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Article

Use of Sunflower and Soybean as Bioindicators to Detect Atrazine Residues in Soils

Uso do Girassol e Soja como Bioindicadores para Detectar Resíduos de Atrazine em Solos

NOVAIS, J.[1]
MENDES, K.F.[2*]
INOUE, M.H.[1]
MACIEL, C.D.G.[3]
HELVIG, E.O.[1]
STANIESKI, C.M.[1]

ABSTRACT - Atrazine is selective for maize, sugarcane and sorghum and may interfere with successor crops such as sunflower and soybean. Despite this problem, there are few studies about the residual effect of this herbicide in these crops. Thus, the objective of the research was to evaluate the residual effect of atrazine through bioavailability of the herbicide in sandy and clayey soils, using soybean and sunflower as bioindicators. The design was completely randomized with four replications and a 2 × 7 + 1 factorial scheme, that is, two doses (2,000 and 2,500 g ha^{-1}), seven application times (0, 15, 30, 45, 60, 75 and 90 days between application (DBA) of herbicide and bioindicator sowing) and one control without application. Dry mass and chlorophyll content of the bioindicators were evaluated at 20 days after sowing. The accumulation of dry mass of the bioindicators in the two types of soil was reduced at 90 DBA. The chlorophyll content at 0 DBA was zero for soybean and sunflower, due to the reduction of dry mass by approximately 100%. It was concluded that the residual effect of atrazine at a dose of 2,500 g ha^{-1} was higher in clayey soils due to the low bioavailability of the product in the soil solution. Therefore, it is recommended to sow sunflower and soybean in both soils after 90 DBA, because atrazine interferes with the chlorophyll content and percentage of dry mass reduction.

Keywords: residual effect, herbicide, bioassay method, soil texture.

RESUMO - A atrazine é seletiva para milho, cana-de-açúcar e sorgo e pode interferir em culturas sucessoras, como girassol e soja. Apesar da problemática, são poucos os estudos acerca do efeito residual desse herbicida nessas culturas. Assim, o objetivo desta pesquisa foi avaliar o efeito residual da atrazine por meio da biodisponibilidade do herbicida em solos de textura arenosa e argilosa, utilizando-se a soja e o girassol como bioindicadores. O delineamento foi inteiramente casualizado com quatro repetições e esquema fatorial 2 × 7 + 1, isto é, duas doses (2.000 e 2.500 g ha^{-1}), sete épocas de aplicação (0, 15, 30, 45, 60, 75 e 90 Dias Entre a Aplicação – DEA do herbicida e a semeadura dos bioindicadores) e uma testemunha, sem aplicação. A massa seca e o teor de clorofila dos bioindicadores foram avaliados aos 20 dias após a semeadura. O acúmulo de massa seca dos bioindicadores nos dois tipos de solo foi reduzido até os 90 DEA. O teor de clorofila, ao 0 DEA, foi zero para a soja e girassol, em virtude da redução de massa seca em aproximadamente 100%. Concluiu-se que o efeito residual da atrazine foi maior no solo de textura argilosa com dose de 2.500 g ha^{-1}, devido à baixa biodisponibilidade do produto na solução do solo. Portanto, recomenda-se a semeadura do girassol e da soja em ambos os solos, após os 90 DEA, pois ocorre interferência nos teores de clorofila e no percentual de redução de massa seca.

Palavras-chave: efeito residual, herbicida, método de bioensaios, textura do solo.

*** Corresponding author:**
<kfmendes@ufv.br>

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[1] Universidade do Estado de Mato Grosso, Tangará da Serra-MT, Brasil; [2] Universidade Federal de Viçosa, Viçosa- MG, Brasil; [3] Universidade Estadual do Centro-Oeste do Paraná, Guarapuava-PR, Brasil.

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INTRODUCTION

Weed control is mainly carried out through herbicides applied directly to the soil or to the aerial part of the plant. Regardless of the place of application, the final or transient fate of herbicides is the soil compartment (Mancuso et al., 2011). Therefore, the environmental behavior of these products will depend on their physicochemical characteristics and the retention, transformation and transportation processes that occur in the environment, as well as on the edaphoclimatic conditions and production system adopted (Niekamp and Johnson, 2001).

Included in the abovementioned aspects is the residual effect, commonly known as carryover. Directly, herbicides cause the phytotoxication of successor crops, reducing productivity due to their residual potential. An herbicide is considered residual when it preserves the integrity of its molecule in the environment for longer (Hinz, 2001; Mancuso et al., 2011).

