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SELECTION OF INDICATOR SPECIES OF THE TEMBOTRIONE SORPTION IN SOILS WITH DIFFERENT ATTRIBUTES

Seleção de Espécie Indicadora da Sorção do Tembotrione em Solos com Diferentes Atributos

ABSTRACT - Studies on herbicide behavior in soils may be performed using biological and chemical methods. The efficiency of the biological method depends on the indicator species sensitivity to low herbicide concentrations in the soil solution. Among the herbicides commonly used in Brazil for corn cultivation, tembotrione stands out. In the last agricultural seasons, the intoxication of some crops when cultivated in succession to corn has been reported, which may be attributed to tembotrione recommendations without the knowledge of their interactions with colloids of tropical soils. In this research, we selected an indicator plant species of tembotrione residues in the soil after sorting 12 species. The sorption of this herbicide was estimated in two Oxisols (Latossolo Amarelo and Latossolo Vermelho-Amarelo, Brazilian Soil Classification) and in a Histosol (Organossolo, Brazilian Soil Classification) with different attributes. Initially, after sorting the 12 plant species, the two most efficient in detecting tembotrione in the soil were selected. In the second stage, the most sensitive species was selected. In the third stage, tembotrione sorption was assessed in the three soils by using the selected species. Among the 12 species, *Beta vulgaris* and *Brassica oleracea* var. *capitata* were the most efficient in detecting tembotrione at low concentrations in the soil, being *B. vulgaris* (beet) the most sensitive. Thus, due to its ease of cultivation, high sensitivity, and fast initial growth, beet can be used as an indicator species of tembotrione presence in the soil solution. The sorption of this herbicide was higher in the Histosol and it is directly related to the organic matter content.

Keywords: bioassay, environmental impact, herbicides, technical efficiency.

RESUMO - Estudos sobre o comportamento de herbicidas no solo podem ser realizados utilizando métodos biológicos e químicos. A condição para o método biológico ser eficiente depende da sensibilidade da espécie indicadora às baixas concentrações do herbicida na solução do solo. Entre os herbicidas comumente usados no Brasil para a cultura do milho, destaca-se o tembotrione; nas últimas safras, tem-se relatado intoxicação de algumas culturas quando cultivadas em sucessão a esta cultura. Esse fato pode ser atribuído a recomendações do tembotrione sem o conhecimento de suas interações com os coloides dos solos tropicais. Nesta pesquisa, selecionou-se uma espécie vegetal indicadora de resíduos do tembotrione no solo, após a triagem de 12 espécies. Estimou-se em seguida a sorção desse herbicida em Latossolo Amarelo, Organossolo e Latossolo Vermelho-Amarelo com diferentes atributos. Inicialmente, após a triagem das 12 espécies vegetais, selecionaram-se as duas mais eficientes em detectar o tembotrione no solo. Na segunda etapa, foi selecionada a espécie mais sensível. Na terceira, foi avaliada a sorção do tembotrione nos três solos utilizando a espécie selecionada.

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*Das 12 espécies avaliadas, **Beta vulgaris** e **Brassica oleracea** var. **capitata** foram as mais eficientes em detectar o tembotrione em baixas concentrações no solo, sendo **B. vulgaris** (beterraba) a mais sensível. Conclui-se que a beterraba, pela sua facilidade de cultivo, alta sensibilidade e rápido crescimento inicial, pode ser usada como espécie indicadora da presença do tembotrione na solução do solo. A sorção desse herbicida foi maior no Organossolo e está diretamente relacionada ao teor de matéria orgânica.*

Palavras-chave: bioensaio, impacto ambiental, herbicidas, eficiência técnica.

INTRODUCTION

The chemical method of weed control is the most used for all crops in Brazil, both by large and small farmers. The high efficiency of herbicides and low costs of the chemical method, when compared to other methods, have been some of the advantages of this technology. However, the use of herbicides without the knowledge on their behavior in the environment can reduce control efficiency and increase the risk of environmental contamination, especially of surface and ground water (Pires et al., 2005; Andrade and Stigter, 2009), as well as compromise their agronomic efficiency (Celis et al., 2005).

