FLUXOFENIM USED AS A SAFENER ON SORGHUM SEED FOR S-METOLACHLOR HERBICIDE

FLUXOFENIM EM SEMENTES DE SORGO COMO PROTETOR AO HERBICIDA S-METOLACHLOR

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ABSTRACT: This study aimed to evaluate the efficiency of protective fluxofenim seed treatment of sorghum hybrids DKB510 and SCG340, in order to increase the selectivity to the herbicide S-metolachlor applied pre-emergence and to determine the activity of detoxification enzyme glutathione S-transferase (GST). This work was divided into two stages. The first step consisted of field evaluation of the effectiveness of the shield to reduce visual symptoms of plant injury caused by the herbicide and the second determined the activity of GST. It was compared the susceptibility of sorghum seeds to the herbicide by means of visual assessment of injuries at 3, 7, 15 and 30 days after emergence (DAE), root dry weight and shoot at 10 DAE, and determination of activity GST. The treatments were: application of the safener dose at 0 and 40 mL per 100 kg of seed, and spraying of the herbicide S-metolachlor at rates of 1,440 and 2,880 g a.i. ha^{-1}, and a control without herbicide. The safener use to seed treatment for both sorghum hybrids (DKB510 and SCG340) increased tolerance to the herbicide S-metolachlor in two doses, and the best results were obtained at a dose of 1,440 g ha^{-1}. The GST enzyme activity showed an increase when using the fluxofenim prior to application of the herbicide S-metolachlor at a dose of 1,440 g ha^{-1} for the two hybrids.

KEYWORKS: Herbicide. Detoxification. Selectivity. *Sorghum bicolor.* S-tranferase.

INTRODUCTION

The slow development of the sorghum plants (*Sorghum bicolor* L. Moech.) in the early stages of growth makes it susceptible to interference of weeds, since the later ones exhibit fast germination and emergence, as they can use the resources of the environment in advance. Sorghum plants are generally little tolerant to graminicides herbicides of pre-emergence application and the damage caused by the application of these herbicides can be severe.

Among the pre-emergence applied herbicides, metolachlor, belonging to the group of acetanilide, has been widely studied, but its action mechanism is not fully known (KARAM, 2003). The acetanilides have been described as inhibiting the synthesis of lipids, fatty acids, leaf waxes, terpenes, flavonoids, proteins and cell division and also by interfering in the hormonal regulation (LIEBL, 1995). Thus the acetanilides are growth inhibitors of the apical and root meristem, the sensitive plants are killed before the emergency, without inhibiting the germination of seeds and immediate halt of growth. On the other hand, the root growth is less sensitive than the shoot growth (VIDAL, 1997).

A mechanism that can check for levels of weed resistance to herbicides is the metabolism of the herbicide, which occurs when the resistant plant has the capacity to break down the herbicide molecule faster than the sensitive plants, making it inactive by hydrolysis or oxidation. The glutathione S-transferases (GST) are considered as detoxification enzymes and were discovered by their ability to metabolize a wide variety of toxic exogenous compounds (xenobiotics) via conjugation with glutathione (MANNERVIK; DANIELSON, 1988; XU et al., 2002). The conjugates are generally inactive, more hydrophilic, less mobile in the plant and more susceptible to processes that lead to their detoxification (EDWARDS et al., 2005).

Some chemical agents, known as safeners, can be used to reduce the intoxication of plants by herbicides through a physiological or molecular mechanism, without interfering in weed control. Significant changes in the increased tolerance of some crops conferred by safeners to the use of certain chemical groups of herbicides can be achieved either through the use of safener during seed treatment, as in mixing the active ingredient in the herbicide applied pre or post-emergence through a single commercial product.

Safeners of various chemical classes have been used to improve herbicide tolerance in corn,

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sorghum, rice, and other cereals. The fluxofenim is a safener derivative of oxime ether (DAVIES; CASELEY, 1999; HATZIOS, 2000), used commercially as sorghum safener in some countries, and its use leads to a reduction in intoxication of S-metolachlor herbicide to the crop (ROSINGER; KÖCHER, 2007).

