassay. The more commonly used indicator species are cucumber, oats, barnyard grass, sunflower, tomato, barley, sorghum, crab grass (*Cenchrus sanguinalis*), yellow foxtail (*Setaria glauca*) *etc*. The ideal test species must however be determined from preliminary experiments with the herbicides under study (Rao, 2000).

Email: aaryanarayana@gmail.com

In a bioassay, indicator species is grown in herbicide treated soil or in a solution of the herbicide extracted qualitatively from soil or plant tissue. This response is compared with that shown by similar plants grown in untreated soil or extract containing known concentration of the same herbicide selected. This gives responses of the sensitive indicator species ranging from nil to complete death, as the herbicide concentration is increased. Once the dose response curve is established, with known concentration of herbicides, the plant response in soil containing unknown herbicide residue is compared with this curve and the quantity of residue is determined.

Since flucetosulfuron is comparatively a newly developed herbicide and its indicator plant is not yet identified, the first step of the experiment was identification of indicator plant for flucetosulfuron. Then with the identified indicator species, the residue of the herbicide in treated plots could be quantified using dose response curve already developed in the first part. Henceforth, the major objective of the study was to identify the suitable indicator plant for flucetosulfuron and determination of herbicide residue in the flucetosulfuron treated plots.

MATERIALS AND METHODS

The experiment was conducted as two parts; First part for the determination of suitable indicator plant for flucetosulfuron and the second part consists of assessment of herbicide residue in post experiment soil using the selected indicator plant.

The pot culture experiments were conducted in the Department of Agronomy, College of Agriculture Vellayani, Thiruvananthapuram and field experiments were conducted in the farmers' field during *Kharif* and *Rabi* seasons of 2016-'17 in Kalliyoor Panchayat, Nemom block, Thiruvananthapuram district, Kerala, India (8.4455^0 N and 76.9918^{0}E). The soil was Typic haplaustalf under the order *Alfisols.*

Part I: Maize, cucumber, sunflower and barnyard millet , proven indicator plants for bioassay of sulfonylurea herbicides, were taken as the test crops. Separate experiments were done in Completely Randomized Design (CRD), for each test crop. Nine different concentrations of flucetosulfuron *viz*., 0, 0.01, 0.05, 0.1, 0.5, 1, 10, 50 and 100 µL L^{-1} constituted the treatments which were replicated thrice. Herbicide free sand was collected, washed thoroughly and air dried. Sand was taken in plastic pots of 500 mL capacity with 250 g sand in each pot separately. Sand in each pot was fortified with different concentrations of flucetosulfuron. Ten seeds of each test species were dibbled in each plastic pot at uniform depth of 2 cm. At 4 DAS, germination count was taken and plants were thinned to three per pot in order to avoid competition. The moisture content of the pots was maintained at field capacity. At 14 DAS, the plants were uprooted from each pot using a sharp knife, without causing any damage to the roots. Observations were taken on shoot length, root length, fresh and dry weight of shoot. Then the plants were dried in hot air oven at 60^{0}C to constant weight and the shoot dry weight was recorded. The data were subjected to statistical analysis and regression equations were developed. For selecting the most sensitive indicator plant, regression models, both quadratic ($Y= a+ bX + cX^2$) and logarithmic linear regression ($Y= a+b \ln(X)$) equations were fitted and the best was found to be the logarithmic linear regression model, which was used. The test crop which showed the highest R^2 value for all the tested parameters was selected as the best indicator plant and the parameter which showed the highest R^2 value was selected as the best parameter for the bioassay of flucetosulfuron. The response curves were also developed for the tested parameters of the best indicator plant (Rao, 2000).