Tembotrione belongs to the chemical group of tricetones and it has been widely used in Brazil to control weeds in corn. It presents a solubility of 28 g L⁻¹, pKa of 3.2, and Koc of 66 mL g⁻¹ (USEPA, 2007). This herbicide has been used in the post-emergence of crops and weeds and has a more efficient control action on weeds of the family Poaceae. Tembotrione has a systemic action on plants and acts to inhibit the biosynthesis of 4-hydroxyphenylpyruvate dioxygenase (HPPD), an important enzyme in the carotenoid synthesis route, which is essential in protecting the plant against high light intensity. In sensitive plants treated with this herbicide, a reduction of carotenoid concentration is observed in the leaves. Under this condition, chlorophyll and photosynthetic membranes are degraded by photo-oxidation, which results in the appearance of intoxication symptoms characterized by an intense whitish coloring of leaves, evolving to necrosis and plant death (USEPA, 2007).

Due to the relatively recent use of tembotrione in Brazil, little is known about its behavior in soils under tropical conditions. The knowledge of retention, transport, and transformation processes of tembotrione in the soil can contribute to guarantee its recommendations from an agronomic and environmental point of view. In these studies, several analytical methods can be adopted, such as biological (Pereira et al., 2016), radioisotopic, and chromatographic (Passos et al., 2013), or by the association of two or more methods (Silva et al., 2007). One of the difficulties to study tembotrione behavior in the soil using biological methods is the lack of information of indicator plant species capable of detecting its low concentrations in the soil solution.

The use of the biological method has some advantages in relation to others, among which the low cost stands out and, in some situations, it may be more sensitive than the chemical method to detect low concentrations of herbicides in the soil (Sandín-España et al., 2011). However, the efficiency of the biological method depends on the correct selection of the plant species used as an indicator for the herbicide.

Thus, the aim of this study was to select a plant species indicator of tembotrione residues in the soil, as well as estimate its sorption in Brazilian soils with different attributes (two Oxisols and a Histosol).

MATERIAL AND METHODS

Soil preparation and herbicide application

Soil samples were collected at a depth from 0 to 20 cm in areas with no pesticide use history, in the following sites: Oxisol (Latossolo Amarelo, LA, Brazilian Soil Classification) from Sooretama, ES; Histosol (Organossolo, Brazilian Soil Classification) from Venda Nova do Imigrante, ES; and Oxisol (Latossolo Vermelho-Amarelo, LVA, Brazilian Soil Classification) from Rio Paranaíba, MG.

These samples were characterized by chemical and physical attributes (Tables 1 and 2). Washed sand was also used as an inert substrate. To make the sand inert, it was incubated with HCl solution for 24 hours and then for another 24 hours with NaOH solution. Subsequently, a sequential washing with water was performed until reaching a pH of 7.0 in order to eliminate organic residues.

Table 1 - Results of chemical and physical analysis of soils

Soil	pH	P	K	Ca ²⁺	Mg ²⁺	Al ³⁺	H+Al	(t)	V	m	OM
	(H ₂ O)	(mg dm ⁻³)									(%)
LVA ⁽¹⁾	6.50	2.60	39.00	1.20	0.40	0.00	2.64	1.70	39.00	0.00	2.18
LA ⁽²⁾	6.30	9.60	110.00	2.90	1.00	0.00	1.32	4.18	76.00	0.00	2.20
Histosol ⁽³⁾	5.00	18.10	185.00	5.10	3.00	0.60	26.64	9.17	25.00	31.00	20.20
	Coarse sand	Fine sand	Silt	Clay	Textural class						
	(dag kg ⁻¹)										
LVA	10.00	33.00	16.00	41.00	Clay						
LA	60.00	19.00	1.00	20.00	Sandy loam						
Histosol	14.00	20.00	30.00	36.00	Clay loam						