Safeners may also increase the use by a variety of mechanisms, by a greater number of active ingredients on crops, by the increased activity of P-450 enzymes (BURTON; MANESS, 1992), GSTs (MOZER et al., 1983; IRZYK; FUERST, 1993), cysteine synthase (HIRASE; MOLIN, 2001), glycosyltransferase (LAMOUREUX; RUSSNESS, 1992) and by raising the levels of glutathione (FARAGO et al.,1994). The carrier vacuolar activities are increased by the safeners (HATZIOS; BURGOS, 2004), besides increasing the rates of GSH conjugation to the herbicides applied in the crop, accelerating the speed of the detoxification process of the herbicides in plants (RIECHERS et al., 1996).

The understanding of the functioning and interaction between seed safeners and herbicides is essential to improve the efficiency of herbicides in weed control and selectivity of these molecules to agricultural crops in order to avoid the appearance of resistant weeds due to intensive spraying of herbicides with the same action mechanisms. Therefore, the objective of this study was to evaluate the effect of using fluxofenim as a safener to the application of different doses of S-metolachlor herbicide and its reflection on the activity of GST enzyme in sorghum crop.

MATERIAL AND METHODS

This study was carried out in two stages; the first one was made in the field in order to visually evaluate the plants according to toxicity caused by the application of S-metolachlor herbicide and compare the seed treatments with fluxofenim on sorghum plants. The second stage was carried out in the laboratory to verify the safener effect on the GST enzyme.

The experiments, one for each hybrid of sorghum for evaluation of injuries, were carried out and evaluated at Experimental Farm, as a field search, belonging to Syngenta Seeds in Uberlândia city, State of Minas Gerais, Brazil, located at Latitude 18° 91’ 86’’S, Longitude 48°17’19’’ WGr., altitude of 925 meters and, set up in 2006. The soil is classified as Red-yellowish Latossol and chemical and structural analysis is presented in Table 1. The evaluated sorghum hybrids were DKB510 and SCG340, produced by the seed companies Monsanto and Dow AgroSciences, respectively.

Table 1. Chemical components of soil fertility and soil structural analysis of the experimental area, Uberlândia/MG, Brazil.

Chemical analysis												
pH	Pmeh^{-1}	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	SB	t	T	V	m	M.O.
Water	mg dm^{-3}		Mmol$_c$ dm^{-3}							%		dag kg^{-1}
6.4	53.7	135	40	16	0	20	59	60	80	75	0	3.1

Textural analysis			
Coarse Sand	g kg^{-1}	36	Very Clayey
Fine Sand		69	
Slime		270	
Clay		624	

The field study was carried out in completely randomized block design with three replications, and the treatments were arranged in subdivided plots. The main plot received the application of S-metolachlor herbicide (Dual Gold^{TM}) at doses of 0, 1,500 and 3,000 mL ha^{-1} and the subplots received the application of fluxofenim safener (Concep^{TM}) at doses 0 and 40 mL per 100 kg seeds (Table 2). Twenty seeds were sown per meter at five cm depth and the fertilization used in seeding was performed with 400 kg ha^{-1} of 8-28-16 (N,P,K). Five hours before the S-metolachlor spraying was made an irrigation of 30 mm, which was repeated twice a week during the experiment. The experimental plots were 3.0 m long and 6 crop rows, spaced 0.7 m, and subplots consisted of three rows treated with safener and three rows without treatment.

Table 2. Description of treatments used in pre-emergence and seed treatment for growing sorghum. Uberlândia/MG, Brazil.

Major Plotl		Subplot
S-metolaclor[1] Dose	Dual Gold[2] Dose	fluxofenim[3] Dose
0	0	0
		40
1,440	1,500	0
		40
2,880	3,000	0
		40

[1] Dose in g a.i. ha^{-1}; [2] Dose in mL c.p. ha^{-1}; [3] Dose in mL per 100 kg seeds.

The seed treatment with fluxofenim at at 40 mL per 100 kg seeds of the commercial product was made the day before sowing. The S-metolachlor spraying was held in pre-emergence at rates of 1,500 mL ha^{-1} and 3,000 mL ha^{-1} using a CO_2 pressurized backpack sprayer equipped with flat fan nozzles XR 11002VS, spaced 0,5 m. The spray volume was 200 L ha^{-1} with working pressure of 2.82 bar.

