Sensitivity of forage turnip to the herbicide tepraloxydim¹

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ABSTRACT

The objective of this study was to evaluate the responses of *Raphanus sativus* to the application of the herbicide tepraloxydim by analyzing photosynthetic and biometric characteristics of the plants. The experiment was conducted in a greenhouse and treatments were instituted when the plants had five expanded leaves. The herbicide tepraloxydim was applied at doses of 0, 75, 100, and 125 g of active ingredient per hectare (g a.i. ha⁻¹). The herbicide dose of 125 g a.i. ha⁻¹led to a small decrease in the photosynthetic rate, water use efficiency, effective quantum yield of PSII, rate of electron transport, and the concentration of chloroplastidic pigments in the leaves of *R. sativus*. On the other hand, there was a small increase in CO₂ concentration in the substomatal chamber. The number of branches, leaves, flowers, plant height and dry mass of the stem and flowers were reduced more prominently in response to herbicide doses than the photosynthetic characteristics. Therefore, treatment with tepraloxydim inhibits the growth and formation of the leaves, branches and flowers of *R. sativus* at the stage of development analyzed. However, doses of 75, and 100 g a.i. ha⁻¹ neither compromise the photosynthetic apparatus nor the stability of cell membranes.

Keywords: acetyl-coenzyme A carboxylase; herbicide; photosynthesis; Raphanus sativus; selectivity.

INTRODUCTION

The forage turnip (*Raphanus sativus* L.), which belongs to the Brassicaceae family is usually used in crop rotation since it has high potential in recycling nutrients, mainly phosphorus and nitrogen (Crusciol *et al.*, 2005; Heinz *et al.*, 2011). This species also tolerates adverse environmental conditions, such as low precipitation and high temperatures (Abdel *et al.*, 2014; Chen *et al.*, 2014).

Considering that about 36% of the dry matter of the forage turnip seed is composed of lipids (de Souza *et al.*, 2010), this species has is a potential source of raw material for the production of biodiesel. The cost of producing biodiesel from the forage turnip is lower than the costs

from other traditional crops, as for example, the soybean (Chammoun *et al.*, 2013). In addition, the by-product of forage turnip obtained from the extraction of oil from its seeds may be used in animal feed (de Souza *et al.*, 2010). Thus, the fore mentioned characteristics demonstrate that the cultivation of forage turnip can be promising and guarantee profitability to the farmer.

In general, the maximum productivity potential of crops depends on weed management because competition with the weed community limits access to water, nutrients, and light, causing productivity losses (Rigoli *et al.*, 2008). Although it has great potential in the agricultural scenario, the crope of the forage turnip is still little explored and to

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date there are no registered herbicides for forage turnip cultivated, only for the management of the genotype that is weed.

As a dicotyledonous, an alternative for the management of the weed community in the production of forage turnip may be the use of graminicides of the aryloxyphenoxypropionate (APP) and cyclohexanedione (CHD) classes (Belkebir & Benhassaine-Kesri et al., 2013), which both inhibit Acetyl-Coenzyme A Carboxylase (ACCase) (Kaundun, 2014). However, several species among the dicotyledons are susceptible to ACCase inhibitors, such as colza and crambe (Belkebir & Benhassaine-Kesri, 2013; Concenço et al., 2014). These species, as well as the forage turnip, belong to the Brassicaceae family. Studies with soybean plants (Belkebir et al., 2006), peanut (Fayez et al., 2014), melon and cucumber (Vidal et al., 2000), and some species of the family Geraniaceae also demonstrated the sensitivities of these species to this class of herbicides (Christopher & Holtum, 2000).

Plant susceptibility to ACCase inhibitor herbicides can be observed by physiological changes, such as decreased photosynthetic rate and water use efficiency and disturbances in electron transport at the photochemical stage of photosynthesis and membrane permeability (Dayan & Watson, 2011; Dayan & Zaccaro, 2012; Concenço *et al.*, 2014). ACCase is responsible for the formation of malonyl-CoA, which is required in the synthesis of fatty acids and secondary metabolites, such as suberins and flavonoids (Kaundun, 2014). Two forms of ACCase are generally found in plants: one heteromeric form in plastids and the other homomeric form in the cytoplasm (Sasaki & Nagano, 2004).