Among the residual herbicides recommended for corn, sorghum and sugarcane is atrazine (Coutinho et al., 2005). This herbicide belongs to the chemical group of photosystem II inhibitors or Hill synthesis inhibitors (Oliveira Júnior, 2011; Rodrigues and Almeida, 2018). It is a selective herbicide of systemic action and is used in pre- or early emergence, mainly for the control of dicotyledonous weeds (Carvalho et al., 2010).

Residual atrazine is influenced by soil texture; more clayey soils have a higher capacity to retain the molecule in colloids. Organic matter (OM) content is the main regulator of the binding and residue formation of this herbicide (Javaroni et al., 1999). Its persistence is considered high in both soil (Janaki et al., 2012) and groundwater (Schwab et al., 2006), and it is degraded by a variety of organisms that make up soil micro- and macrofauna (Jablonowski et al., 2010). The half-life ($t_{1/2}$) of atrazine in soil varies according to its sorption to OM (Daniel et al., 2002; Lima et al., 2010; Mudhoo and Garg, 2011), crop characteristics, soil pH and the history of herbicide use (Popov et al., 2005; Jablonowski et al., 2010; Vonberg et al., 2014).

Numerous studies have shown the sensitivity of crops to the carryover effect of atrazine, which significantly reduces vetch, clover and bean biomass, and minimizes the emergence of barley and oats by 20% and 16%, respectively (Palhano et al., 2018). Sunflower (*Helianthus annuus* L.) is also sensitive when sown shortly after application of atrazine to the predecessor crop (Brighenti et al., 2002). The presence of atrazine in the soil decreases carrot shoot dry mass (Bontempo et al., 2016). Cornelius and Bradley (2017) also found that residual atrazine reduces dry ryegrass biomass by 37% to 51%.

To determine the carryover and persistence of herbicides in the environment, we used the bioassay technique, based on the biological response of the plant to the presence of herbicide. It is an easily reproducible, low-cost method that allows the detection of biologically active residues. Thus, various means of evaluation were employed, such as the percentage germination, weight or size of plant parts, and phenological changes, estimated visually or by objective measurements (Horowitz, 1976; Vouzounis and Americanos, 2002; Pintar et al., 2017).

In general, atrazine is one of the most widely used herbicides in maize for broadleaf weed control. However, due to its persistence in the environment, phytointoxication problems may occur in subsequent crops such as soybean and sunflower, interfering with development and thereby reducing their productivity. Knowing the importance of the Brazilian agricultural sector and the scarcity of research on residual atrazine in Cerrado soils, there is an interest in evaluating the interval between atrazine application and soybean and sunflower sowing required to avoid phytotoxic effects. Therefore, the objective of this research was to evaluate the residual effect of atrazine through herbicide bioavailability in sandy and clayey soils, using soybean and sunflower as bioindicators.

MATERIAL AND METHODS

Two experiments were carried out, one with the soybean bioindicator and the other with sunflower, in clayey soils classified as Red Latosol and sandy soils classified as Quartzarenic Neosol. Soil samples were taken over 6 months from a chemical-free area located in Tangará da Serra-MT, Brazil. They were collected at a depth of 0–10 cm, sifted for removal of vegetable debris and stored in 2 dm^3 pots. The physicochemical attributes of the soils used are described in Table 1.

Table 1 - Physicochemical attributes of clay and sandy soils of Tangará da Serra, MT, Brazil. 2016