Evaluations of the dry mass of shoots and roots were performed at 10 days after emergence (DAE) of plants, and present plants were collected in 1m of row per plot. The effects of treatments on sorghum plants were visually assessed at 3, 7, 15, 30 DAE of plants, using a percentage scale of grades, in which 0 represents the absence of symptoms and 100 death of plants (SBCPD, 1995). The evaluations were initiated at the moment the control plants without chemical treatment (herbicide or safener) had an emergency over 70% of visible plants.

Samples of the plant shoots for each experimental unit of the study were collected at 15 days after application (DAA) of the treatment and 10 DAE of plants, when they were between 2 to 4 fully expanded leaves for determination of GST activity. Only a few treatments were selected for this stage and are described in Table 3.

Table 3. Treatments selected for the determination of GST activity in sorghum. Uberlândia/MG, Brazil.

S-metolaclor[1] Dose	Dual Gold[2] Dose	fluxofenim[3] Dose
0	0	0
0	0	40
1,440	1,500	0
1,440	1,500	40

[1] Dose in g a.a.i. ha^{-1}; [2] Dose in mL c.p. ha^{-1};[3] Dose in mL per 100 kg seeds.

After the collection of the seedlings, the shoot samples were washed with distilled water and dried superficially with sheets of paper towels. 1g samples were placed in plastic bags properly labeled and wrapped in aluminum foil. Then, these samples were frozen in liquid nitrogen (-190° C) and stored in a freezer at a temperature of -85° C for later determination of GST. Measurements were performed at the Laboratory of Xenobiotics from the Department of Chemistry and Biochemistry of the Institute of Biosciences, UNESP, Botucatu/SP.

For extraction of GST was used the method proposed by Ekler et al. (1993), the GST enzyme activity was determined according to the method proposed by Wu et al. (1996), and the molar extinction coefficient of 9.6 mmol cm^{-1} (HABIG; JAKOBY, 1981) was used to calculate the enzyme activity. The specific activity of GST was expressed in nmol min^{-1} mg^{-1} protein. The total soluble protein content in the extracting buffer used in the extraction of GST was estimated by the method of Lowry et al. (1951). Bovine serum albumin (BSA) was used as standard protein. The absorbance readings were performed in a spectrophotometer at a wavelength of 660 nm.

The experimental delimitation adopted for this step of the study was completely randomized with 12 replications, and treatments were arranged in a 2x2 factorial design, corresponding to two doses of the herbicide (0 and 1,500 ml ha^{-1}) and two doses of safener (0 and 40 mL per 100 kg seed).

All results obtained in this study were submitted to analysis of variance by "F" test and the treatment averages compared by "t" test ($p<0.05$).

RESULTS AND DISCUSSION

Table 4 presents the results of phytotoxicity caused by chemical treatments (safener and herbicide) on plants of the hybrid sorghum DKB510 in different periods of time after the initial of the emergency. Note that the seed treatment with fluxofenim at 3 DAE, isolated from the application of S-metolachlor herbicide, caused a phytotoxicity, which was characterized by a delayed initial emergence of seedlings; but it was not affected when compared to the chemical untreated control plant. In this and subsequent evaluation, plants that received isolated seed treatment did not show foliar symptoms of poisoning (such as height reduction) when compared to the control plant (without safener or herbicide).

Table 4. Phytotoxicity of S-metolachlor herbicide in DKB510 sorghum plants treated or non-treated with fluxofenim safener in different periods of time. Uberlândia/MG, Brazil.

S-metolachlor Dose (g a.i. ha^{-1})	% Phytotoxicity							
	3 DAE		7 DAE		15 DAE		30 DAE	
	with[1]	without[1]	with[1]	without[1]	with[1]	without[1]	with[1]	without[1]
0	9.0 Aa	0.0 Aa	3.3 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa
1,440	11.7ABa	43.3 Bb	6.7 Aa	41.7 Bb	1.7 Aa	26.7 Bb	0.0 Aa	28.3 Bb
2,880	23.3 Ba	68.3 Cb	21.7 Ba	81.0 Cb	25.0 Ba	70.0 Cb	7.7 Ba	78.3 Cb
F Herbicide (H)	148.71**		129.00**		160.56**		234.14**	
F Safener (S)	64.99**		232.69**		84.00**		658.28**	
F (H) x (S)	33.69**		84.54**		26.14**		254.84**	
C.V. (%)	38.9		37.4		48.2		32.7	
l.s.d.	11.85		8.49		10.79		5.45	

[1]Addition or non-addition of fluxofenim safener; ** Significant at the level of 1% probability; Averages followed by same small letter in the row and capital letter in the column, do not statistically differ among them by t test ($p<0.05$).
DAE – days after emergence.