Part II: Field experiment were conducted for two seasons *viz.*, *Kharif* and *Rabi* seasons of 2016-'17 with 12 treatments under Randomised Block Design, *viz.*,

T_1 : Flucetosulfuron @ 20 g ha^{-1} at 2-3 DAS,
T_2 : Flucetosulfuron @ 25 g ha^{-1} at 2-3 DAS,
T_3 : Flucetosulfuron @ 30 g ha^{-1} at 2-3 DAS,
T_4 : Flucetosulfuron @ 20 g ha^{-1} at 10-12 DAS,
T_5 : Flucetosulfuron @ 25 g ha^{-1} at 10-12 DAS,
T_6 : Flucetosulfuron @ 30 g ha^{-1} at 10-12 DAS,
T_7 : Flucetosulfuron @ 20 g ha^{-1} at 18-20 DAS ,
T_8 : Flucetosulfuron @ 25 g ha^{-1} at 18-20 DAS ,
T_9 : Flucetosulfuron @ 30 g ha^{-1} at 18-20 DAS ,
T_{10}: Bispyribac sodium @ 25 g ha^{-1} at 15 DAS,
T_{11}: Hand weeding at 20 and 40 DAS and
T_{12} : Weedy check.

For the determination of herbicide residue in post experiment soil, the soil samples were collected at a depth of 0-15 cm from each treatment after the harvest of the crop and kept in a suitable container. Seeds of the best indicator plant selected *i.e.*, sunflower was sown in container at the rate of 10 seeds pot^{-1}. At 4 DAS, germination count was taken and plants were thinned to three per pot in order to avoid competition. The moisture content of the pots was maintained at field capacity. At 14 DAS, the plants were uprooted from each pot using a sharp knife, without causing any damage to the roots. Observations were taken on shoot length, root length, fresh and dry weight of shoot. Then the plants were dried in hot air oven at 60^{0}C to constant weight and the shoot dry weight was recorded.These values were compared with the standard curve developed in the first part of the experiment.

Table 1: Effect of different concentrations of flucetosulfuron on growth parameters of tested indicator plants

Treatments	Growth parameters																	
	Barnyard millet					Cucumber				Sunflower				Maize				
	G (%)	SL (cm)	RL (cm)	SFW (g)	SDW (g)	G (%)	SL (cm)	RL (cm)	SFW (g)	SDW (g)	G (%)	SL (cm)	RL (cm)	SFW (g)	SDW (g)	G (%)	SL (cm)	RL (cm)	SFW (g)	SDW (g)
T_1(0 µL L^{-1})	78.33	16.76	8.60	0.30	0.078	73.33	6.63	2.91	1.05	0.085	40.00	8.50	1.78	0.81	0.072	93.33	21.82	13.02	1.37	0.051
T_2(0.01µLL^{-1})	58.33	15.44	7.57	0.22	0.061	70.00	5.93	1.81	0.84	0.059	36.67	7.20	1.77	0.80	0.051	83.33	17.64	11.51	1.02	0.040
T_3(0.05 µLL^{-1})	41.67	5.64	3.63	0.07	0.020	66.67	5.32	1.67	0.43	0.014	36.67	6.60	1.63	0.64	0.041	76.67	17.79	5.86	0.82	0.033
T_4(0.1 µLL^{-1})	41.67	6.36	5.60	0.07	0.015	60.00	4.79	1.32	0.34	0.014	36.67	5.67	1.77	0.58	0.039	76.67	17.86	6.62	0.38	0.026
T_5(0.5 µLL^{-1})	23.33	2.51	3.13	0.07	0.010	50.00	2.86	1.07	0.22	0.012	26.67	4.93	1.47	0.48	0.032	73.33	15.94	4.83	0.09	0.005
T_6(1 µLL^{-1})	6.67	0.92	1.67	0.05	0.007	46.67	2.41	0.65	0.20	0.009	26.67	3.60	0.77	0.24	0.007	40.00	8.78	3.84	0.06	0.004
T_7(10 µLL^{-1})	16.67	0.47	0.61	0.03	0.004	30.00	1.56	0.37	0.14	0.007	26.67	3.33	0.63	0.22	0.003	23.33	6.84	3.25	0.05	0.003
T_8(50 µLL^{-1})	6.67	0.16	0.20	0.02	0.004	20.00	1.31	0.25	0.12	0.005	20.00	2.40	0.55	0.15	0.002	13.33	2.39	2.52	0.03	0.001
T_9(100 µLL^{-1})	6.67	0.11	0.09	0.01	0.001	13.33	0.23	0.15	0.09	0.004	16.67	0.60	0.17	0.12	0.002	10.00	2.80	0.98	0.02	0.000
SEm(±)	*	**0.602**	**0.274**	**0.019**	**0.005**	*	**0.402**	**0.204**	**0.080**	**0.004**	*	**0.709**	**0.093**	**0.082**	**0.006**	*	**1.007**	**0.571**	**0.112**	**0.004**
LSD (0.05)		**1.032**	**0.814**	**0.032**	**0.014**		**1.195**	**0.605**	**0.238**	**0.013**		**2.106**	**0.275**	**0.244**	**0.018**		**2.990**	**1.696**	**0.333**	**0.013**