Analyses carried out in the Laboratory of Soil Analysis Viçosa according to the methodology of the Brazilian Agricultural Research Corporation, Embrapa (1997); (t) = effective cation exchange capacity; V = base saturation; m = Al³⁺ saturation; OM = organic matter; ⁽¹⁾ Oxisol (Latossolo Vermelho-Amarelo, Brazilian Soil Classification) from Rio Paranaíba, MG; ⁽²⁾ Oxisol (Latossolo Amarelo, Brazilian Soil Classification) from Sooretama, ES; ⁽³⁾ Histosol (Organossolo, Brazilian Soil Classification) from Venda Nova do Imigrante, ES.

A CO₂ pressurized sprayer equipped with a boom with two TT 110 02 nozzles spaced 0.50 m and with a volume of 150 L ha⁻¹ was used for herbicide application.

Pre-selection of the indicator plant of tembotrione residues

The species used in this study are described in Table 2.

The experiment was carried out in a completely randomized design in a 5 x 12 factorial scheme with four replications. Factor A consisted of tembotrione doses (0, 2.63, 5.25, 7.89, and 10.50 g ha⁻¹) and factor B of tested plant species (*Brachiaria decumbens*, *Crotalaria juncea*, *Glycine max*, *Phaseolus vulgaris*, *Helianthus annuus*, *Sorghum bicolor*, *Hibiscus esculentus*, *Citrullus lanatus*, *Cucurbita maxima*, *Beta vulgaris*, *Brassica oleracea* var. *capitata*, and *Capsicum annuum*).

Pots with a volumetric capacity of 0.12 dm³ were filled with washed sand and then the herbicides were applied. Subsequently, the substrate was homogenized and transferred back into the pots. Sowing was conducted with five seeds/achenes at 1 cm depth. After emergence, the number of plants was standardized in three seedlings per pot. Plant shoot was collected at 21 days after emergence (DAE) and dried in an oven at 70 °C until constant weight.

The two most sensitive species were selected through the dose-response model of dry matter accumulation of plant shoot as a function of the increase in herbicide dose.

Indicator plant selection of tembotrione

After selecting the two most sensitive species, an experiment was carried out in a completely randomized design in a 2 x 11 factorial scheme with four replications. Factor A consisted of plant

Table 2 - Species used for the selection tests of bioindicator species of tembotrione residues

Scientific name	Common name
<i>Brachiaria decumbens</i>	Brachiaria
<i>Crotalaria juncea</i>	Sunn hemp
<i>Glycine max</i>	Soybean
<i>Phaseolus vulgaris</i>	Common beans
<i>Helianthus annuus</i>	Sunflower
<i>Sorghum bicolor</i>	Sorghum
<i>Hibiscus esculentus</i>	Okra
<i>Citrullus lanatus</i>	Watermelon
<i>Cucurbita maxima</i>	Squash
<i>Beta vulgaris</i>	Beet
<i>Brassica oleracea</i> var. <i>capitata</i>	Cabbage
<i>Capsicum annuum</i>	Bell pepper

species (*B. vulgaris* and *B. oleracea*) and factor B of tembotrione doses (0, 10.08, 20.16, 30.24, 40.32, 50.40, 60.48, 70.56, 80.64, 90.72, and 100.80 g ha⁻¹).

The herbicide was applied in trays with 10 cm height, filled with samples of an Oxisol (LA). After herbicide application, the soil sample contained in each tray was homogenized and placed in containers with a volume of 0.12 dm³, followed by sowing the species to be assessed as indicators. Five seeds/achenes were sown at 1 cm depth. After emergence, three seedlings were standardized per pot. At 21 DAE, the intoxication index was visually assessed, assigning scores from zero (absence of intoxication) to 100 (plant death). Subsequently, shoot and roots of plants were collected and taken to a forced air ventilation oven at 70 °C until constant weight.

