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Residues of atrazine and tembotrione in the soil affect the initial growth of beets[1]

Resíduos de atrazine e de tembotrione no solo afetam o crescimento inicial da beterraba

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Abstract - The cultivation of beets is frequently done in a crop rotation system with cereals such as corn. In this case, herbicides with residual effect applied during corn growth can harm the establishment and growth of beets grown in succession. Therefore, three experiments were carried out in a greenhouse to evaluate the residual effect of atrazine and tembotrione, in isolation or in a mixture, on the initial growth of beets. The treatments consisted of six dosages corresponding to 0, 10, 20, 30, 40 and 50% of the recommended dosage. The evaluations were on: plant intoxication, shoot dry matter and SPAD index. Atrazine and tembotrione had a negative effect on the initial growth of beets, presenting reduction in the values of shoot dry matter and SPAD index, as well as increase in the intoxication as the dosage also increased. When the herbicides were applied in a mixture, the effect was enhanced, causing the death of plants in the highest dosages (40 and 50%). Due to these results, the cultivation of beets after the application of atrazine and/or tembotrione in corn crops may become impracticable due to the carryover potential in these situations.
Keywords: *Beta vulgaris*; carryover; herbicides

Resumo - O cultivo da beterraba é frequentemente realizado em sistema de rotação de culturas com cereais como o milho. Neste caso, herbicidas com efeito residual aplicados durante o cultivo do milho podem prejudicar o estabelecimento e o crescimento da beterraba cultivada em sucessão. Diante disso, três experimentos foram conduzidos em casa de vegetação para avaliar o

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efeito residual dos herbicidas atrazine e tembotrione, isolados ou em mistura, sobre o crescimento inicial da beterraba. Os tratamentos consistiram de seis doses correspondentes a 0, 10, 20, 30, 40 e 50% da dose recomendada. As avaliações realizadas foram: intoxicação das plantas, matéria seca da parte aérea e índice SPAD. O atrazine e tembotrione provocaram efeito negativo sobre o crescimento inicial da beterraba, apresentando redução nos valores de matéria seca da parte aérea e do índice SPAD, bem como aumento da intoxicação com o incremento das doses. Quando os herbicidas foram aplicados em mistura o efeito foi potencializado, causando a morte das plantas nas maiores doses (40 e 50%). Em função desses resultados, o cultivo de beterraba após a aplicação de atrazine e, ou tembotrione na cultura do milho pode tornar-se inviável devido ao potencial de *carryover* nessas situações.
Palavras-chaves: *Beta vulgaris*; *carryover*; herbicidas

Introduction

Table beets (*Beta vulgaris* L.), commercially grown in Brazil, is a highly important vegetable crop both for the country's food and economy (Tivelli et al., 2011). It presents high contents of iron, besides having sugars, proteins, mineral salts and other beneficial elements that give it an excellent nutritional value. The Brazilian average productivity is between 30 and 40 tons of tuberous roots per hectare; the states of São Paulo, Minas Gerais, Paraná, Santa Catarina and Rio Grande do Sul are the greatest producers (Aphortesp, 2015).

The short cycle of beets is what makes it commonly inserted in a system of succession of crops with cereals, such as corn (Riddle et al., 2013) and other vegetable crops, seeking a better use of the resources in the area and, mainly, the breaking of pathogens and weeds cycle (Tivelli et al., 2011). Crop rotation has been very used in Brazil, but there is a lack of papers in the literature on the residual effect of herbicides used in the previous crops on the sensitive vegetable crops grown in succession, such as beets.

The residue of herbicides in the soil can cause undesirable effects in subsequent crops, named carryover, which is due to the ability to retain integrity of molecules and, consequently, their chemical, physical and functional characteristics in the environment (Mancuso et al., 2011). When the herbicides reach the soil, they are subject to the sorption, transformation and transportation processes, and these compounds can persist for either a short period or for months or years, in the case of those highly persistent (Filizola et al.,2002). The persistence of herbicides may vary according to the physical-chemical characteristics of the molecule and the soil, soil humidity, microbial activity and climatic conditions (Rodrigues and Almeida, 2011).