Note: G%- Germination percentage, SL- Shoot length, RL- Root length, SFW- Shoot fresh weight, SDW- Shoot dry weight

RESULTS AND DISCUSSION

The effect of different concentrations of flucetosulfuron on different growth parameters of the test crops, *viz.*, germination percentage, shoot length, root length, shoot fresh weight and shoot dry weight are given in table 1. Data on germination percentage was not statistically analysed since gradual variation was not observed in the data corresponding to different concentrations of flucetosulfuron in the pot.

Barnyard millet

As the concentration of flucetosulfuron increased, shoot length, root length, shoot fresh weight and shoot dry weight reduced significantly. Logarithmic linear regression equations were developed for the above parameters of barnyard millet.

Cucumber

Significant reduction was observed in the shoot length, root length, shoot fresh weight and shoot dry weight with increase in concentration of flucetosulfuron, in the case of cucumber also. Logarithmic linear regression equations were developed for each tested parameter of the crop.

Sunflower

As the concentration of flucetosulfuron increased, a corresponding decrease was observed in the shoot length, root length, shoot fresh weight and shoot dry weight of sunflower also. Logarithmic linear regression equations were developed for the above said parameters of sunflower.

Maize

Similar to other test crops, in maize also significant reduction was observed in parameters *viz*., shoot length, root length, shoot fresh weight and shoot dry weight with increase in concentration of flucetosulfuron. Logarithmic linear regression equations were developed for the above said parameters of maize also.

Table 2: R^2 values of different parameters of tested indicator plants, Y= a + b ln (X) for identifying the most sensitive indicator plant for flucetosufuron

Crop	R^2 values for different parameters			
	Shoot length	Root length	Shoot Fresh weight	Shoot Dry weight
Barnyard millet	0.7067	0.8632	0.6662	0.6085
Cucumber	0.9377	0.9456	0.7390	0.5455
Sunflower	0.9462	0.9280	0.9200	0.8813
Maize	0.9364	0.8304	0.7084	0.7927

Table 3: Residual effect of flucetosulfuron on growth parameters of sunflower (*Kharif* and *Rabi* seasons of 2016-2017)

Treatments	First crop					Second crop				
	Growth parameters of sunflower					Growth parameters of sunflower				
	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
T_1	43.33	6.63	1.57	0.61	0.046	36.67	7.20	1.30	0.69	0.054
T_2	36.67	5.83	1.23	0.79	0.048	43.33	6.37	1.33	0.70	0.057
T_3	36.67	6.07	1.77	0.54	0.040	40.00	6.23	1.13	0.80	0.058
T_4	43.33	6.97	1.53	0.81	0.047	40.00	6.00	1.80	0.63	0.053
T_5	40.00	5.53	1.30	0.84	0.052	40.00	6.53	1.43	0.83	0.052
T_6	50.00	5.30	1.40	0.63	0.043	36.67	6.23	1.70	0.65	0.047
T_7	36.67	6.07	1.63	0.91	0.053	46.67	6.47	1.07	0.80	0.046
T_8	30.00	6.30	1.47	0.84	0.052	33.33	6.33	1.63	0.79	0.059
T_9	40.00	5.70	1.70	0.95	0.055	36.67	6.13	1.40	0.76	0.055
T_{10}	46.67	6.13	1.47	0.78	0.055	33.33	6.23	1.63	0.90	0.055
T_{11}	33.33	6.10	1.53	0.71	0.054	40.00	6.47	1.80	0.84	0.053
T_{12}	46.67	6.10	1.60	0.69	0.061	33.33	6.33	1.60	0.91	0.066
SEm(±)	**5.500**	**0.765**	**0.208**	**0.090**	**0.0054**	**4.532**	**0.546**	**0.220**	**0.102**	**0.0056**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