In this way, enzyme inhibitor herbicides can be used safely with dicotyledons because these herbicides inhibit only the homomeric form of ACCase. The opposite occurs with the species of the Poaceae family since they have only the homomeric form of ACCase, both in the cytoplasm and in the plastids. Since about 80% of the ACCase activity in the leaves occurs in plastids, inhibition of this enzyme leads to the death of species of the family Poaceae (Délye, 2005).

The herbicide tepraloxydim (an ACCase inhibitor), belongs to the chemical group of CHD - (EZ)–(RS) -2–{1-[(2E)-3-chloroallyloxymino] propil}-3-hydroxy-5-perhydropyran-4-ylcyelohex-2-em-1-one, has a mechanism of systemic action. This herbicide is recommended in postemergence for some dicotyledons, such as soybeans, cotton, and common beans (maximum dose of 0.5 liter per hectare) (Rodrigues & Almeida, 2011). However, the occurrence of sensitivity in dicotyledon species to ACCase inhibitors (Belkebir & Benhassaine-Kesri, 2013; Concenço *et al.*, 2014) reinforces the need for studies on the selectivity

of the crop of interest for proper management of grasses before specific herbicides can be recommended and thus avoid losses for farmers (Vidal *et al.*, 2000).

Considering that the forage turnip is a member of the Brassicaceae, as are the dicotyledons crambe and colza, which have been shown to be sensitive to ACCase inhibitors (Belkebir & Benhassaine-Kesri, 2013; Concenço et al., 2014), we evaluated the hypothesis that the herbicide tepraloxydim, representative of the chemical group of CHD, in the doses of 75, 100, and 125 g a.i. ha¹ may cause damage the photosynthetic and biometric characteristics of *R. sativus* plants. Therefore, the objective of this study was to evaluate the responses of *R. sativus* plants to the application of the herbicide tepraloxydim by analyzing photosynthetic characteristics, membrane permeability, and biometric measurements.

MATERIAL AND METHODS

Plant Material and Experimental Conditions

The experiment was conducted under a randomized block design in a greenhouse. Seeds of Raphanus sativus (cultivar CATIAL 1000) were sown in a mixture of two parts of dystroferric red latosol with one part fine sand, with the purpose of having one plant per pot. Each pot containing 4 dm³ of substrate, 2 g of dolomitic limestone were added, with a relative neutralization power of 100%. Subsequently, fertilization was carried out with 2.8 g of urea, 1.7 g of mono-ammonium phosphate, 2 g of potassium chloride, 0.9 g of magnesium sulfate, 0.02 g of copper sulfate, 0.14 g of zinc sulfate, and 0.03 g of boric acid. The liming and adduction were performed based on the results of the chemical and physical analyses of the substrate, which presented the following composition: pH H₂O - 5.8; P = 0.9 mg dm^{-3} ; $K = 9 \text{ mg dm}^{-3}$; $Ca = 0.59 \text{ cmol dm}^{-3}$; Mg = 0.17 $cmol_{2}dm^{-3}$; $Al = 0.05 cmol_{2}dm^{-3}$; $H + Al = 1.8 cmol_{2}dm^{-3}$; S $= 0.8 \text{ mg dm}^{-3}$; B = 0.1 mg dm⁻³; Cu = 0.5 mg dm⁻³; Fe = 118 $mg dm^{-3}$; $Mn = 16.7 mg dm^{-3}$; $Zn = 0.2 mg dm^{-3}$; Na = 1.8 mgdm⁻³; base saturation = 30%; cation exchange capacity = 2.6 cmol dm-3; organic matter = 6.2%; clay = 38.5%; silt = 7.5%; and sand = 54%.

The herbicide tepraloxydim was applied at 26 days after sowing *R. sativus* (4 to 5 leaves). The treatments consisted of the following doses: 0 (only water), 75, 100, and 125 grams of active ingredient per hectare (g a.i. ha⁻¹). These doses correspond to 0, 75, 100, and 125%, respectively; of the recommended dose of the product for the bean, cotton, and soybean crops, as well as *R. sativus*, all of which are dicotyledons. Mineral oil was used in the spray mixture at the concentration of 0.5%. For application, a sprayer was used with constant pressure, maintained by compressed CO₂, with a bar containing four spray nozzles and nozzle series. The volume of the spray mixture used was 200 L.ha⁻¹.