In face of the complex dynamics of the herbicides in the soil, many farmers do not know the effects caused by the carryover of herbicides. This can affect the growth, productivity, and quality of vegetable crops that are commercialized (Hutchinson et al., 2007; Robinson and Mcnaughton 2012; Sousa et al., 2012; Riddle et al., 2013). Beets are highly sensitive to soil residues, the reason why they are used as a bioindicator species in trials with different herbicides (Szmigielski et al., 2009; Santos et al., 2013; Silva et al., 2014). The growth of beets is usually carried out in succession to the corn crop (Soltani et al., 2005), in which atrazine and tembotrione are frequently applied.

Atrazine (2-chloro-4- (ethylamino) -6- (isopropylamino) -s-triazine) is an herbicide from the group of triazines, a photosystem II inhibitor, recommended for the control of several eudicots weeds and some types of grass in corn crops (Rodrigues and Almeida, 2011). Atrazine's half-life is approximately 75 days, classified as moderately persistent (IUPAC, 2015a). Nakagawa et al. (1995) found residues of this herbicide up to 180 days after application.

Tembotrione 2- [2-chloro-4-Methylsulfonyl-3- [(2,2,2-trifluoroethoxy) methyl] -benzyl] -1,3-cyclohexanedione), a member of the triketones chemical group, inhibits synthesis of carotenoids through direct action on the HPPH enzyme (4-hydroxyphenylpyruvate dioxygenase). It is effective in the control of several eudicots and monocots. According to tembotrione degradation studies carried out in other countries, the dissipation period of 90% (DT90) is 105 days in field conditions, while in laboratory conditions, the tembotrione DT90 got to 262 days (IUPAC, 2015b). However, Silva et al. (2014) evaluated the residual effect of tembotrione in a clay Red-Yellow Latosol, in tropical conditions, and observed that, even 150 days after the application of the herbicide on the soil, the emergence of seedlings of beets was unfeasible.

Therefore, the residual effect of herbicides applied on corn may be harmful to beets crops, causing risks and compromising the rotation/succession system of the area. Moreover, there is no information regarding the residual effect and damage potential for sensitive substitute crops, such as beets on the Brazilian labels of atrazine and tembotrione commercial products, increasing the difficulty in minimizing such problems. Therefore, the objective was to evaluate the residual effect of atrazine and tembotrione, applied in isolation or in a mixture, on the initial growth of beets.

Material and Methods

Three experiments were carried out in a greenhouse. Vases with volumetric capacity of 3.5 dm³ were filled with a sample taken from the 0-20 cm layer of a Red-Yellow Latosol. The planting fertilization was carried out based on the results of soil analysis (Table 1).

Table 1. Chemical characterization of the soil used in the experiment.

pH	S.O.M.	P-rem	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	$CEC_{pH7,0}$
(H_2O)	dag dm^{-3}	mg L^{-1}	mg dm^{-3}		cmol$_c$ dm^{-3}				
5.2	3.9	12.9	8.4	56	2.9	0.7	0.41	7.4	11.14

Each experiment corresponded to the evaluation of an isolated herbicide, namely: Experiment 1: atrazine (Atrazina Nortox 500 SC, 500 g L^{-1}, SC, Nortox), Experiment 2: tembotrione (Soberan, 420 g L^{-1}, SC, Bayer) and Experiment 3: (atrazine + tembotrione). The design adopted was entirely randomized with five replicates. The treatments consisted of six dosages of atrazine (0, 250, 500, 750, 1000 and 1250 g ha^{-1} a.i.), of tembotrione (0, 10, 20, 30, 40 and 50 g ha^{-1} a.i.) and of the mixture (0, 250+10, 500+20, 750+30, 1000+40 and 1250+50 g ha^{-1} a.i. of atrazine and tembotrione, respectively). Each dosage corresponded to 0, 10, 20, 30, 40 and 50% of the recommended dosage for corn crops.