The percentage inhibition of shoot length, root length, shoot fresh weight and dry weight of sunflower corresponding to different concentrations of the herbicide flucetosulfuron is given in figures 1-4.

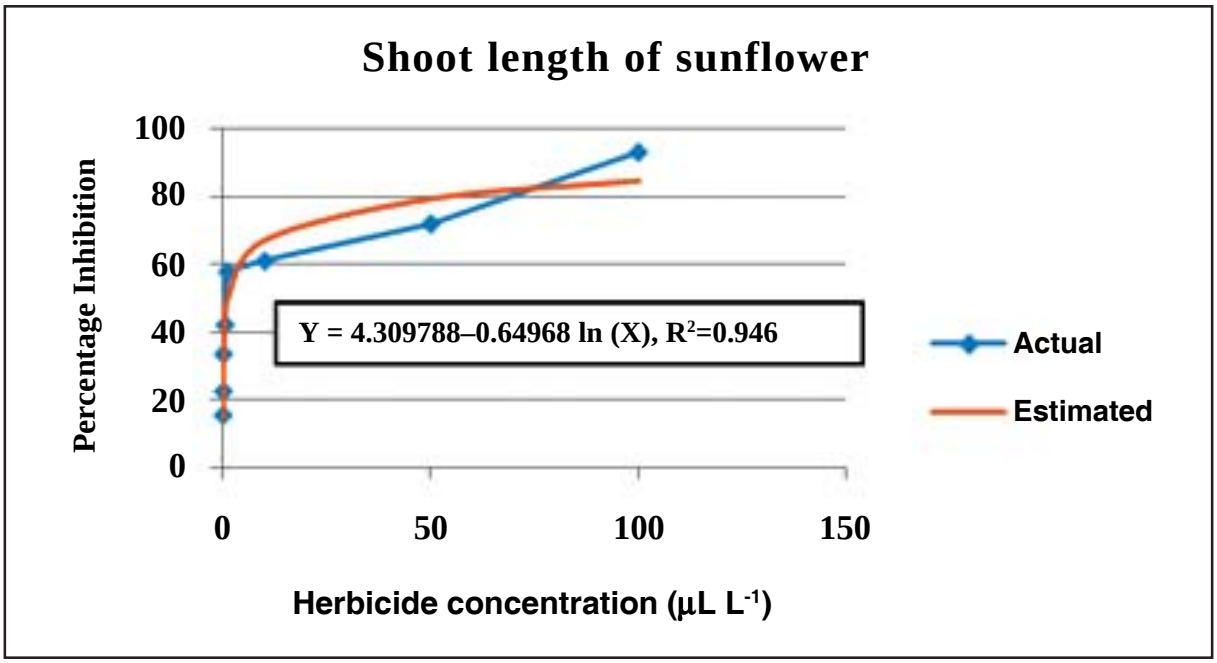

Fig. 1: Percentage growth inhibition in the shoot length of sunflower as influenced by the herbicide flucetosulfuron