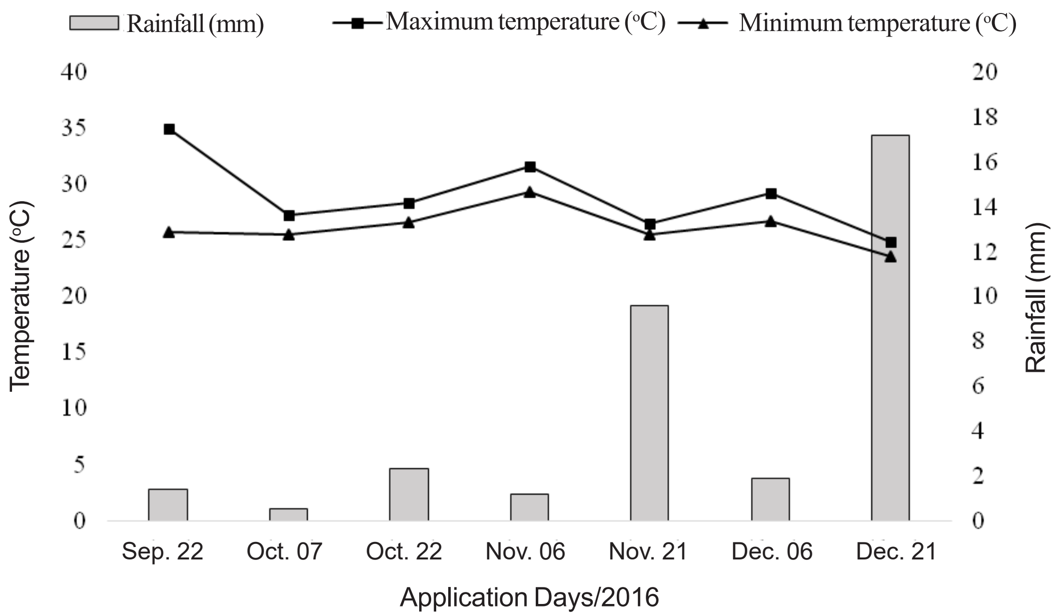
Soil	pH		Al^{3+}	$H^+ + Al^{3+}$	$Ca^{2+} + Mg^{2+}$	Ca^{2+}	K^+
	$CaCl_2$	H_2O	($cmol_c\ dm^{-3}$)				
Clay[1]	6.10	6.30	0.00	2.80	6.75	4.86	0.50
Sandy[2]	6.10	6.50	0.00	0.50	1.26	0.77	0.23

P	OM	CEC	BS	Sand	Silt	Clay
($mg\ dm^{-3}$)	($g\ dm^{-3}$)	($cmol_c\ dm^{-3}$)	(%)	($g\ kg^{-1}$)		
2.30	41.00	10.05	72.14	523.00	130.00	357.00
10.60	6.00	1.99	74.87	846.00	63.00	91.00

[1] Latossolo Vermelho (Oxisol); [2] Neossolo Quartzarênico (Entisol). Source: Plante Certo Laboratory, Várzea Grande, MT, Brazil.

The experimental design was completely randomized with four replications, in a 2 × 7 + 1 factorial scheme, evaluating two doses of atrazine (2,000 and 2,500 g ha^{-1}) in clay and sandy soils, seven times of application (0, 15, 30, 45, 60, 75 and 90 days between application (DBA) of herbicide and bioindicator sowing) and a control without application. The doses were defined based on the average dose used by the producers in the state of Mato Grosso.

Applications were made with a CO_2-based costal sprayer equipped with four XR110.02-type tips, spaced 0.50 m apart, with a spray volume equivalent to 200 L ha^{-1} and 300 kPa working pressure. At the time of application, the meteorological data (maximum and minimum temperature and rainfall) provided by the National Institute of Meteorology (INMET) were verified; they are described in Figure 1.



Source: INMET (2016).

Figure 1 - Maximum and minimum temperature and rainfall on the days of atrazine application.

For the experiments to be performed simultaneously, atrazine applications started at 90 DBA (last period evaluated) and continued at 15 day intervals up to 0 (zero) DBA (first period evaluated); shortly thereafter, the soybean and sunflower seeds were sown manually. In each pot, 10 seeds of each crop (bioindicator) were used. After sowing, the pots were irrigated manually with a 15 mm blade until 20 days after sowing (DAS). No thinning was performed, leaving 20 plants in the pots: 10 soybean and 10 sunflower.

Dry mass and chlorophyll content were evaluated at 20 DAS for both bioindicators. For the dry mass parameter, three whole plants per repetition were submitted to a temperature of 70 °C for 48 h in a greenhouse, enough time to reach the constant dry mass of the bioindicators. Subsequently, the samples were weighed to determine the percentage reduction in relation to the control treatment without herbicide application. To measure the chlorophyll content, expressed as the dimensionless Falker chlorophyll index (FCI), three plants per repetition were evaluated using a ClorofiLOG 1030® (Falker CFL, Porto Alegre, RS, Brazil).

The collected data were submitted to analysis of variance (ANOVA) and significance by the F test ($p < 0.01$ and 0.05) and, when significant, adjusted in regression equations, using SISVAR 5.6® software (Ferreira, 2014).