Considering the depth of 0-10 cm of a hectare and the soil density of 1 g dm^{-3}, the dosages corresponded to 0, 250, 500, 750, 1000 and 1250 ppb of atrazine; 0, 10, 20, 30, 40 and 50 ppb of tembotrione; and 0, 250+10, 500+20, 750+30, 1000+40 and 1250+50 ppb of atrazine + tembotrione mixture, respectively. The herbicides were mixed to the soil with the help of a 25 rpm mixer.

After the application of the herbicides and homogenization, ten seeds beets Maravilha cultivar were sown in 04/09/2015, leaving four seedlings after thinning. The cultivation time was 40 days after the emergence date. At 10, 20, 30 and 40 days after emergence (DAE), visual symptoms of intoxication were evaluated, following the scale proposed by SBCPD (1995), in which 0% represented the absence of symptoms and 100% the death of the plant. At 40 DAE, an evaluation was done on the SPAD index on the leaf blade with FALKER clorofiLOG. On the day of the harvest, the shoot of plants was collected and took to dry in a forced-air circulation greenhouse at 70 ºC, until it reached constant mass. After that, the shoot dry matter was obtained.

The data was submitted to the ANOVA, and the effect of the dosages was analyzed by regression ($p<0.05$). The choice of models linear, sigmoidal and exponential was based on the significance of the regression coefficients, on the biological phenomenon and on the determination coefficient.

Results and Discussion

Atrazine carryover

Atrazine caused low intoxication on beet plants, regardless of the dosage, in the evaluation at 10 DAE, with maximum intoxication of approximately 5% in the highest dosage (Figure 1A). The biological activity of an herbicide in the plant happens according to the absorption, translocation, metabolism and sensitivity of the plant to this herbicide and/or its metabolites (Silva et al., 2007).

There was an increase in intoxication with the increase in the dosage at 20 DAE (Figure 1B). At 30 and 40 DAE (Figures 1C and 1D, respectively) the plants presented 12.7 and 30.0% of intoxication, respectively, in the dosage of 1250 ppb. The gradual increase in intoxication can be related to the higher exposure and absorption of the herbicide present in the soil with the growth of the roots.