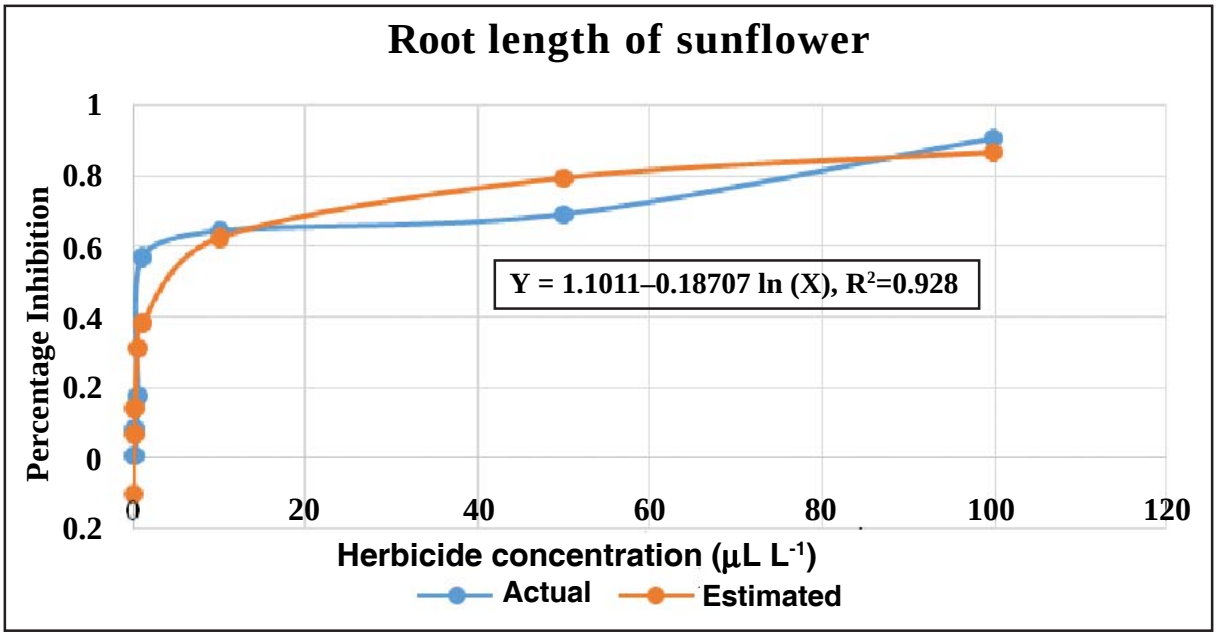

Fig. 2: Percentage growth inhibition in the root length of sunflower as influenced by the herbicide flucetosulfuron