The gas exchanges were measured at 3, 7 and 11 days after application of the herbicide (DAAH). The chlorophyll *a* fluorescence image and the concentration of chloroplastidic pigments were analyzed at 11 DAAH, whereas the biometric characteristics of the plants were determined at 12 DAAH.

Gas exchange

Gas exchange from R. sativus plants was measured in fully expanded leaves to determine the net photosynthetic rate $(A, \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1})$, stomatal conductance $(g_s, \text{mol H}_2\text{O m}^{-2} \text{ s}^{-1})$, transpiration rate $(E, \text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1})$ and ratio between internal (C_i) and external (C_a) CO₂ concentrations (C_i/C_a) . The water use efficiency (WUE) was calculated as the ratio between A and E. The parameters were measured using an infrared gas analyzer (IRGA, model LI6400xt, Li-Cor, Nebraska, EUA). The measurements of A, g_s , E, and C_i/C_a were performed between 8:00 and 11:00 am under constant photosynthetically active radiation $(PAR, 1000 \mu \text{mol photons m}^{-2} \text{ s}^{-1})$ and temperature $(25 \, ^{\circ}\text{C})$.

Chlorophyll a fluorescence

The data and images of chlorophyll a fluorescence were measured and obtained in the same leaf of the photosynthesis using a modulated imaging-PAM fluorometer (Heinz, Walz, Effeltrich, Germany). For the measurements, leaves were initially dark-acclimated for 40 min so that the reaction centers were fully opened to obtain the minimal (F_0) and maximal chlorophyll fluorescence $(F_{\rm M})$. From these values, the potential quantum yield of PSII $[F_V/F_M = (F_M - F_0)/F_M]$ was calculated according to Genty et al. (1989). After sample illumination, saturation pulses were applied to determine the light-acclimated variables: the quantum yield of photochemical energy conversion in PSII (Y_{II}), the quenching of regulated (Y_{NPO}) , and the nonregulated (Y_{NO}) non-photochemical dissipation. The Y_{II} was also used to estimate the apparent electron transport rate ($ETR = Y_{II} \times PAR \times A_{leaf} \times 0.5$) (Bilger et al., 1995), where PAR is the photon flux (μ mol m⁻² s⁻¹) on the leaves, A_{leaf} is the amount corresponding to the fraction of incident light absorbed by the leaves, and 0.5 is the excitation energy fraction directed to the PSII (Laisk & Loreto, 1996).

Photosynthetic pigments

Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were measured according to Costa *et al.* (2014). Three leaf discs (0.6 cm²) were immersed in 5 mL of a dimethyl sulfoxide (DMSO) solution saturated with calcium carbonate and incubated at 65<°C for 24 hours. Absorbance was measured at 665.1, 649.1, and 480.0 nm in a UV-VIS

spectrophotometer (model 60S, Thermo Scientific, Madison, USA), and the concentrations were calculated according to Wellburn (1994) and expressed by the leaf area.

Rate of electrolyte leakage

Leaf discs (0.6 cm⁻²) were immersed in ultrapure water, and the initial conductivity was measured after 24 h. The samples were then kept at 100 °C for 1 h, and the final conductivity was evaluated. The electrical conductivity of the leaf sample was measured using a conductivity meter (CD-850 model, Instrutherm, Brazil), and the rate of electrolyte leakage (%) was calculated according to Silva *et al.*, (2014).

Biometric analyses

The plants were measured to determine their height (H, m), number of leaves (NL), number of nodes (NN), number of branches (NB), number of flowers (NF), and stem diameter (SD, mm). Leaves, stems and flowers were separated, packed in paper bags and dried in a forced-air-circulation oven (65 °C) for 48 h to obtain leaf dry matter (LDM, g), stem dry matter (SDM, g), and flowers dry matter (FDM, g).

Statistical analyses

The obtained data were submitted to analysis of variance and to the adjustment of the logistic nonlinear regression model, represented by the following formula: $\hat{y} = a/(1+(x/b)c)$, where a = value of the analyzed variable in the lowest dose of the herbicide, b = dose of the herbicide responsible for the 50% decrease of the analyzed variable (I_{50}), and c = slope of the curve around the I_{50} (de Souza *et al.*, 2000). For the variables that did not fit the logistic model, a linear model was used. Action Stat 3 software was used for the statistical analysis of the data, and the graphics were made using the software SigmaPlot V.10 (SPSS Inc., USA).