The data of accumulated shoot (SDM), root (RDM), and total dry matter (DMTotal) and intoxication were used to select the most sensitive species by using the herbicide dose-response models.

With the values of SDM, RDM, DMTotal, and intoxication, a non-linear log-logistic model was fitted, as proposed by Seefeldt et al. (1995) and adapted to determine the most sensitive variable to the herbicide:

$$Y = \frac{D}{1 + \frac{X^b}{C_{50}}} \quad (\text{eq. 1})$$

where D is the maximum level of the dose-response curve, b is the curve slope around the C_{50} , and C_{50} is the dose-response corresponding to a reduction of 50% in the shoot dry matter of the indicator plant or 50% of intoxication.

Tembotrione sorption in different soils

The experiment was performed in a completely randomized design in a 4 x 11 factorial scheme with four replications, in which factor A consisted of the assessed substrates (Oxisols, Histosol, and washed sand) and factor B of applied tembotrione doses (0, 10.08, 20.16, 30.24, 40.32, 50.40, 60.48, 70.56, 80.64, 90.72, and 100.80 g ha⁻¹). *B. vulgaris* was the bioindicator species used.

The herbicide was applied in a 10 cm high tray filled with soil samples. After application, the soil sample contained in each tray was homogenized and placed in containers with a volume of 0.12 dm³. The indicator plant was then sown. After emergence, three seedlings were standardized per pot. At 21 days after emergence (DAE), plants were assessed regarding herbicide intoxication. Subsequently, plant shoot was collected and taken to a forced air ventilation oven at 70 °C until constant weight.

The data of accumulated shoot dry matter (SDM) were fitted to a non-linear log-logistic model, as proposed by Seefeldt et al. (1995) (Equation 1).

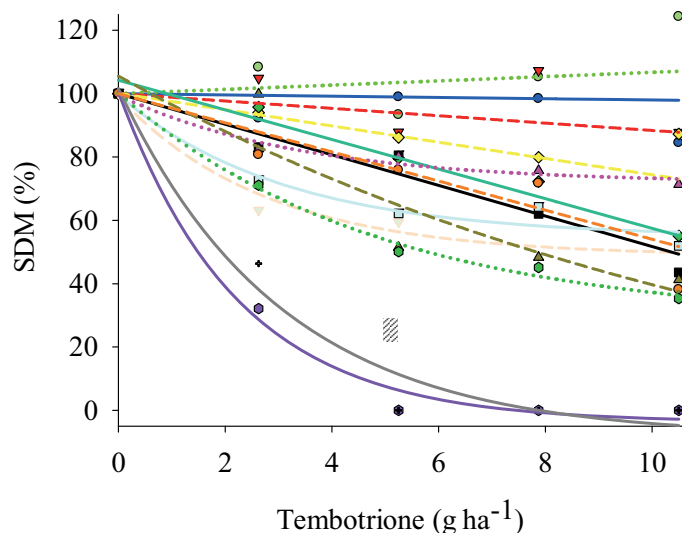
From the data obtained from C_{50} for each soil and sand, the soil adsorption ratio (AR) was calculated in relation to the response obtained in the sand of the indicator species:

$$AR = \frac{C_{50} \text{ SOIL} - C_{50} \text{ SAND}}{C_{50} \text{ SAND}} \quad (\text{eq. 2})$$

RESULTS AND DISCUSSION

Pre-selection and selection of indicator plant

Cabbage and beet were the most sensitive species to tembotrione when grown in sand in the pre-selection experiment (Figure 1).