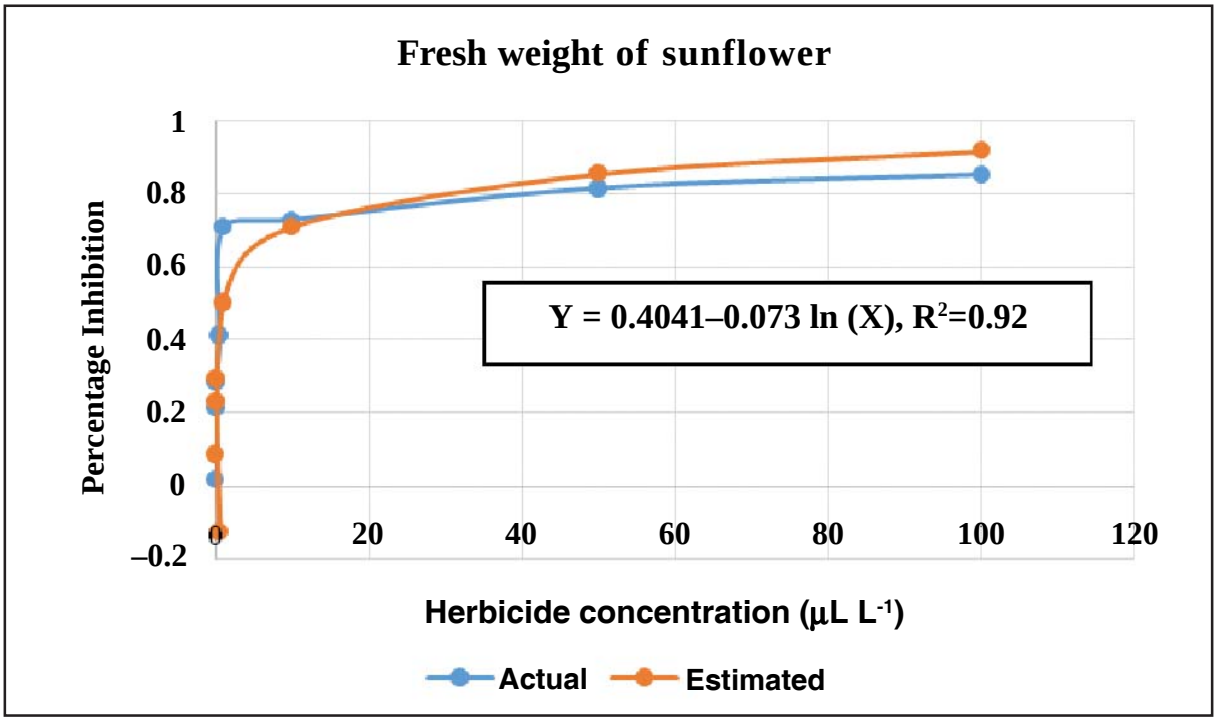

Fig. 3: Percentage growth inhibition in the shoot fresh weight of sunflower as influenced by the herbicide flucetosulfuron

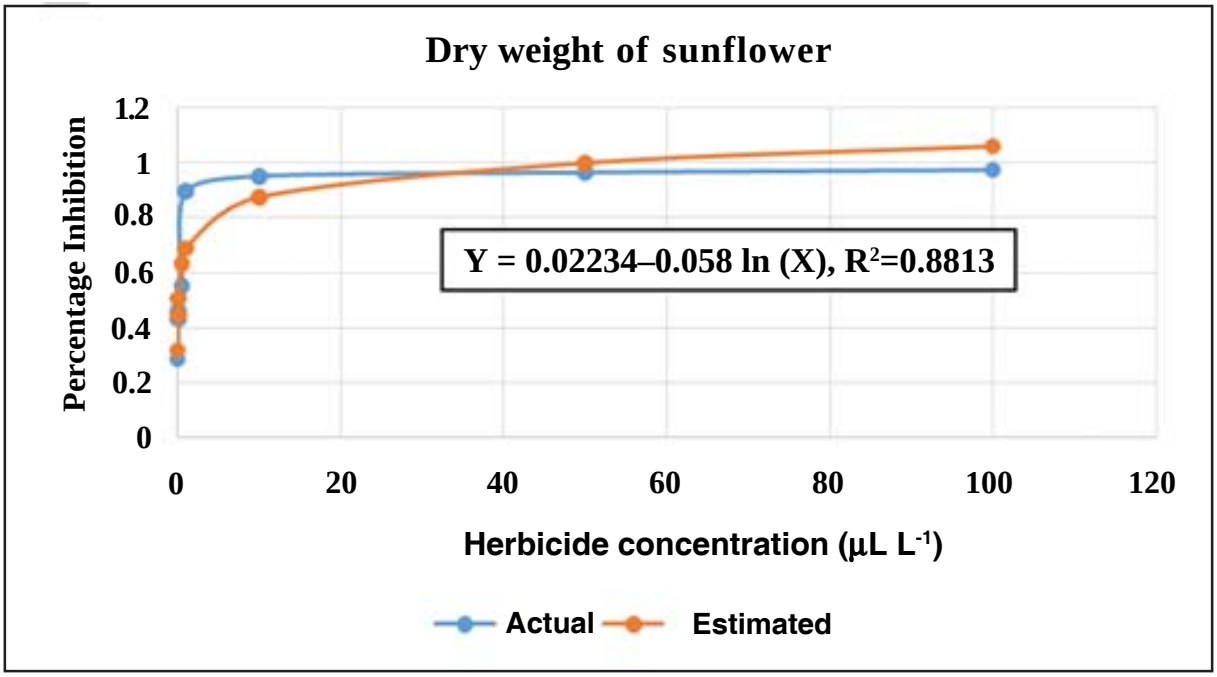

Fig. 4: Percentage growth inhibition in the shoot dry weight of sunflower as in fluenced by the herbicide flucetosulfuron

