Indian Journal of Weed Science 40 (3&4) : 166-169, 2008

Using chlorophyll fluorescence to study the effect of sulfosulfuron and surfactants on little seed canary grass

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ABSTRACT

Chlorophyll fluorescence has been used widely to detect the effect of herbicides in crops and weeds as it is a simple, sensitive and non-destructive method. Among the chlorophyll fluorescence parameters, maximum quantum efficiency, Fv/Fm, can be used to study the effects of herbicides as well as to monitor the development of herbicide resistance in weeds. In the present study, an attempt was made to assess the effectivity of sulfosulfuron, a sulfonylurea herbicide, in controlling *Phalaris minor,* a notorious weed of wheat crop in the Indo-Gangetic plains. Sulfosulfuron is very effective in controlling the isoproturon resistant population of *P. minor*. Differences in Fv/Fm values were observed among the control and treated plants within a week after treatment.

Key words: Chlorophyll fluorescence, Maximum quantum efficiency, Sulfosulfuron, *Phalaris minor.*

Screening for herbicide efficacy in the green house or in the field is usually a lengthy process. A wide range of analytical methods is available to detect phytochemical changes, such as chromatographic techniques coupled with various detectors (Bringmann *et al.* 1999). However, these methods are time-consuming owing to sample preparation, including extraction and filtration, are complicated to perform and require expensive apparatus. Several novel methods have been implemented in an attempt to shorten the evaluation time. If it was possible to apply a cost effective method shortly after exposure to herbicide, the cost of screening experiments would be reduced. One of the most frequently used method for monitoring the status of photosynthetic apparatus in plants involves measurement of *in vivo* chlorophyll fluorescence. This parameter is effective in providing a snapshot of the physiological status of the plant being exposed to various stress factors. It can also be used for the measurement of plant response to herbicides with various modes of actions including the PS-II inhibitors. It has potential to be used as a tool to indicate future death of grass species due to herbicide exposure by observing early-warning effects.

Wheat is one of the world's most extensively cultivated food crops. In India, wheat is the second key staple food after rice. Acclimatization of high yielding dwarf varieties of wheat has made a remarkable increase in wheat production, particularly in the Indo-Gangetic plains. However, these high yielding varieties are raised under resource rich environment they face a problem of severe competition from weeds. Among weed flora that infests the wheat crop, *Phalaris minor* (common name: little seed canary grass), is one of the most important noxious weed infesting wheat crop in north-western Indo-Gangetic plains of India. It has a fast initial growth and hence suppresses the growth of wheat crop to a greater extent. Isoproturon, a urea herbicide was effectively used to control *P. minor* for quite a long time till reports of resistance against herbicide were reported from Punjab and Haryana (Malik and Singh, 1995). Due to resistance, the control of *Phalaris minor* dropped from an impressive 78 % to a bleak 27% within a time span of 3 years (1990-93), causing yield loss to the tune of 40-60% in affected areas. The affected area ranges between 0.8 and 1.0 million hectares in N-W India, mostly contained in the states of Punjab (0.3 m ha) and Haryana (0.5-0.6 m ha). Sulfosulfuron, a sulfonylurea herbicide, has successfully been used by the farmers in affected areas to control the isproturon resistant *P.Minor*. This herbicide acts as an inhibitor of acetolactate synthase (ALS) or AHAS, inhibiting the biosynthesis of essential amino acids viz., valine, leucine, isoleucine and hence, stops cell division. After some period, reports of regeneration of *P. minor* in sulfosulfuron treated plots. There are reports of resistance to the particular herbicide in North Korea (Foes *et al.* 1999). In present investigation an effort has been made to develop a fast and rapid method for detection of the effect of sulfosulfuron, a sulfonylurea herbicide, on *Phalaris minor* and wheat.

MATERIALS AND METHODS

The experiment was carried out at the crop research centre (CRC), Govind Ballabh Pant University of Agriculture, Pantnagar during the winter season of 2004-2005. Wheat variety 2382 was used in the experiment. Twelve treatments of the herbicide sulfosulfuron, consisting of three different doses *viz.,* half (12.5 g/ha), normal (25 g/ha), and double dose of herbicide (50 g/ha) along with surfactants Safal or Active-80 at1250 ml/ha, were applied to the field 35 days after sowing (DAS). The experiment was laid out in a randomized block design (RBD) with three replications. The spraying was done manually with knapsack sprayer fitted with flat fan nozzle delivering 500 l/ha. Chlorophyll fluorescence yield from wheat and *Phalaris minor* leaves was measured at 0, 24, 48, 96h, and 15 days after herbicide application, using a portable chlorophyll fluorometer (Handy–PEA, Hansatech Instruments, King's Lynn, Norfolk, UK), which emits a light of 650 nm wavelength with an intensity of 3000 µmol photons/ m^2s. The measurement was done on dark adapted leaves.