It was also observed that in the plots of land that did not receive seed treatment with fluxofenim, all tested herbicide doses were harmful to the emergence of plants and that these plants showed visual symptoms of intoxication; and from 3 DAE these treatments had values superior to the control plant. In the last assessment, at 30 DAE, the levels of observed injury were unacceptable in any of the tested herbicide doses, indicating a high sensitivity of the plants to the product, and the highest dose (2,880 mL ha^{-1}) was the most phytotoxic. S-metolachlor herbicide is a selective product to various crops (cotton, sugar cane and corn) applied in pre-emergence and presents high effectiveness on grass. In Brazil, it is registered for the utilization on the corn crop and its maximum recommended dose is 1,750 mL p.c. ha^{-1} (1,680 mL a.i. ha^{-1}). The product does not contain register for sorghum crop due to its toxicity. The toxic effect of this group of herbicide can be observed after germination of seedlings, characterized by the non-opening the coleoptile and by the wrinkling of the definitive leaves, caused by the minor growth of the central vein in relation to leaf growth (KARAM, 2003).

The interaction between the seed treatment with the safener and the application of S-metolachlor herbicide (1,440 mL ha^{-1} and 2,880 mL. ha^{-1}), in pre-emergence of DKB510 sorghum, reduced the phytotoxicity symptoms. In the plots of land where seeds were treated with fluxofenim there was an increase in selectivity of the product to sorghum seedlings. At 3 DAE was observed a greater harm (mainly reflected in the delay of emergency), but the 1,440 mL ha^{-1} showed similar values to the control plant. At 15 DAE, it has been observed on the plots that received S-metolachlor at 1,440 mL ha^{-1}, the visual symptoms of injury were no longer observed. It was found that the control plant (with safener but without herbicide) at 1,440 mL ha^{-1} in plots treated with fluxofenim were similar during evaluations of 3, 7, 15 and 30 DAE, which showed that the product helped in the reduction of injuries caused by the herbicide. It is noteworthy that, regardless of the dose of applied herbicide, the plots that received treated seeds with fluxofenim had less severe injuries.

The S-metolachlor at 2,880 mL ha^{-1} was more toxic to the crop even in the presence of the safener. However, the presence of seed treatment was important to increase the selectivity, and the absence of this treatment would prevent the use of S-metolachlor in sorghum. Fuerst and Gronwald, (1986), Gronwald et al. (1987) and Alla and Hassan (1998) indicated the viability of using safeners in the treatment of sorghum seeds to protect against the effects of metolachlor, presumably by inducing the synthesis of new GST enzymes responsible for catalysis and detoxification of the herbicide.

Table 5 shows the results of the dry mass weight from the shoot and the root, harvested at 10 days after emergence. The absence of seed treatment in the plots that received herbicide spraying in pre-emergence provided plants with a reduced accumulation of shoot and root dry mass.

Table 5. Values of shoots and roots dry mass of DKB510 sorghum treated or not with the fluxofenim safener, 10 days after emergence. Uberlândia/MG, Brazil.

S-metolachlor Dose (g a.i ha^{-1})	Shoot Part (g)		Root (g)	
	with[1]	without[1]	with[1]	without[1]
0	360.00 Aa	400.00 Aa	266.67 Aa	256.67 Aa
1,440	326.66 Aa	300.00 Aba	240.00 Aa	206.67 Aba
2,880	340.00 Aa	170.00 Ba	223.33 Aa	143.33 Ba
F Herbicide (H)	79.87**		107.60**	
F Safener (S)	12.20**		40.26**	
F (H) x (S)	17.18**		10.09**	
C.V. (%)	31.7		19.5	
l.s.d.	200.35		86.83	

[1] Adding fluxofenim safener or not; ** Significant at 1% probability; Averages followed by same small letter in the row and capital letter in the column, do not statistically differ among them by t test (p<0.05).