—●— Cucumber	$\hat{Y} = 99.9875 - 0.1979 * x \quad r^2 = 0.72$
—●— <i>Brachiaria decumbens</i>	$\hat{Y} = 100.0122 + 0.6720 * x \quad r^2 = 0.95$
—▲— Sunn hemp	$\hat{Y} = 48.7349 + 50.1687 * \exp(-0.3586 * x) \quad R^2 = 0.86$
—▼— Soybean	$\hat{Y} = 100.0068 - 1.1619 * x \quad r^2 = 0.99$
—■— Common beans	$\hat{Y} = 99.9893 - 4.8240 * x \quad r^2 = 0.99$
—□— Sunflower	$\hat{Y} = 54.7907 + 44.8454 * \exp(0.3246 * x) \quad R^2 = 0.96$
—◇— Sorghum	$\hat{Y} = 100.0802 - 2.5684 * x \quad r^2 = 0.99$
—◆— Okra	$\hat{Y} = 104.2221 - 4.6736 * x \quad r^2 = 0.99$
—▲— Watermelon	$\hat{Y} = 71.7694 + 27.7468 * \exp(0.2907 * x) \quad R^2 = 0.97$
—▲— Squash	$\hat{Y} = -17.4280 + 122.9978 * \exp(0.0767 * x) \quad R^2 = 0.85$
—●— Tomato	$\hat{Y} = -3.7763 + 104.2767 * \exp(0.4441 * x) \quad R^2 = 0.99$
—●— Beet	$\hat{Y} = -8.3042 + 109.8817 * \exp(0.3277 * x) \quad R^2 = 0.97$
—+— Cabbage	$\hat{Y} = 99.9853 - 4.5937 * x \quad r^2 = 0.99$
—●— Bell pepper	

Figure 1 - Percentage of shoot dry matter (SDM) of *Cucumis sativus*, *Brachiaria decumbens*, *Crotalaria juncea*, *Glycine max*, *Phaseolus vulgaris*, *Helianthus annuus*, *Sorghum bicolor*, *Hibiscus esculentus*, *Citrullus lanatus*, *Cucurbita maxima*, *Beta vulgaris*, *Brassica oleracea* var. *capitata*, and *Capsicum anuum* grown in sand samples after the application of different doses of tembotrione at 21 DAE.

The higher sensitivity of cabbage and beet to tembotrione (Figure 1) may be attributed to their lower ability to degrade the herbicide. For some herbicides, their movements in more tolerant species may be restricted, with a more markedly degradation and metabolism (Flessner et al., 2011). In addition, there may be different vascular tissue arrangements, presence of interstitial meristems, metabolism, and exudation through the root system (Guerra et al., 2014). In studies on the selection of watermelon varieties tolerant to clomazone, an herbicide with the same mechanism of action of tembotrione, a great genetic variability was observed between the most tolerant and susceptible accessions (Howard et al., 2011), which may have allowed separating these two species by genetic similarity. However, the highest beet sensitivity to tembotrione when compared to cabbage may be due to its higher carotenoid content. In fact, tembotrione inhibits the HPPD enzyme, interrupting carotenoid biosynthesis, which leads to the bleaching of the foliage of treated plants and, in the case of sensitive plants, to death (Dayan et al., 2007).

When comparing the two species selected in the preliminary experiment, we observed that the beet is more sensitive to tembotrione (Figure 2).

The highest reduction of beet shoot due to tembotrione is related to its mechanism of action, as previously mentioned. Carotenoids are essential in dissipating excess energy in chlorophyll after light excitation. This excess energy promotes oxidative effects on chlorophyll and