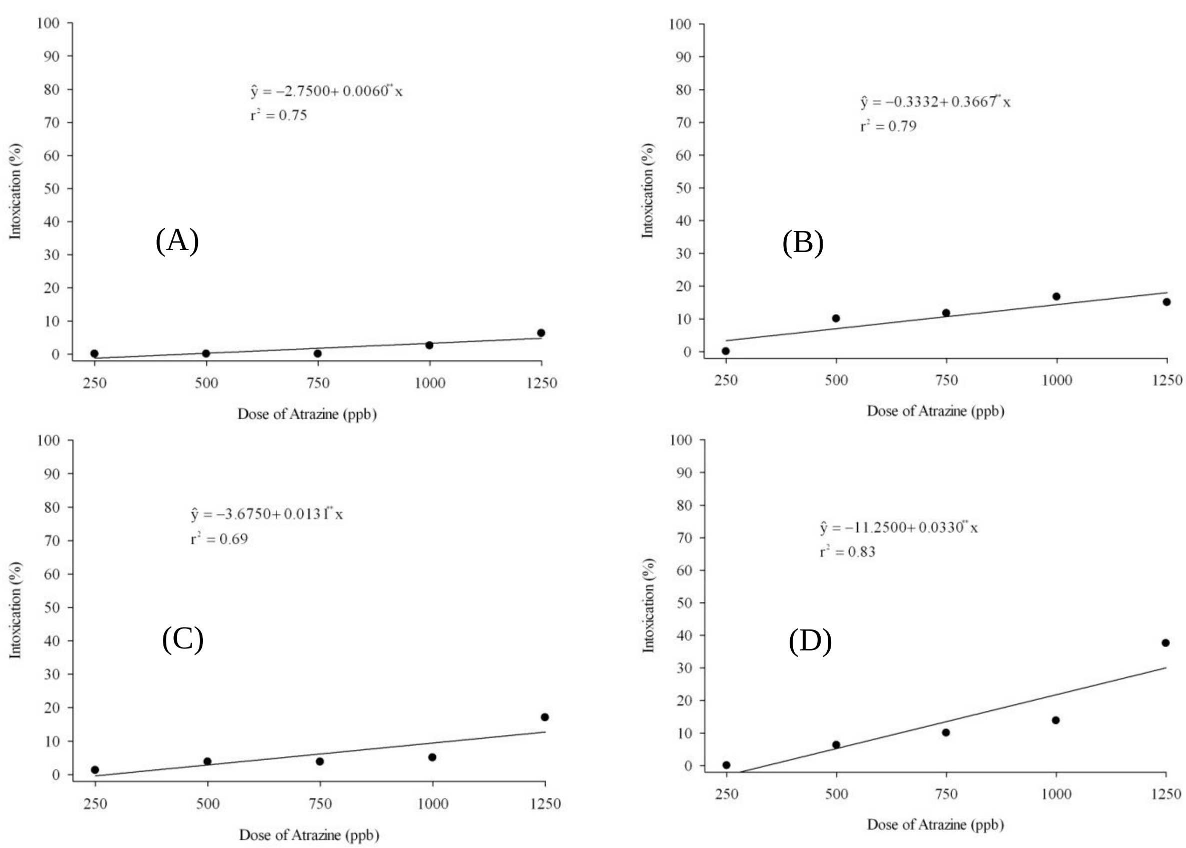

Figure 1. Intoxication of beet plants cultivated in soil with increasing dosages of atrazine in different evaluation days (A) 10 DAE, (B) 20 DAE, (C) 30 DAE and (D) 40 DAE.

The SPAD index was linearly reduced with the increase in the dosage of the herbicide (Figure 2A), which is directly related to the symptoms caused on the plant by atrazine, characterized by chlorosis of the leaves, resulting in lower green intensity. The SPAD index (Soil Plant Analysis Development) analyzes, in real time, the intensity of green in the leaves, and has positive and significant correlation with the chlorophyll content in several annual and vegetable crops (Wood et al., 1993). The inhibition of photosystem II caused by this herbicide results in the production of oxygen reactive species and in the destruction of chlorophyll in the treated plants, also justifying the reduction of the leaf blade SPAD index.

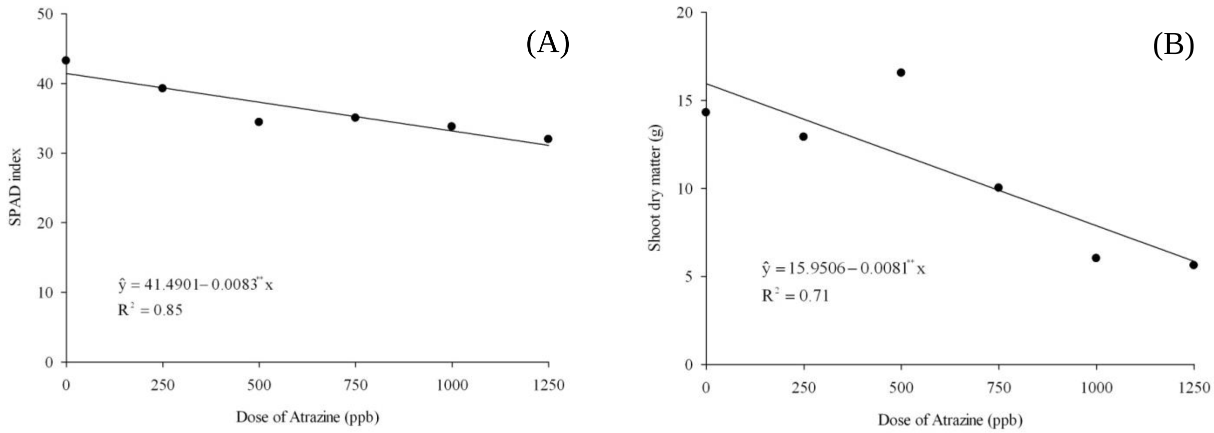

Figure 2. SPAD index (A) and shoot dry matter (B) of beet plants cultivated in soil with different dosages of atrazine.