The results show that S-metolachlor at 2,880 mL ha^{-1}provided approximately 60% reductions in the shoot dry mass and 45% in the root dry mass; and the 1,440 mL ha^{-1}, even after shown lower values (25% less shoot and 19% less root) was similar to the results of the plot without herbicide spraying.

The dry mass accumulation in both shoot and roots was similar when seeds were treated with the safener, regardless of the used herbicide dose, compared to the control (with safener and without herbicide). Despite the observed difference between treatments with and without safener, with greater accumulation of root dry mass for treatments with safener (mainly to the plots subjected to 2,880 mL ha^{-1}), the values were similar between the treated or non-treated seeds, regardless of the used dose.

The results observed for SCG340 sorghum (Table 6) were similar to the DKB510 one. There was a beneficial effect of seed treatment with the fluxofenim safener in reducing the phytotoxicity caused by the herbicide. It was observed at 3 DAE, that the use of the safener by itself also resulted in a slight phytotoxicity compared to the untreated control plant (safener or herbicide) and the observed symptoms in both sorghum hybrids were similar and momentary.

Table 6. Phytotoxicity of S-metolachlor herbicide in SCG340 sorghum plants, treated or not with the fluxofenim safener in different periods of time. Uberlândia/MG, Brazil.

S-metolachlor dose (g a.i. ha^{-1})	% Phytotoxicity							
	3 DAE		7 DAE		15 DAE		30 DAE	
	with[1]	without[1]	with[1]	without[1]	with[1]	without[1]	with[1]	without[1]
0	5.7 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa
1,440	12.7 Aa	60.0 Bb	9.0 Ba	78.3 Bb	1.0 Aa	56.7 Bb	0.0 Aa	53.3 Bb
2,880	25.0 Ba	88.3 Cb	20.0 Ba	91.7 Cb	24.3 Ba	87.7 Cb	7.7 Ba	88.3 Cb
F Herbicide (H)	463.99**		309.05**		229.84**		234.14**	
F Safener (S)	173.17**		112.65**		1481.97**		658.28**	
F (H) x (S)	61.46**		315.14**		375.11**		254.84**	
C.V. (%)	17.7		8.7		7.7		9.7	
l.s.d.	11.27		5.83		4.37		4.80	

[1] Adding fluxofenim safener or not; DAE – days after emergence; ** Significant at 1% probability; Averages followed by same small letter in the row and capital letter in the column, do not statistically differ among them by t test ($p<0.05$).

The notes of phytotoxicity again responded to the increase of the herbicide dose, and the plots without the safener seed treatment had higher injury. The application of the 1,440 mL ha^{-1} at 3 DAE has caused phytotoxicity of 61% and at the end of the evaluation period presented values above 50% of injury. Also, the spraying of the 2,880 mL ha^{-1} has caused injuries over 85%. It should be noted that the SCG340 hybrid showed greater sensitivity to the herbicide, compared to DKB510 hybrid.

In the plots that received the seed treatment with the safener, when comparing the different doses of tested herbicides, it can be noted that the application of the 1,440 mL ha^{-1} showed a higher selectivity to the crop, presenting visual notes of injury around 12% at 3 DAE and at the end of the evaluation period, at 30 DAE, the plants had fully recovered. The 2,880 mL ha^{-1} dose can be considered very high, because in spite of seed treatment with fluxofenim, there were elevated intoxication values by the crop.

Yet, it can be seen in Table 6 that up to 15 DAE the values of phytotoxicity for the highest dose were close to 25%, while the lowest dose was less than 5% of intoxication. The treatment of seeds with no safener at any tested dose, again led to lack of selectivity, precluding the use of this herbicide to the crop.

The accumulation of dry matter in shoots was not influenced by doses of herbicide when the seeds were treated with the safener, and there were similar values to the control plant with the safener (Table 7). Failure to use the safener resulted in decreasing values of dry matter when increasing the dose of the herbicide. The 2,880 mL ha^{-1} dose provided a 60% reduction in dry mass accumulation in the shoots compared to the control plant without chemical treatment, while the 1,440 mL ha^{-1} dose provided a 30% decrease.

Table 7. Values of shoots and roots dry mass of SCG340 sorghum, treated or not with fluxofenim safener, 10 days after emergence. Uberlândia/MG, Brazil.