Among the indicator plants screened *viz.*, barnyard millet, cucumber, sunflower and maize, sunflower was selected as most suitable indicator plant for identifying the residue of flucetosulfuron in soil, since it recorded the highest R^2 value for shoot length (0.9462), fresh shoot weight (0.9200) and dry shoot weight (0.8812). Regression equations were developed for these parameters by plotting the values against the herbicide concentrations in logarithmic scale. Shoot length was selected as the best parameter for the detection of herbicide residue in soil, as it recorded the highest R^2 value (0.9462) among the tested parameters (Table-2) and the log linear regression equation, Y= 4.309788 – 0.64968 ln (X), R^2= 0.946 was developed. Cucumber was reported as the most sensitive indicator plant for bromacil (Leela, 1981); fluchloralin and pendimethalin (Jayakumar *et al*., 1985). For flucarbazone and sulfentrazone herbicides, sugar beet was identified as the most suitable indicator plant (Szmigielski *et al*., 2012). Sunflower was identified as the most sensitive indicator plant for residue studies of trisulfuron (Hernandez *et al*., 2001), chlorimuron and metsulfuron (Castro *et al*., 2002). Alonso-prados *et al*. (2002) also reported the sensitivity of sunflower to sulfonylurea residues. Several bioassay methods for sulfonylurea herbicides have been reported using lentil (*Lens culinaris* Med.), lettuce (*Lactuca sativa* L.), sunflower (*Helianthus annuus* L.), corn (*Zea mays* L.), pea (*Pisum sativum* L.) and lupin (*Lupinus angustifolius* L.). In some studies, plant height or dry or fresh weight has been found to be a sensitive response parameter to sulfonylurea exposure (Stork and Hannah, 1996). However, according to Yadav (2006), cucumber was the most sensitive indicator plant and its shoot length was identified as the best parameter to estimate the residue of pyrazosulfuron ethyl in soil. The percentage reduction in shoot length, root length, shoot fresh weight and shoot dry weight is given in fig.1-4.

Residual effect of flucetosulfuron in post-harvest soil

The effect of residual flucetosulfuron in post-harvest soil on growth parameters of sunflower is given in table-3. The results revealed that there was no significant difference among the treatments on the different growth parameters compared *viz.*, germination percent, shoot length, root length, shoot fresh weight and shoot dry weight indicating that the herbicide flucetosulfuron does not leave residues in the soil after the crop. Henceforth, application of flucetosulfuron will not cause any harmful effects on the succeeding crop. The results are in conformity with the findings of Raj (2016) according to whom the herbicide mixtures *viz.*, byspiribac sodium+ metamifop and penoxulam + cyhalofop butyl did not have any toxic residual effect in wet seeded paddy soil. Shaban *et al.* (2016) reported that maize fields treated with Acetochlor, Sulcotrione, Metribuzin and Pendimethalin at different rates could be sown with wheat or faba bean after harvest without any harmful effect. Naik *et al.* (2004) evaluated the residual effect of the pre-emergence herbicides *viz.*, alachlor, oxyfluorfen, metalochlor and butachlor applied to garlic on cucumber. The germination of cucumber seeds was not affected by the herbicides and root and shoot lengths did not significantly vary with the herbicide level, suggesting that the herbicides did not persist in the soil after harvest of garlic.

Herbicide residue analysis is getting more importance nowadays, in the aspect of environmental safety. Bioassay is one of the most important, efficient and cost effective techniques for assessing herbicide residue in soil. The indicator plant used for bioassay must be sensitive to minute quantities of the herbicide and for each herbicide the suitable indicator plant should be standadized. For the herbicide flucetosulfuron, the recent addition in the array of sulfonyl urea herbicides for rice, sunflower was identified as the best indicator plant among the tested crops, *viz.*, barnyard millet, cucumber, sunflower and maize, and shoot length of sunflower was adjudged as the best parameter to assess the herbicide residue in soil. From the results obtained after the bioassay of each season field experiment using sunflower, it is clear that there is no residual toxicity of flucetosulfuron in post experiment soil since the growth parameters of sunflower was not significantly influenced by residual flucetosulfuron. Since the new generation herbicide flucetosulfuron leaves no toxic residue in the wetland paddy fields, it can be recommended for use in rice based cropping system.