RESULTS AND DISCUSSION

The application of the herbicide tepraloxydim at the lowest doses did not cause visual damage to the leaves of *R. sativus* plants (Figures 1A, B, and C). However, the dose of 125 g a.i. ha⁻¹ affected the formation of new leaves, evidenced by the leaf wrinkling symptoms (Figure 1D). These symptoms of wrinkling on young leaves, observed at seven DAAH, suggest that the synthesis of lipids, which are essential constituents of membranes, was limited. Thus, heteromeric ACCase probably did not supply all the acetyl-CoA carboxylation to malonyl-CoA that was required, compromising leaf growth zones by inadequate formation of cell membranes and organelles (Délye, 2005).

The probable inhibition of the homomeric ACCase by the herbicide tepraloxydim also compromised the growth and development of *R. sativus*. Therefore, the formation of new structures, such as branches and flowers, and the height of the plants were inhibited by the application of the different doses of the herbicide (Figure 2).

The numbers of leaves (NL), branches (NB) and flowers (NF) as well as the dry matter weights of the stems (SDM), flowers (FDM), and the plant height (H) all decreased as a function of the doses of the herbicide tepraloxydim (Figure 2A, C, G, D, H, and F). As indicated by the lower values of I₅₀, which represent the dose of the herbicide responsible for the 50% decrease of the analyzed variable (de Souza et al., 2000), that the most sensitive biometric characteristics were FDM (83.01 g a.i. ha⁻¹), NB (97.06 g a.i. ha⁻¹), and H (107.75 g a.i. ha⁻¹). However, it was not possible to adjust leaf dry matter (LDM) to the regression model and R. sativus stem diameter (SD) did not differ as a function of the herbicide dose (Figure 2B and E). In this sense, Vidal et al. (2000) also observed reductions in the biometric characteristics of cucumber and melon plants. The leaf area and total dry matter of the plants were reduced in response to application of the ACCase inhibitor herbicide fluazifop-p-butyl (Vidal et al., 2000). The decrease of LDM (approximately 50%) in Brassica napus plants treated with the herbicide sethoxydim was also reported by Belkebir & Benhassaine-Kesri (2013). However, in this work with R. sativus plants, there was no change in LDM.

There were a reduction in the chlorophyll and carotenoid content in *R. sativus* in response to the doses of the herbicide tepraloxydim (Figure 3). This supports the hypothesis that this herbicide restricted the biosynthesis of lipids, which are essential constituents of membranes, including chloroplast membranes. Corroborating this hypothesis, Fayez *et al.* (2014) reported that the herbicide fluazifop-p-butyl also reduced the concentration of chloroplastidic pigments from leaves of newly formed peanut plants. Similarly, the chlorophyll content of leaves of *B. napus* was reduced in response to treatment with the

herbicide sethoxydim (Belkebir & Benhassaine-Kesri, 2013). Although there was a reduction in the chloroplast pigment content in *R. sativus* in this study, this was not enough to trigger chlorosis and foliar necrosis, which was observed in peanut plants (Fayez *et al.*, 2014).

In addition to the biometric variables and the chloroplast pigment content, the parameters of gas exchanges photosynthetic rate (A), instantaneous water use efficiency (WUE), internal concentration of $CO_{2}(C_{2})$, and the ratio between the internal and external CO, concentration (C/C_a) differed as a function of the doses of tepraloxydim (Figure 4A, D, E, and F). There was also a difference as a function of the DAAH factor since the measurements were performed at 3, 7, and 11 DAAH. These variations in A, g_s , WUE and transpiration rate (E) are due exclusively to the environmental conditions at the time of analysis and to the differentiation of the plant material as a function of the phenological stage change (Figure 5A, B, C, and D). Variables A and WUE decreased in response to increased herbicide rates and C_i , and C/C_a increased. However, these changes were not of high order, with the decrease and increase more accentuated only at the higher dose of 125 g a.i. ha⁻¹.