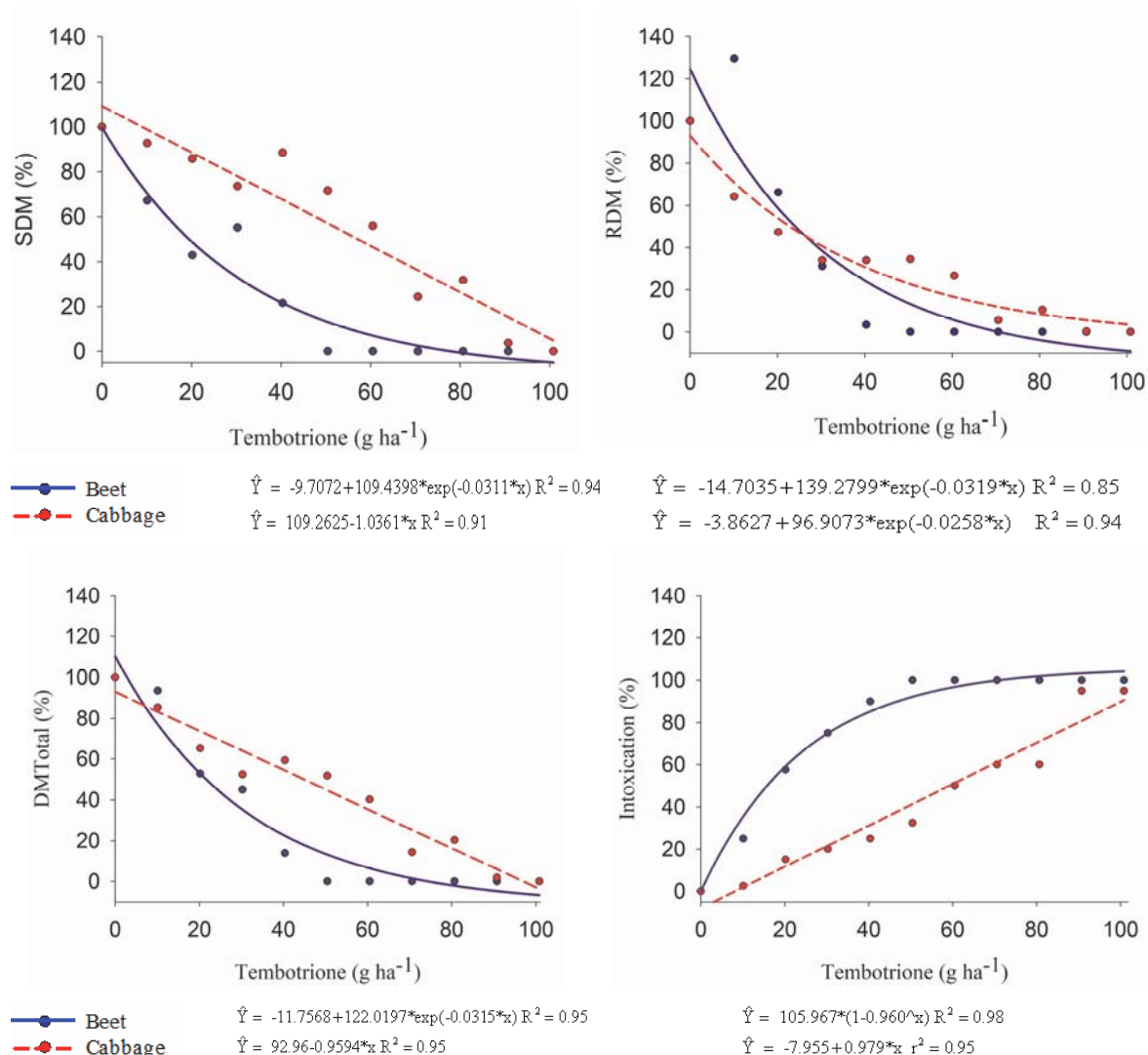


Figure 2 - Percentage of shoot (SDM), root (RDM), and total dry matter (DMTotal) and intoxication (%) of beet and cabbage plants grown in Oxisol (LA) samples after tembotrione application at 21 DAE.

photosynthetic membranes, causing bleaching in young tissues and subsequent necrosis of photosynthetic tissues, which leads to the death of the sensitive plant submitted to the application (Hess, 2000). However, excess energy does not occur in non-photosynthetic tissues, such as in root system (Table 3), and the effects are not readily observed in these organs.