With the increase in the dosages of atrazine in the soil, there was a linear reduction of shoot dry matter of beets (Figure 2B). The presence of 1250 ppb of atrazine in the soil caused a reduction of 63% in this variable (Figure 2B). Marchesan et al. (2011), when evaluating other species of vegetable crops, also found decreasing levels of dry matter as the dosage of atrazine in the soil was increased.

Tembotrione carryover

For tembotrione, regardless of the evaluation time, there was an increase in intoxication with the increase of the dosage (Figure 3), getting to much higher levels than the ones observed on atrazine.

At 10 and 20 DAE, the dosage of 40 ppb caused intoxication close to 60 and 70%, respectively, with 90% intoxication in the highest dosage. In the evaluations at 30 and 40 DAE, the intoxication increased up to the dosage of 50 ppb, leading to the death of the plants. In these evaluations, the intoxication verified in the smallest dosage was equal to or higher than 50% (Figure 3). Such results show the residual effect of tembotrione in the soil, agreeing with the paper by Blanco and Caetano (2008), who used the bioassay methodology with the beets and, with the results, inferred that tembotrione, regardless of the dosage, persisted until 55 days after the treatment.

The SPAD index was reduced starting from 20 ppb (Figure 4A), and the dosage of 36.6 ppb of tembotrione was responsible for reducing this variable in 50%. The action mechanism of tembotrione happens by the inhibition of the synthesis of carotenoids, causing leaf chlorosis until albinism in the plants. In the present study, chlorosis was observed on the leaves, causing reduction of the green color intensity.

The shoot dry matter of beet plants reduced with the increase of the dosages of tembotrione in the soil (Figure 4B), showing the effect of the herbicide on the growth of the plant, which reduced 77% of the accumulation of the shoot dry matter and in the highest dosages caused the death of the plant. Plants intoxication and reductions of dry matter and productivity of beets were seen in studies carried out in Canada, whether one year after the application of mesotrione (70 to 560 g ha^{-1}) or growing the vegetable crops right after the application (7 to 56 g ha^{-1} a.i.) (Riddle et al., 2013).