S-metolachlor Dose (g a.i. ha^{-1})	Shoot Part (g)		Root (g)	
	with[1]	without[1]	with[1]	without[1]
0	280.00 Aa	296.67 Aa	266.67 Aa	393.33 Aa
1,440	260.00 Aa	203.33 Ba	240.00 Aa	176.67 Bb
2,880	280.00 Aa	120.00 Cb	223.33 Aa	186.67 Ba
F Herbicide (H)	99.45**		13.62**	
F Safener (S)	145.16**		113.20**	
F (H) x (S)	85.77**		13.17**	
C.V. (%)	30.0		91.1	
l.s.d.	74.16		183.35	

[1] Adding fluxofenim safener or not;** Significant at 1% probability; Averages followed by same small letter in the row and capital letter in the column, do not statistically differ among them by t test (p<0.05).

Increasing the dose of the herbicide also ordered a reduction in the root dry mass, regardless of receiving the seed treatment, and the 2,880 mL ha^{-1} dose behaved similarly in relation to the seeds treatment or not. The seed treatment with the safener provided a difference of 15% of roots dry mass in relation to 1,440 mL a.i. ha^{-1} dose and the control plant (with safener), while the same dose compared to the control plan (without safener) had a decrease of 45%. These values agree with the observed visual symptoms of injury presented in Table 6.

For sorghum crop the absence of seed treatment with the safener can take the crop to reach intolerable levels of injury and that would compromise the development of plants, reducing the shoots and roots dry mass. It could be noted that the safener has increased the tolerance of the crop, regardless of the hybrid to S-metolachlor herbicide in any of the tested doses, but the selectivity was higher at the lowest dose of the herbicide (1,440 mL ha^{-1}).

The corresponding values of GST activity in sorghum are presented in Table 8, when the seeds received treatment with the safener and were subjected or not to treatment with S-metolachlor herbicide, compared to the untreated control plant (nor safener or herbicide). The increase in activity of the enzyme glutathione S-transferase in plants is directly responsible for the efficiency of some crops become less sensitive to certain phytotoxic herbicides. In the presented results were demonstrated that treatment of sorghum seeds with the safener led to lower plant injury caused by the low selectivity of S-metolachlor herbicide compared to plots that did not receive seed treatment.

It can be seen in Table 8, despite no significant differences, that the seed treatment by itself increased by 13% the GST enzyme activity when compared to the untreated control plant, and when plants received only the herbicide spraying, the enzyme activity decreased by 26%. S-metolachlor herbicide is not registered for sorghum crop due to damage of injury that can occasionally be observed. The decrease in GST activity when there was no protection by treating seeds with fluxofenim highlights the deficiency of the crop in increasing the activity of the enzyme sufficiently to metabolize the herbicide at levels that allow their lower toxicity to plants. However, when the plants of sorghum received seed treatment with the safener and were subjected to herbicide spraying, GST enzyme activity increased 20% compared to the control plant without any treatment and 62% when compared to the untreated seed plot but with herbicide application. Although not significantly different, pre-treatment with the safener caused an increase in GST activity in the presence of the herbicide for sorghum.

Table 8. Activity of glutathione S-transferase, in the shoots of sorghum plants after seed treatment with fluxofenim safener and submitted to S-metolachlor herbicide.

S-metolachlor Dose (g a.i. ha^{-1})	GST Activity (nmol min^{-1} mg^{-1} of protein)	
	with[1]	without[1]
0	6.9341	6.0877
1,440	7.3392	4.5217
F Herbicide (H)	0.301^{ns}	
F Safener (S)	3.000^{ns}	
F (H) x (S)	0.868^{ns}	
C.V. (%)	58.9	

[1] Adding fluxofenim safener or not; [ns] not significant at 5% probability by t test.

Grass species, such as corn and sorghum, have little tolerance to thiocarbamates herbicides (EPTC) and chloroacetamide (metolachlor and alachlor). However, pre-treatment with safeners, such as flurazole, dichlormid, benoxacor and naphtali anhydride increase plant tolerance to these herbicides by inducing selectively the GST activity and, thus, raise the rate of detoxification via conjugation (GRONWALD et al., 1987; IRZYK et al., 1995).