Tembotrione sorption in different soils by indicator plant

Among the analyzed variables, shoot dry matter was the most adequate to study tembotrione sensitivity in species since it presented a higher sensitivity to the treatment, evidenced by the lower C_{50} value (Table 3).

The highest reduction of tembotrione was observed in the Histosol, as evidenced by the values of C_{50} and adsorption ratio of 97.38 g ha⁻¹ and 34.54, respectively (Figure 3 and Table 4). In the Oxisols (LVA and LA), on the other hand, C_{50} values were 16.78 and 19.21 g ha⁻¹, and the adsorption ratio was 5.12 and 6.01, respectively.

The highest tembotrione sorption to Histosol (Figure 3 and Table 4) is due to its higher organic matter content (Table 1), higher specific surface area, and adsorption sites available in this soil (Kearns et al., 2014). In general, the interaction of herbicides with soil colloids occurs through surface interactions, such as hydrogen bonds, and Van der Waals interactions, among

others (Clausen et al., 2001; Kovaios et al., 2006; Vivian et al., 2007), being able to bind to available hydroxyl and carboxylic groups (Liao et al., 2014). In addition to clay and organic matter contents, the herbicide may be more sorbed depending on the stage of soil organic matter decomposition (Li et al., 2003; Si et al., 2006; García-Jaramillo et al., 2014).

The lowest tembotrione sorption in the Oxisols (LVA and LA) (Figure 3 and Table 4) is due to the high contents of sand and low contents of organic matter and clay (Table 1). This relationship with soil texture has also been observed in previous studies with imazapyr (Firmino et al., 2008). A probable obstruction of available clays by a high amount of amorphous compounds of Fe groupings that support oxidic minerals, such as hematite, may occur. These compounds can be aggregated to clay bonding sites, reducing the effective cation exchange capacity (CEC) of soils (Stipicevic et al., 2014), as well as porosity and specific surface area (Paul et al., 2010). This phenomenon may still be associated with a low organic matter content in these Oxisols (Li et al., 2003).

Table 3 - Values of C_{50} calculated as a function of the percentage of shoot (SDM), root (RDM) and total dry matter (DMTotal) of beet plants grown in Oxisol (LA) samples after tembotrione application at 21 DAE and their respective equations

LA	Equation	C_{50} (g ha ⁻¹)
SDM	$\hat{Y} = \frac{(98.570)}{1 + \frac{X^{1.272}}{19.21}} R^2 = 0.93$	19.21
RDM	$\hat{Y} = \frac{(114.777)}{1 + \frac{X^{4.349}}{22.663}} R^2 = 0.97$	22.66
DMTotal	$Y = \frac{(100.388)}{1 + \frac{X^{2.679}}{23.262}} R^2 = 0.98$	23.26
Intoxication	$Y = \frac{(106.135)}{1 + \frac{X^{2.156}}{19.55}} R^2 = 0.99$	19.55