The percentage dry matter reduction was adjusted in a first-order kinetic model ($C = C_0 e^{kt}$, where C is the inhibition of bioindicator dry mass by the herbicide at time t (%); C_0 is the initial inhibition of herbicide (%); t is the incubation time; and e k is a dissipation rate constant (day^{-1}); then, k values were used to calculate $t_{1/2}$ as follows: $t_{1/2} = \ln 2/k$.

Regarding chlorophyll content, nonlinear regression was used, modeling an exponential equation ($y = a \times (1 - \exp(-b \times x))$ and a polynomial equation ($y = y_0 + a \times x$) using Sigma Plot software (version 10.0.1, 2007 for Windows; Systat Software Inc., Point Richmond, CA, USA).

RESULTS AND DISCUSSION

There was significant interaction ($p < 0.01$) between the bioindicators and the residual effect of atrazine for the percentage of dry plant mass reduction (Figure 2). Up to 90 DBA, a reduction in the dry mass of sunflower bioindicators of more than 40% was found in clay soil and of soybean in clay and sandy soils. The reduction in sunflower in sandy soil was below this value, at doses of 2,000 (28.63%) and 2,500 g ha^{-1} (29.93%) at 90 DBA (Figure 2A). Sunflower behavior in sandy soil was due to root depth, which is greater in sandy than in clay soils (Ungaro et al., 2009). Brighenti et al. (2000) stated that the selectivity of an herbicide depends on its position in the soil profile, so that exposure of the product to sunflower roots may result in a reduction of crop dry biomass.

The largest reduction (~100%) of sunflower dry mass occurred at 0 DBA in clay soil for both doses, due to the direct effect of the herbicide, causing no germination of the bioindicator. Values for sunflower in the sandy soil, at the doses of 2,500 and 2,000 g ha^{-1}, were reduced by 69.51% and 65.95%, respectively (Figure 2A). This resulted from the higher OM content (41.00 g dm^{-3}) of clay soil compared to sandy soil (6.00 g dm^{-3}) (Table 1). Atrazine molecules tend to be more

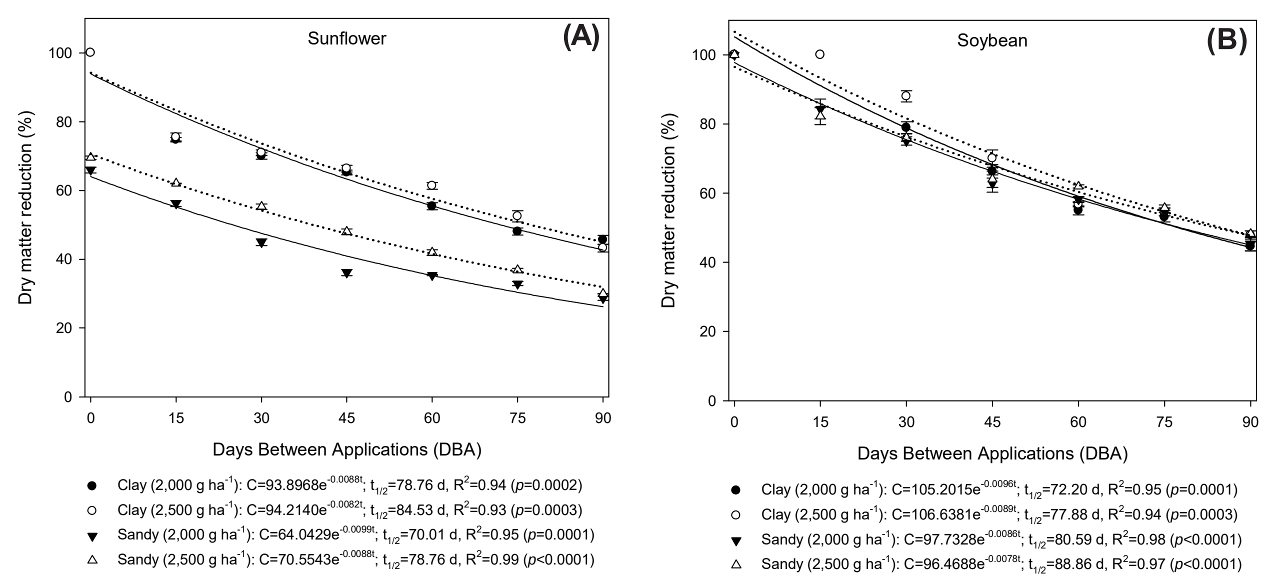


Figure 2 - Percentage reduction of sunflower (A) and soybean (B) dry matter relative to control, at 0, 15, 30, 45, 60, 75 and 90 Days Between Application (DBA) of atrazine and bioindicator sowing.

entrapped in soils with a higher OM content (Procopio et al., 2001; Correia and Langenbach, 2006).