RESULTS AND DISCUSSION

In *P. minor* leaves, F_v/F_m values recorded immediately after herbicide application were similar in both control and herbicide treated plants when used with surfactant 'Safal' (Table 1). Thereafter, at 24 and 48h, a significant decrease in F_v/F_m was observed (0.760 and 0.717, respectively) at higher dose of the herbicide (50 g/ha) as compared to the control plants (0.816). Beyond that, at 96 h and 15d F_v/F_m values were greatly reduced, at normal (25 g/ha) and double dose (50 g/ha) of the herbicide. The values, at 96h were 0.685, 0.650 and at 15d 0.665, 0.546 at normal and double dose of the herbicide, respectively, compared to 0.807 for control plants. In case of wheat plants, no significant changes in F_v/F_m values were observed in different treatments at different time intervals.

Table 1. Maximum quantum efficiency (F_v/F_m) of *Phalaris minor* and wheat plants at different time intervals after application of sulfosulfuron with surfactant, Safal (S_1).

Doses of sulfosulfuron	*Phalaris minor*					Wheat				
	0h	24h	48h	96h	15d	0h	24h	48h	96h	15d
Control	0.826	0.816	0.798	0.807	0.791	0.826	0.771	0.811	0.821	0.802
Conrotl+S_1	0.822	0.789	0.783	0.801	0.810	0.826	0.790	0.806	0.783	0.787
12.5+ S_1	0.815	0.786	0.777	0.760	0.671	0.820	0.773	0.790	0.787	0.796
25+S_1	0.802	0.780	0.749	0.685	0.665	0.816	0.781	0.777	0.800	0.796
50+S_1	0.809	0.760	0.717	0.650	0.546	0.821	0.783	0.783	0.798	0.790
LSD(P=0.05)	0.026	0.044	0.050	0.0220	0.0250	0.500	0.544	0.020	0.500	0.0140

h - hour

With the second surfactant i.e. Active-80, F_v/F_m values of *Phalaris minor* leaves did not differ significantly up to 48 h among all the treatments (Table 2). Thereafter, at 96 h and 15 days, significant decreases in F_v/F_m values (0.670-0.697 and 0.610-0.673, respectively) were observed at normal and double doses of the herbicide. In case of wheat plants, however, F_v/F_m values were similar in both control and treated plants at all stages of development.

According to the Bjorkman and Demming (1987), or Frachbout and Leipner (2003) if F_v/F_m values are in between 0.700-0.830, efficiency of PS-II is not affected. Because of its robustness, F_v/F_m has been widely used to quantify stress-induced perturbations in the photosynthetic apparatus, since a decrease in F_v/F_m may be caused by development of slowly quenching processes and damage to PS-II reaction centre, both of them reducing the maximum quantum efficiency of PS-II photochemistry. In the present study, reduction in F_v/F_m values in *P. minor* plants at 48h-15d after herbicide application indicates injury to the photosynthetic apparatus. There are evidences that many inhibitors of metabolic processes that are not directly involved in photosynthetic metabolism can modify fluorescence kinetics (Blowers 1989, Percival and Baker 1991). Kirkwood *et al.* 2000 also reported similar findings and found that glyphosphate caused large changes in F_v/F_m values after 1 day.

Table 2. Maximum quantum efficiency (F_v/F_m) of *Phalaris minor* and wheat plants at different time intervals after application of sulfosulfuron with surfactant, Active-80 (S_2).