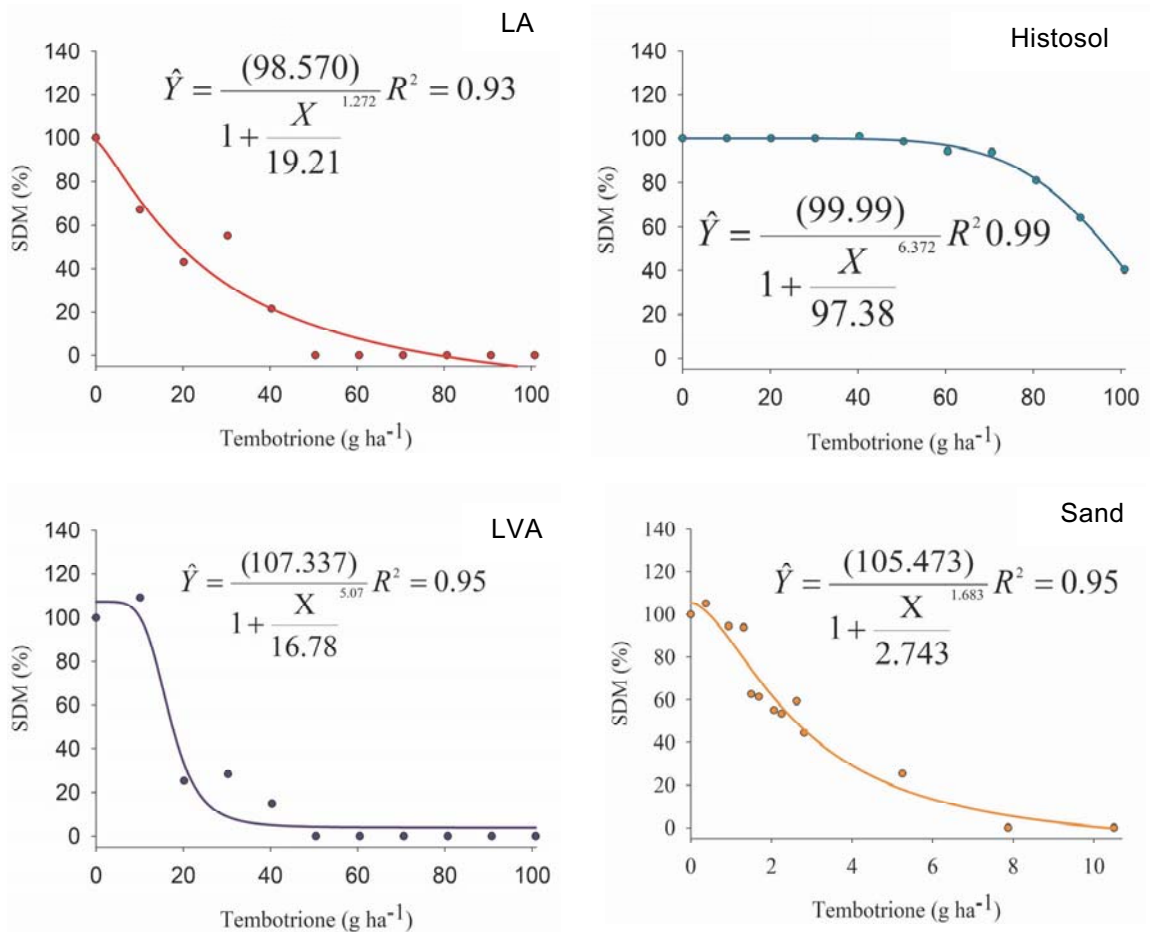


Figure 3 - Percentage of shoot dry matter (SDM) of beet plants grown in samples of an Oxisol (LA), Histosol, Oxisol (LVA), and sand after tembotrione application at 21 DAE.

Table 4 - Values of C_{50} calculated as a function of the percentage of shoot dry matter (SDM) of beet plants and adsorption ratio (AR) of tembotrione in samples of an Oxisol (LA), Histosol, Oxisol (LVA), and sand at 21 DAE

Soil	C_{50}	AR
LA ⁽¹⁾	19.21	6.01
Histosol ⁽²⁾	97.38	34.54
LVA ⁽³⁾	16.78	5.12
Sand	2.74	1.00

⁽¹⁾ Oxisol (Latosolo Amarelo, Brazilian Soil Classification) from Sooretama, ES; ⁽²⁾ Histosol (Organossolo, Brazilian Soil Classification) from Venda Nova do Imigrante, ES; ⁽³⁾ Oxisol (Latosolo Vermelho-Amarelo, Brazilian Soil Classification) from Rio Paranaíba, MG.

Beet (*B. vulgaris*) can be used as an indicator species of tembotrione presence in the soil solution due to its high sensitivity, ease of cultivation, and fast initial growth. Tembotrione sorption was higher in the Histosol and it is directly related to the organic matter content.

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