Corroborating these data, Robinson (2008) observed a 66.6% reduction in the dry weight of carrot shoots 12 months after application when studying atrazine carryover in sandy loam soil.

At a dose of 2,500 g ha^{-1}, atrazine applied to clay soil provided, at 15 and 30 DBA, a reduction of 70.97% and 75.51% in sunflower dry matter, respectively (Figure 2A). As the period after application increased, the residual effect of atrazine on the soil decreased.

In soybean, there was a reduction in dry mass accumulation of approximately 100% at 0 and 15 DBA for the doses applied in the clay soil. In the sandy soil, the same value was only observed at 0 DBA (Figure 2B); dry matter accumulation decreased as the interval between application and sowing increased.

When analyzing the half-life ($t_{1/2}$) of atrazine for sunflower, greater persistence ($t_{1/2}$ = 84.53 days) of this herbicide was observed in the clay soil at a dose of 2,500 g ha^{-1} (Figure 2A), which explains the largest reduction in dry mass. There was also a similar half-life ($t_{1/2}$ = 78.76 days) for the clay (2,000 g ha^{-1}) and sandy soils (2,500 g ha^{-1}), since at 0 DBA the concentration of the herbicide in sandy soils was close to 70% (Figure 2A).

In relation to soybean, atrazine $t_{1/2}$ differed from that for the sunflower bioindicator, the highest value ($t_{1/2}$ = 88.86 days) being recorded for 2,500 g ha^{-1} in sandy soil, while the lowest value ($t_{1/2}$ = 72.20 days) was found for the clay soil at a dose of 2,000 g ha^{-1} (Figure 2B).

Several studies have already measured atrazine $t_{1/2}$, which can vary from 2 months to 6 years, depending on the conditions of the environment (Ávila et al., 2009). Nakagawa et al. (1995), when evaluating a Dark Red Latosol, found that this value can reach 180 days. Laboratory experiments have demonstrated a $t_{1/2}$ ranging from 5 to 433 days (Charnay et al., 2005; Vryzas et al., 2012).

Chlorophyll content (FCI) showed significant interaction ($p < 0.01$) for both bioindicators, soil doses and textures (Figure 3). Sunflower FCI at 0 DBA was zero at both doses for clay soil, as the initial concentration of atrazine was high for this soil type. It is also worth considering the pre-emergent effect of the herbicide, which inhibited seed germination. In both soils, as the interval between the application of atrazine and sowing of the sunflower bioindicator increased, there was a gradual increase in chlorophyll content, due to a decrease of the residual herbicide in question.

In the sandy soil, the chlorophyll content of the sunflower was about 20 FCI, due to the lower herbicide concentration in relation to the clay soil. From 15 DBA onwards, the chlorophyll content increased exponentially until reaching the maximum growth point at 90 DBA (Figure 3A). It is noteworthy that the control without atrazine application presented an average FCI of 27.55 in the clay soil and 28.43 in the sandy soil, which are higher than those observed in the treatments with herbicide application.

Javaroni et al. (1999) reported an average $t_{1/2}$ of 26 days for atrazine dissipation in a sandy soil, corroborating that observed in the present research, since at 30 DBA the sunflower cultivated in sandy soil had the highest chlorophyll values [22.89 FCI (2,000 g ha^{-1}) and 21.12 FCI (2,500 g ha^{-1})]. In sunflower, $t_{1/2}$ for sandy soil was between 70 and 78 days (Figure 2A).

When analyzing the soybean bioindicator FCI, it was noted that for both soils and doses, the chlorophyll content was zero at 0 DBA, since there was a reduction of dry mass by about 100%. For the sandy soil at the higher dose, the content stabilized at 45 DBA. For clay soil, the highest chlorophyll content was found at 90 DBA (30.73 FCI) and at the lower dose of atrazine: 2,000 g ha^{-1}. The control treatment had a mean FCI of 33.23 in the clay soil and 32.30 in the sandy soil (Figure 3B).