Doses of sulfosulfuron	*Phalaris minor*					Wheat				
	0h	24h	48h	96h	15d	0h	24h	48h	96h	15d
Control	0.826	0.816	0.798	0.807	0.791	0.826	0.771	0.811	0.821	0.802
Control+S_2	0.810	0.812	0.782	0.792	0.785	0.795	0.767	0.805	0. 812	0.795
12.5+ S_2	0.794	0.809	0.780	0.743	0.722	0.810	0.777	0.783	0.796	0.779
25+S_2	0.801	0.789	0.777	0.697	0.673	0.820	0.770	0.787	0.773	0.786
50+S_2	0.806	0.775	0.767	0.670	0.610	0.830	0.765	0.796	0.789	0.790
LSD (P=0.05)	0.026	0.044	0.050	0.022	0.025	0.500	0.544	0.020	0.500	0.0140

h - hour

Dry matter production and per cent regeneration of *P. minor* plants treated with different doses of sulfosulfuron along with two surfactants is presented in Figure 1 and Figure 2, respectively. Weed dry matter was maximum (556.68 and 410 g/m^2) in the control plots. It was reduced significantly when treated with different doses of sulfosulfuron. The dry matter of *P. minor* in sulfosulfuron treated plots ranged between 100-133.3 g/m^2 when used with the surfactant 'Safal' and between 190-113.3 g/m^2 when used with Active-80. However, there were no significant differences in weed dry matter among the different doses of sulfosulfuron. Figure 2 shows regeneration of *P. minor* plants at different doses of herbicide applied with both the surfactants. No regeneration was noticed when surfactant Safal was used. However, with the surfactant Active-80, 20-40% regeneration was noticed at half and normal dose of the herbicide, respectively.

Reduction in dry matter of *P. minor* was observed as a result of treatment with sulfosulfuron. This is in conformity with earlier reports that sulfosulfuron reduces the dry matter of weeds (Brar *et al.* 2002, Saha *et al.* 2003, Singh and Kundra 2003). The present study shows that surfactants play an important role in the effectiveness of herbicide on *P. minor* and different types of surfactants will try to affect its efficacy. In this case, the efficacy of herbicide applied along with sufactant, Safal (S_1) was seem to be higher than when surfactant, Active-80 (S_2) was used.

Regeneration of *P. minor* in sulfosulfuron treated plants has been reported in wheat crop. The present study again confirms that. As regeneration was low or totally absent at higher dose, it indicates that in future, the recommended dose of the herbicide may be increased.

On the basis of data presented in this study , measurement of maximum quantum efficiency (F_v/F_m) as early as between 48-96h of the herbicide application can be used to assess the effectively of sulfosulfuron as well as that of surfactants.

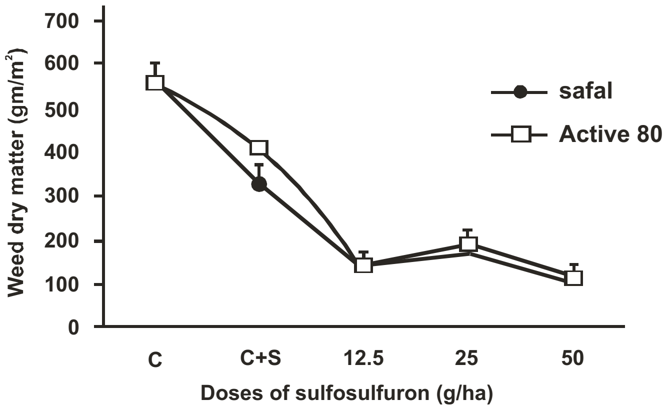

Fig. 1. Dry matter production of *P. minor* treated with different doses of sulfosulfuron along with surfactants, safal (S_1) and active-80 (S_2).

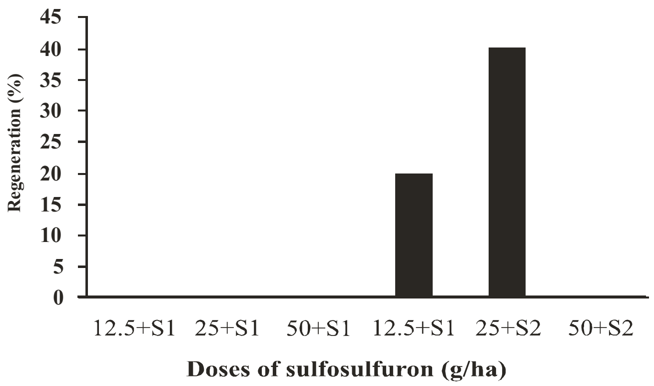

Fig. 2. Regeneration (%) of *P. minor* treated with different doses of sulfosulfuron along with surfactants, safal (S_1) and active-80 (S_2).

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