The evaluated seeds safener in this study was able to generate an increase, although not significantly different in GST activity for the sorghum crop. It's important to highlight that the few data on literature about fluxofenim safener also demonstrate the high affinity between the molecule and S-metolachlor herbicide (DAVIES; CASELEY, 1999; HATZIOS, 2000). This high similarity between the two products, among other factors, may be involved with the synergism results and protection obtained for the sorghum crop.

RESUMO: Este estudo teve como objetivo avaliar a eficiência do protetor fluxofenim no tratamento de sementes de sorgo, híbridos DKB510 e SCG340, com intuito de aumentar a seletividade ao herbicida S-metolachlor aplicado em pré-emergência e determinar a atividade da enzima de detoxificação glutationa S-transferase (GST). O trabalho foi dividido em duas etapas. A primeira etapa constou da avaliação em campo da eficiência do protetor em reduzir sintomas visuais de fitointoxicação causados pelo herbicida e na segunda foi determinada a atividade da GST. Comparou-se a suscetibilidade das sementes de sorgo ao herbicida por meio da avaliação visual de injúrias aos 3, 7, 15 e 30 dias após a emergência (DAE), massa seca de raiz e parte aérea aos 10 DAE, além da determinação da atividade da GST. Os tratamentos utilizados foram: aplicação do protetor na dose de 0 e 40mL por 100 kg de sementes, e a pulverização do herbicida S-metolachlor nas doses de 1.440 e 2.880 mL i.a.ha^{-1}, além de uma testemunha sem herbicida. O delineamento experimental utilizado foi o de blocos casualizados com três repetições. A utilização do protetor no tratamento de sementes para ambos os híbridos de sorgo aumentou a tolerância ao herbicida S-metolachlor nas duas doses utilizadas, sendo os melhores resultados obtidos na dose de 1.440 mL ha^{-1}. A atividade da enzima GST apresentou aumento quando da utilização do fluxofenim anteriormente à aplicação do herbicida S-metolachlor na dose de 1.440 mL. ha^{-1} para os dois híbridos testados.

PALAVRAS-CHAVE: Controle químico. Seletividade. *Sorghum bicolor.* S-tranferase.

REFERENCES

ALLA, M. M. N.; HASSAN, N. M. Efficacy of exogenous GA_3 and herbicide protetors in protection of *Zea mays* from metolachlor toxicity. **Plant Physiology and Biochemistry**, Paris, v. 36, n. 11, p. 809-815, 1998.

BURTON, J. D.; MANESS, E. P. Constitutive and inducible bentazon hydroxylation in shattercane (*Sorghum bicolor*) and Johnsongrass (*S. halepense*). **Pesticide Biochemistry and Physiology**, San Diego, v. 44, n. 1, p. 40-49, 1992.

DAVIES, J.; CASELEY, J. C. Herbicide protetors: a review. **Pesticide Science**, Changai, v. 55, n. 11, p.1043-1058, 1999.

EDWARDS, R.; BRAZIER-HICKS, M.; DIXON, D. P.; CUMMINS, I. Chemical manipulation of antioxidant defences in plants. **Advances in Botanical Research**, Nance, v. 42, n.1, p. 1-32, 2005.

EKLER, Z.; DUTKA, F.; STEPHENSON, G.R. Protetor effects on acetochlor toxicity uptake, metabolism and Glutathione S-transferase activity in maize. **Weed Research**, Osford, v. 54, n. 1, p.96-109, 1993.

FARAGO, S.; BRUNOLD, C.; Kreuz, K. Herbicide safeners and glutathione metabolism. **Physiologia Plantarum**, v. 91, n. 3, p. 537–542, 1994.

FUERST, E.P.; GRONWALD, J. W. Induction of rapid metabolism of metolachlor in sorghum (*Sorghum bicolor*) shoots by CGA-92194 and other antidotes. **Weed Science**, Champaign, v. 34, n. 2, p. 354-361, 1986.

GRONWALD, J.W.; FUERST, E. P.; EBERLEIN, C. V.; EGLI, M. A. Effect of herbicide antidotes on glutathione content and glutathione S-transferase activity of sorghum shoots. **Pesticide Biochemistry and Physiology**, Amsterdam, v. 29, n. 1, p. 66-76, 1987.

HABIG, W.H.; JAKOBY, W. B. Assays for differentiation of glutathione S-transferases. **Methods in Enzymology**, New York, v. 77, n.3, p. 398-405, 1981.