The reduction in A in young leaves of R. sativus in response to ACCase inhibitor action was also reported in Crambe abyssinica plants treated with the herbicides sethoxydim, fluazifop-p-butyl, and clethodim (Concenço et al., 2014). Already Xia et al. (2006) reported a marked decrease (up to 63%) in CO_2 assimilation in cucumber plants treated with the fluazifop-p-butyl and haloxyfop-p-methyl herbicides at doses of about 40 and 27 g a.i. ha⁻¹, respectively. Considering that ACCase inhibitors block lipid synthesis, the action of these herbicides on the photosynthetic process is indirect. This indirect action can be observed in this study, because it required a high dose of the herbicide tepraloxydim to reduce A by 50% in R. sativus, according to the I_{50} of 220.77 g a.i. ha⁻¹.



Figure 1: Symptoms on leaves of *Raphanus sativus* 7 days after application of the herbicide tepraloxydim at doses of 0 (A), 75 (B), 100 (C), and 125 g a.i. ha⁻¹ (D).

The reduction in the WUE in this study is mainly due to the decrease in CO_2 assimilation since E did not differ in response to herbicide doses (Figure 4D and C). Corroborating these results, the accumulation of CO_2 in the substomatal chamber in response to the herbicide, as evidenced by the small increase in C_i and in the C_i/C_a ratio, demonstrates that the limitation in A was due, at least in part, to the impairment of CO_2 assimilation by inhibition of the Calvin-Benson cycle.

The chlorophyll a fluorescence measures are good indicators of herbicide-promoted stresses to the

photosynthetic apparatus (Silva *et al.*, 2014; Lima *et al.*, 2017). In this study, the chlorophyll *a* fluorescence parameters of minimal fluorescence (F_0), relative electron transport rate (ETR), and the effective quantum yield of PSII ($Y_{\rm II}$) of *R. sativus* differed according to the increasing doses of the herbicide tepraloxydim (Figure 6A-B, E-F, and G-H). However, the potential quantum yield of PSII ($F_{\rm V}/F_{\rm M}$), quantum yield of regulated energy dissipation of PSII ($Y_{\rm NPQ}$), and quantum yield of non-regulated energy dissipation of PSII ($Y_{\rm NPQ}$) did not differ (Figure 6C-D, I-J, and K-L). Thus, the $F_{\rm V}/F_{\rm M}$ values, which

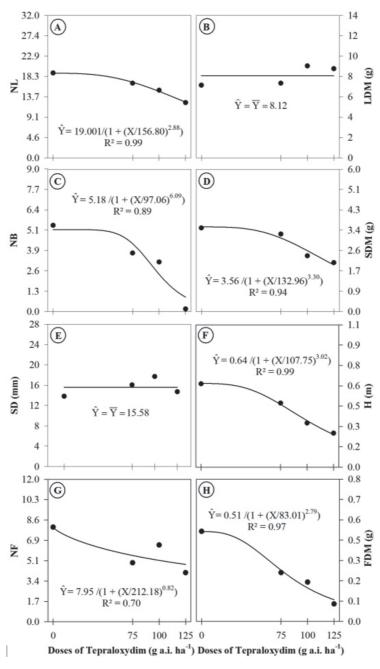


Figure 2: Number of leaves (NL) (A), leaf dry matter (LDM) (B), number of branches (NB) (C), stem dry matter (SDM) (D), stem diameter (SD) (E), plant height (H) (F), number of flowers (NF) (G), and flowers dry matter of (FDM) (H) of *Raphanus sativus* plants treated with different doses of the herbicide tepraloxydim. Data are means of (n = 6).

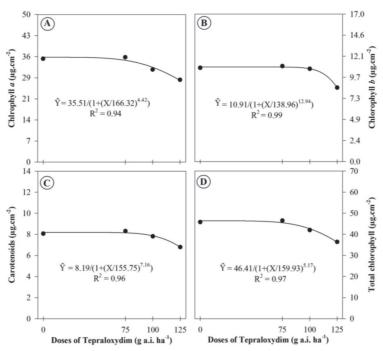


Figure 3: Concentrations of chlorophyll a (A), chlorophyll b (B), carotenoids (C), and total chlorophyll (D) of *Raphanus sativus* plants submitted to different doses of the herbicide tepraloxydim. Data are means of (n = 6).