There was a difference in chlorophyll content ($p < 0.01$) up to 60 DBA, with the higher dose of atrazine in clay soil providing the lowest chlorophyll content in soybean (26.64 FCI) when compared to the higher dose of 2,000 g ha^{-1} in sandy soil (29.11 FCI). Oliveira Júnior (2001), in soils of medium and clay texture, verified that $t_{1/2}$ was 55 days. In this research, the $t_{1/2}$ exceeded 78 days (Figure 2A). Thus, due to the interference caused by the herbicide on the electron

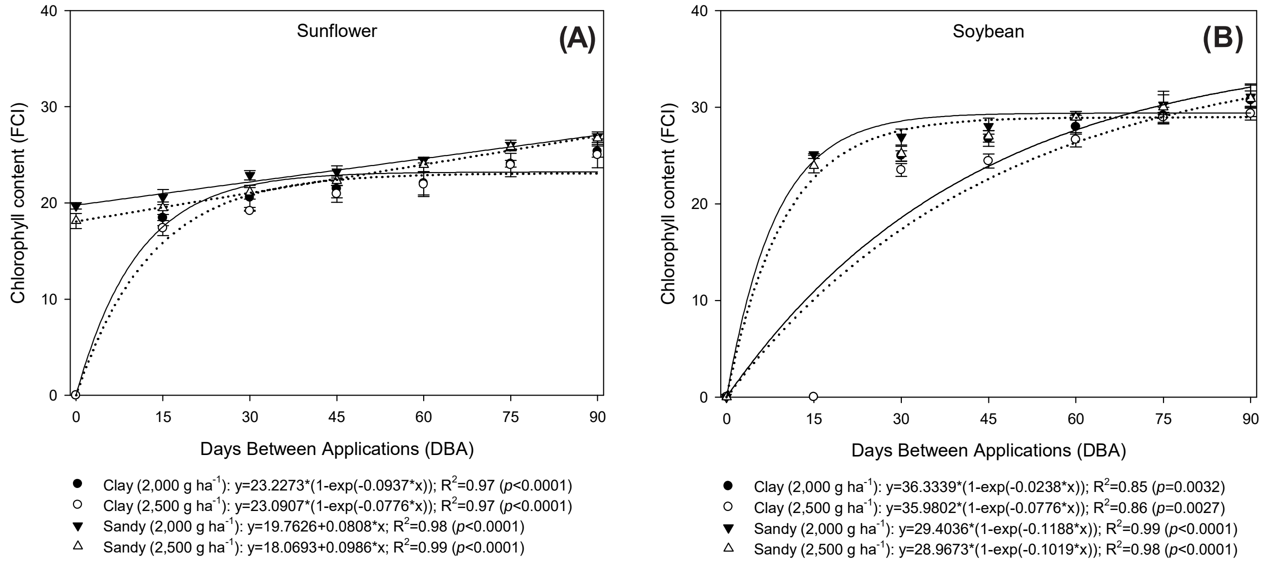


Figure 3 - Chlorophyll content (Falker Chlorophyll Index - FCI) of sunflower (A) and soybean (B) at 0, 15, 30, 45, 60, 75 and 90 Days Between Application (DBA) of atrazine and bioindicator sowing.

transport of plant photosystem II, which acts by blocking electron transport from the action site of plastoquinone Q_A to Q_B, preventing CO_2 fixation and the production of ATP and $NADPH_2$ (Oliveira Júnior, 2011), the lowest chlorophyll value obtained in this research is justified.

Atrazine provided a higher residual effect in clayey soil when compared to sandy soil, resulting in higher percentages of dry matter reduction and lower chlorophyll content for sunflower and soybean bioindicators. Both doses influenced chlorophyll content and percentage decrease of soybean and sunflower dry mass; the higher dose provided a higher percentage of dry mass reduction and lower chlorophyll content in both soil types. Brighenti et al. (2002) found that due to the persistence of atrazine in the environment and its phytotoxic effects on successor crops, a 180 days interval should be adopted between application of the herbicide on maize and the sowing of sunflower.

In addition, sunflower and soybean have been shown to be atrazine-sensitive crops, as the residual effect or bioavailability in the herbicide soil solution interfered with their development, and these crops can be used in bioassays to measure atrazine residue in the soil. Therefore, sowing of both sunflower and soybean is recommended for sandy and clay soils after 90 DBA, because until this time the residue of atrazine interferes with chlorophyll content and percentage of dry mass reduction.

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