REFERENCES

Alonso-Prados, L.J. E,. Hernandez-Sevillano, S., Llanos, M., Villarroya and Garcia-Baudin, J.M. 2002.Effects of sulfosulfuron soil residues on barley(*Hordeum vulgare*), sunflower (*Helianthus annuus*)and common vetch (*Vicia sativa*). *Crop Prot.***21**: 1061-66.

Anwar, M. P., Juraimi, A. S., Puteh, A., Man, A. and Rahman, M. M. 2012. Efficacy, phytotoxicity and economics of different herbicides in aerobic rice. *Acta Agric. Scandin.* **62**: 604-15.

Bhimwal, J. P. and Pandey, P. C. 2014. Bio-efficacy of new herbicide molecules for broad spectrum weed control in transplanted rice (*Oryza sativa* L.). *Bioscan.***9**(4): 1549-51.

Castro, M.C., Bedmar, F., Monterubbianesi, M.G., Peretti, A. and Barassi,C. 2002. Determination of chlorimuron and metsulfuron residues in two soils of Argentina using a rapid seed-bioassay. *J. Env. Biol.*,**23**(4): 353-358.

Günther, P., Pestemer, W., Rahman, A., and Nordmeyer, H. 1993. A bioassay technique to study the leaching behaviour of sulfonylurea herbicides in different soils. *Weed Res*., **33**:177-185.

Hernandez, S.E., Villarroya, M., Alonso, P.J.L., and Garcia, B.J.M. 2001. Bioassay to detect MON-37500 and triasulfuron residues in soils. *Weed Technol*. **15**: 447-452.

Horowitz, M. 1976. Application of bioassay techniques to herbicide investigations. *Weed Res.***16**: 209-215.

Jayakumar, R., Ali, A.M., and Subramanian, S. 1985. Residual effects of dinitroaniline herbicides (fluchloraline and pendimethalin) in irrigated *Arachis hypogaea*var. Pol.2. *Madras Agric. J.***72**:286-288.

Juraimi, A. S., Uddin, Md. K., Anwar, Md. P., Mohamed, M. T. M., Ismail, M. R., and Man, A. 2013. Sustainable weed management in direct seeded rice culture: A review. *Aus. J. Crop Sci*.**7**(7): 989- 1002.

Leela, D. 1981. Biomass for detection of soil residues. *Pesticides* **15**: 24-26.

Naik, A. H. K., Muniyappa, T. V., and Naik, D. C. 2004. Weed control, crop toxicity ratings and quantification of herbicide persistence in alfisols through bioassays. *J. Ecobiol.***16**(3): 201-206.

Raj, S. K. 2016. Herbicide mixtures for weed management in direct seeded puddled rice. *Ph.D. Thesis*, Kerala Agricultural University, Thrissur, 312p.

Rao, V. S. 2000. *Principles of Weed Science*. Oxford and IBH Publishing Co. Pvt. Ltd. pp. 500-503.

Shaban, A.,. Safina, A., Yehia, R., Rasha, G. M., and Abo El-Hassan. 2016. Effect of some herbicides on quality of maize grains and the following winter crops. *Egypt . J Appl. Sci*., **31** (1): 1-14.

Stork, P. and Hannah, M. C. 1996. A bioassay method for formulation, testing and residue studies of sulfonyl urea and sulfonanilide herbicide. *Weed Res.***36**: 271-281.

Szmigielski, A.M., Schoenau, J.J., and Johnson, E.N. (2012) Use of sugarbeet as a bioindicator plant for detection of flucarbazone and sulfentrazone herbicides in soil. Available: http://www.intechnopen.com/download/pdf.2599 [17-11-2016].

Yadav, P.I.P. 2006.Bioefficacy and residual effect of the new generation herbicide pyrazosulfuron ethyl in transplanted rice. *Ph.D. Thesis*, Kerala Agricultural University, Thrissur, 207p.