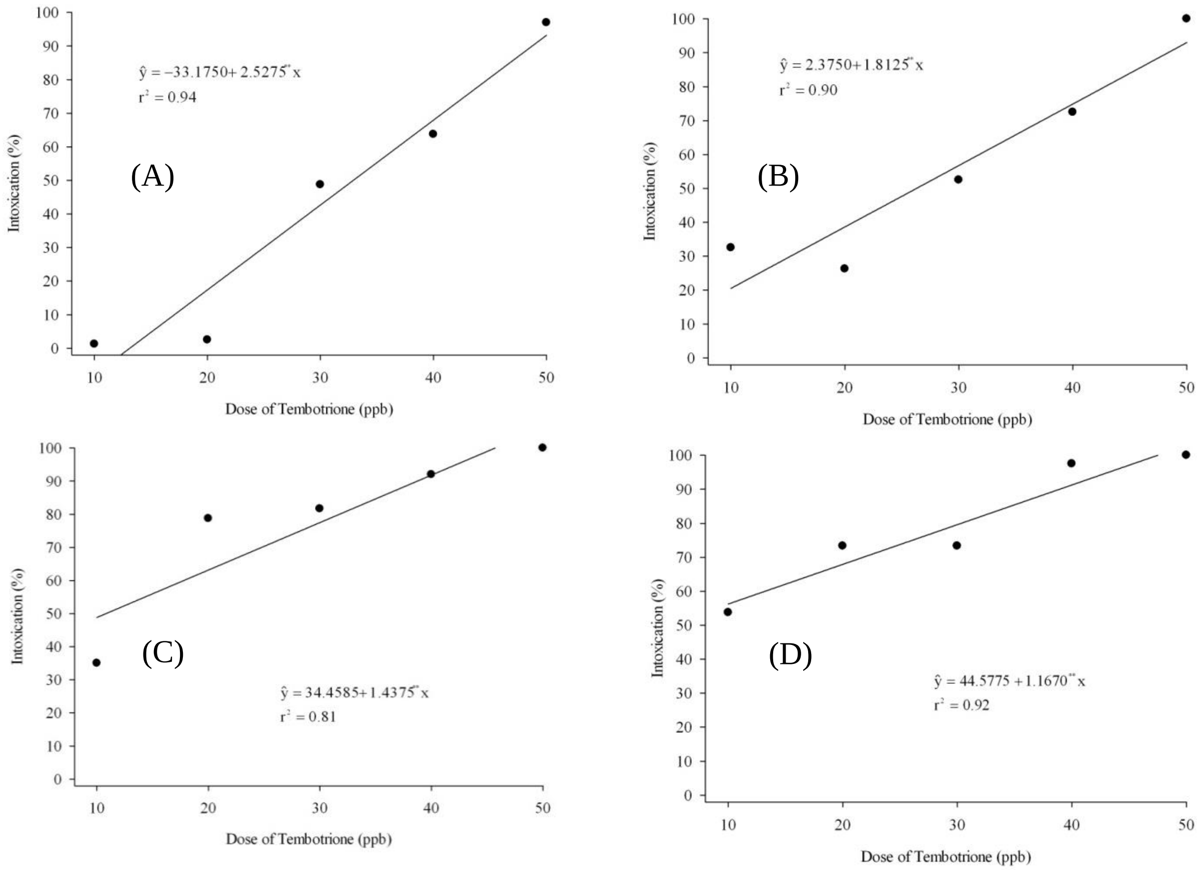

Figure 3. Intoxication of beet plants cultivated in soil with different dosages of atrazine in different evaluation days (A) 10 DAE, (B) 20 DAE, (C) 30 DAE and (D) 40 DAE.

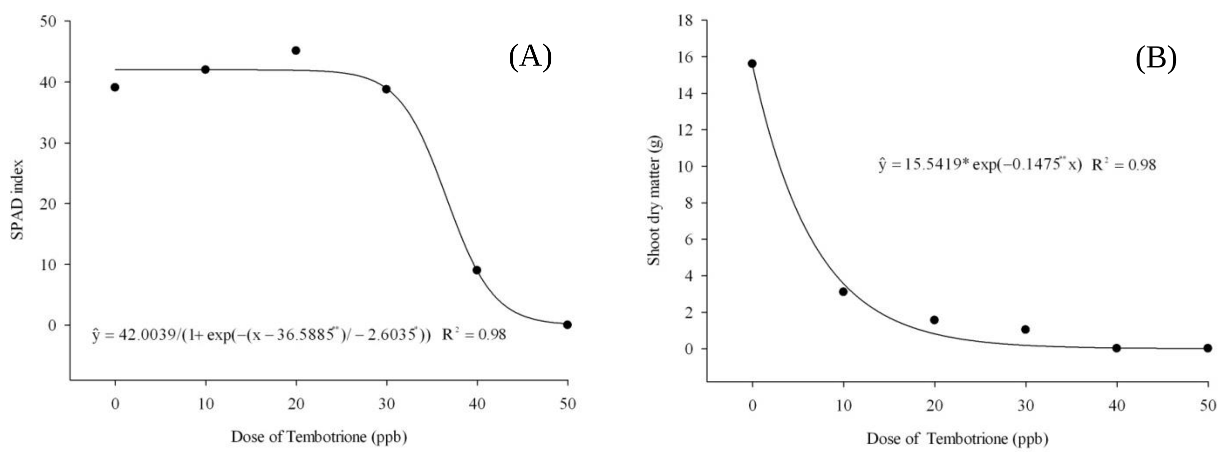

Figure 4. SPAD index (A) and shoot dry matter (B) of beet plants cultivated in soil with different dosages of tembotrione.

Other studies about the carryover of herbicides in the beet crop were carried out in Canada, the USA and in Chile with molecules that inhibit PROTOX (saflufenacil) and ALS (imazosulfuron, imazapyr, imazapic and imazethapyr). In these papers, it was proven that there was residual activity of the herbicides even after one or two years after the application, as well as reduction of growth, productivity, and quality of tubes collected from beets cultivated in the area (Alister and Kogan, 2005; Felix et al., 2012; Robinson and Mcnaughton, 2012).