HATZIOS, K.K. Herbicide protetors and synergists. In: Roberts, T. (Ed.). **Metabolism of Agrochemials in Plants**. Wiley: Chichester, 2000. p.259-294.

HATZIOS, K.K.; BURGOS, N. Metabolism-based herbicide resistance: regulation by protetors. **Weed Science,** Champaign, v. 52, n. 3, p. 454-467, 2004.

HIRASE, K.; MOLIN, W. Effect of Flurazole and Other Safeners for Chloroacetanilide Herbicides on Cysteine Synthase in Sorghum Shoots. **Pesticide Biochemistry and Physiology**, Amsterdam, v. 71, n. 1, p. 116-123, 2001.

IRZYK, H. R.; FUERST, E. P. Purification and characterization of a glutathione S-transferase form benoxacor-treated maize (*Zea mays*). **Plant Physiology**, Waterbury, v. 102, n. 6, p. 803-810, 1993.

IRZYK, H. R., POTTER, S.; WARD, E. A. DNA clone encoding the 27-kilodalton subunits of glutathione S-transferase IV from *Zea mays*. **Plant Physiology**, Waterbury, v. 107, n. 3, p. 311-312, 1995.

KARAM, D. Características do herbicida S-metolachlor nas culturas de milho e sorgo. EMBRAPA/CNPMS: Circular Técnica 36, 2003. p. 65-67.

LAMOUREUX, G. L.; RUSNESS, D. G. The mechanism of action of BAS 145 138 as a safener for chlorimuron ethyl in corn: Effect on hydroxylation, glutathione conjugation, glucoside conjugation, and acetolactate synthase. **Pesticide Biochemistry and Physiology**, Amsterdam, v. 42, n. 1, p. 128-139, 1992.

LIEBL, R. Cell growth inhibitors (cloroacetamides, cabomothioates, napropamide, bensulide). In: LIEBL, R. (Ed.). **Herbicide action**. West Lafayette: Purdue University, 1995. p. 200-224.

LOWRY, O.H.; ROSEBROUGH, N. J.; FARR, A. L. Protein measurement with Folin-phenol reagent. **Journal of Biology and Chemistry**, Rockville, v. 193, n. 2, p. 265-275, 1951.

MANNERVIK, B.; DANIELSON, U. H. Glutathione S-transferase: structure and catalytic activity. **Critical Reviews in Biochemistry and molecular biology**, London, v. 23, n. 3, p. 283-337, 1988.

MOZER, T. J.; TIEMEIER, D. C.; JAWORSKI, E. G. Purifications and characterization of corn glutathione S-transferase. **Biochemistry**, Oxford, v. 22, n. 9, p. 1068-1072, 1983.

RIECHERS, D. E.; YANG, K.; IRZYK, G. P.; JONES, S. S.; FUERST, E. P. Variability of glutathione S-transferase levels and dimethanamid tolerance in protetor-treated wheat and wheat relatives. **Pesticide Biochemistry and Physiology**, Amsterdam, v. 56, n. 1, p. 88-101, 1996.

ROSINGER, C.; KÖCHER, H. Safener for herbicides. In: KRÄMER, W. and SCHIRMEr, U. (ed). **Modern Crop Protection Compounds**. Wiley: Weinheim, 2007. p. 259-282.

SBCPD - Sociedade Brasileira da Ciência das Plantas Daninhas. **Procedimentos para instalação, avaliação e análise de experimentos com herbicidas**. Londrina: SBCPD. 1995. 42p.

VIDAL, R. A. **Herbicidas: mecanismo de ação e resistência de plantas**. Porto Alegre: UFRS, 1997. 165 p.

WU, J.; OMOKAWA, H.; HATZIOS, K. K. Glutathione S-transferase activity in unsafened and fenclorin-safened-rice (*Oryza sativa*). **Pesticide Biochemistry and Physiology**, Amsterdam, v. 54, n. 2, p. 20-229. 1996.

XU, F.; LAGUDAH, E. S.; MOOSE, S. P.; RIECHERS, D. E. Tandemly duplicated Safener-Induced Glutathione *S*-Transferase Genes from *Triticum tauschii* Contribute to Genome- and Organ-Specific Expression in Hexaploid Wheat. **Plant Physiology**, Waterbury, v. 130, n. 3, p. 362-373, 2002.