Atrazine + tembotrione carryover

Regardless of the evaluation time, there was an increase in intoxication with the increase of the dosage of herbicide mixture (atrazine + tembotrione) (Figure 5). Greater efficiency was observed in the mixture (atrazine + tembotrione) when compared to tembotrione in isolation in the control of corn weeds (Zagonel and Fernandes, 2007).

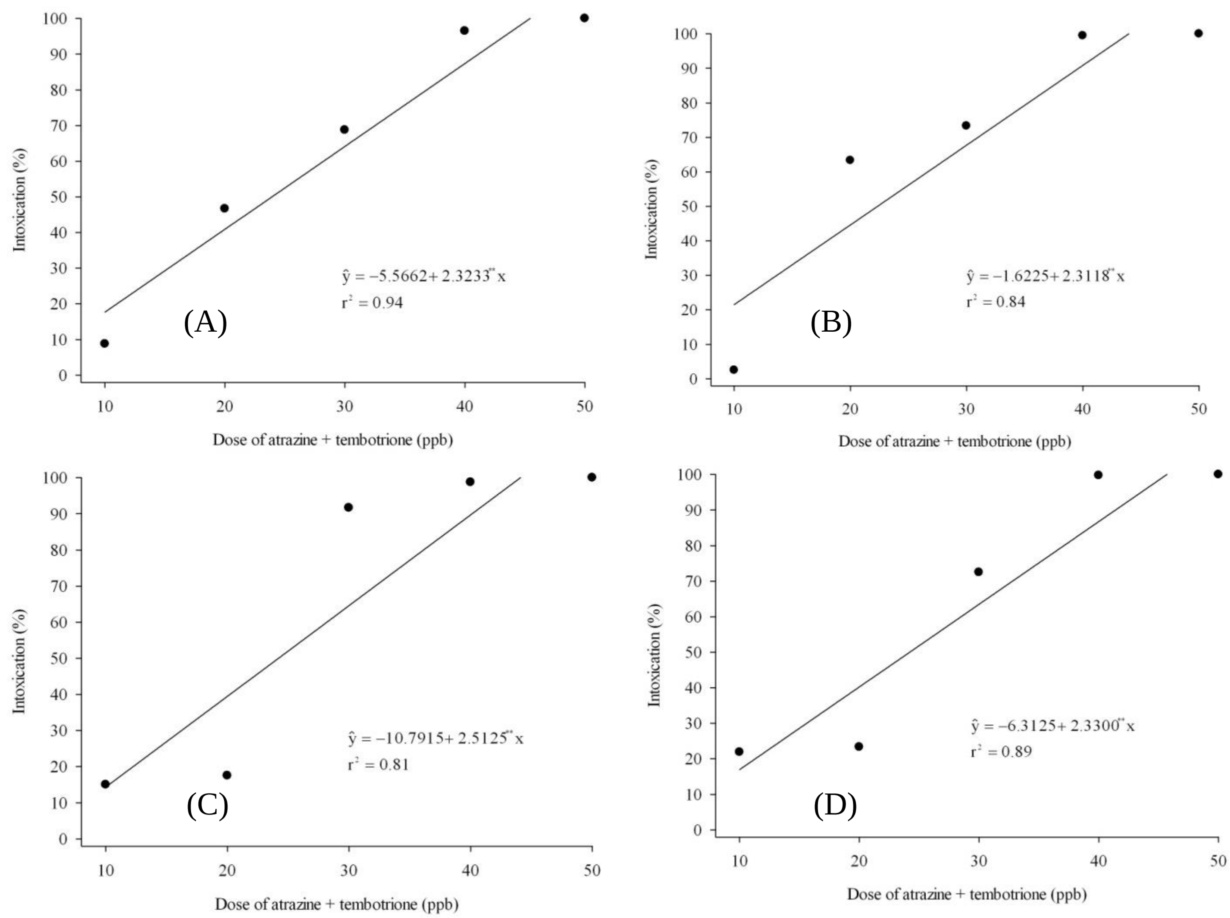

Figure 5. Intoxication of beet plants cultivated in soil with different dosages of the mixture (atrazine + tembotrione) in different evaluation days (A) 10 DAE, (B) 20 DAE, (C) 30 DAE and (D) 40 DAE.

The SPAD index had a drastic decrease with the increase in the dosage starting from 30% (Figure 6A). The mixture of a photosystem II inhibitor herbicide with a carotenoids synthesis inhibitor intensified the visual symptoms of chlorosis on the leaves. There was a reduction in the shoot dry matter with the increase in the dosage of herbicides mixture (Figure 6B), and the maximum dosage caused the death of the plants.

Soltani et al. (2005) observed that isoxaflutole, an herbicide that has the same action mechanism as tembotrione, in the dosages of 105 and 210 g ha^{-1} applied in pre-emergence of the corn crop one year before the cultivation of beets reduced the stand in 33 and 42%, the dry matter in 51 and 57% and the productivity in 40 and 60%, respectively. When assessing the mixture isoxaflutole + atrazine (105 + 1063 and 210 + 2126 g ha^{-1}) the stand reduction was 39 and 58%, the dry matter reduction was 56 and 73%, and productivity decreased 52 and 82%, respectively (Soltani et al., 2005). Such results corroborate the ones found in the present study, proving the increase in the damages caused on beet plants due to the presence of the mixture atrazine + tembotrione in the soil.

The sensitivity of vegetable crops to herbicide residues on the soil reinforces the need to know their application history in the area and the lack of information in the literature, especially on the Brazilian labels of registered products for the rotational crops, regarding the restriction period for sensitive vegetable crops in areas where persistent molecules were applied.

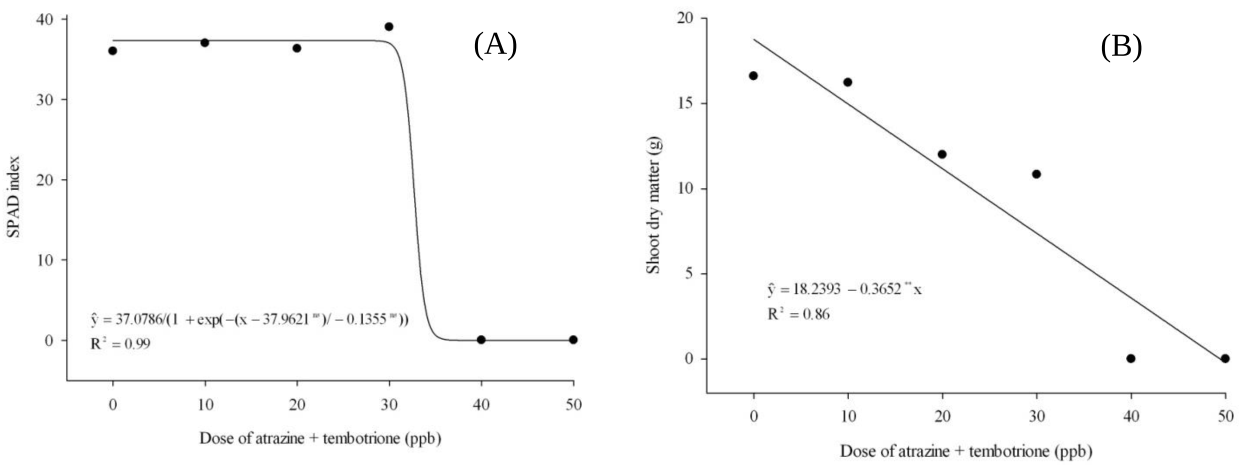

Figure 6. SPAD index (A) and shoot dry matter (B) of beet plants cultivated in soil treated with the mixture (atrazine + tembotrione)

Conclusions

Atrazine and tembotrione residues in the soil, applied in isolation or in a mixture, because negative effects on the initial growth of beets.

The cultivation of beets after the application of atrazine and/or tembotrione in corn crops may become impracticable due to the carryover potential in these situations.

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