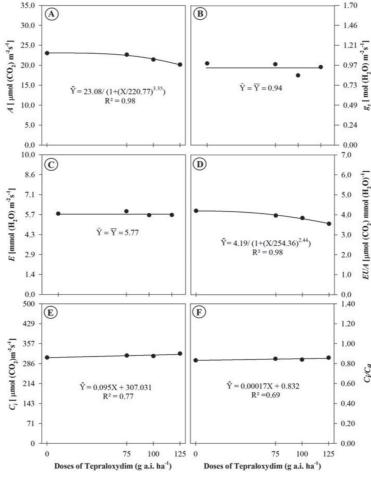


Figure 4: Photosynthetic rate (*A*) (A), stomatal conductance (g_s) (B), transpiration rate (*E*) (C), water use efficiency (*WUE*) (D), CO₂ internal concentration (C_i) (E), and ratio between internal and external CO₂ concentration (C_i / C_a) (F) of *Raphanus sativus* plants submitted to different doses of the herbicide tepraloxydim. Data are means of (n = 18).

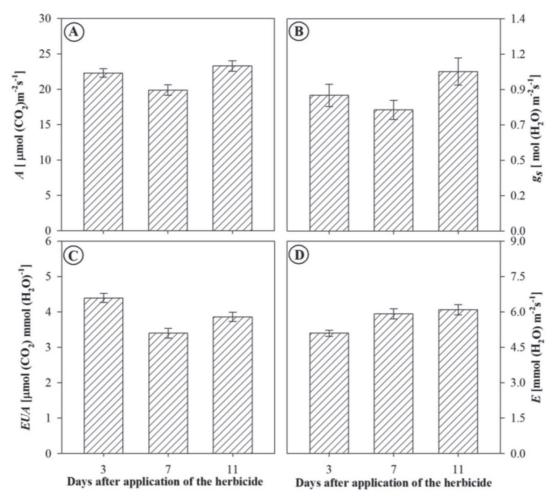


Figure 5: Photosynthetic rate (*A*) (*A*), stomatal conductance (g_s) (*B*), water use efficiency (*WUE*) (*C*), and transpiration rate (*E*) (*D*) of *Raphanus sativus* plants at 3, 7, and 11 days after application of the herbicide tempraloxydim. The bars represent the standard error of the means (n = 24).

remained around 0.8, show that there was no photoinhibition (Maxwell & Johnson, 2000). Similar values for this parameter of $F_{\rm v}/F_{\rm M}$ were visualized by Silva *et al.* (2014) and Moura *et al.* (2018) in *R. sativus* plants, when compared to the control plants, proving that these were ideal values for this species. The increase of F_0 as a function of the increase of the doses of the herbicide tepraloxydim, even in a non-high order, is an indication of the limitations in the transfer of the excitation energy from the antenna pigments to the reaction center (Lima *et al.*, 2017; Batista *et al.*, 2018).

The reduction of A values is related to the reduction of $Y_{\rm II}$ and ETR, since the energy destined for the photochemical dissipation decreased as a function of the increment of the doses of the herbicide tepraloxydim. Thus, the reduction of ATP and NADPH formation was probably the limiting factor for the Calvin-Benson cycle, since there was no stomatal limitation, as evidenced by the g_s values. Unlike $Y_{\rm II}$, the $Y_{\rm NPQ}$ and $Y_{\rm NO}$ remained unchanged, even with increasing $Y_{\rm NPQ}$ at the dose of 125 g a.i. ha⁻¹, this could not be adjusted to the

regression model. $Y_{\rm NPQ}$ and $Y_{\rm NO}$ have the function of dissipating the energy absorbed in the form of heat by the xanthophyll cycle, which is activated by the protonation of the thylakoid lumen (Ruban, 2016). This protonation leads to the de-epoxidation of vialaxanthin, forming anteraxanthin, which forms zeaxanthin (Kromdijk *et al.*, 2016; Mathur *et al.*, 2018). This dissipation of energy is a way of protecting the photosynthetic apparatus against the excess energy of excitation in general and the formation of reactive oxygen species in particular (Cardona *et al.*, 2018).

The rate of electrolyte liberation (*REL*) of *R. sativus* plants was not altered by the herbicide tepraloxydim (Figure 7). This is an indication that there was no damage at the membrane level (Duke & Kenyon, 1993). Thus, corroborating the chlorophyll a fluorescence measurements, in which there was no reduction of the $F_{\rm v}/F_{\rm M}$ values, thereby indicating the stability of PSII and the chloroplast membranes.

CONCLUSION

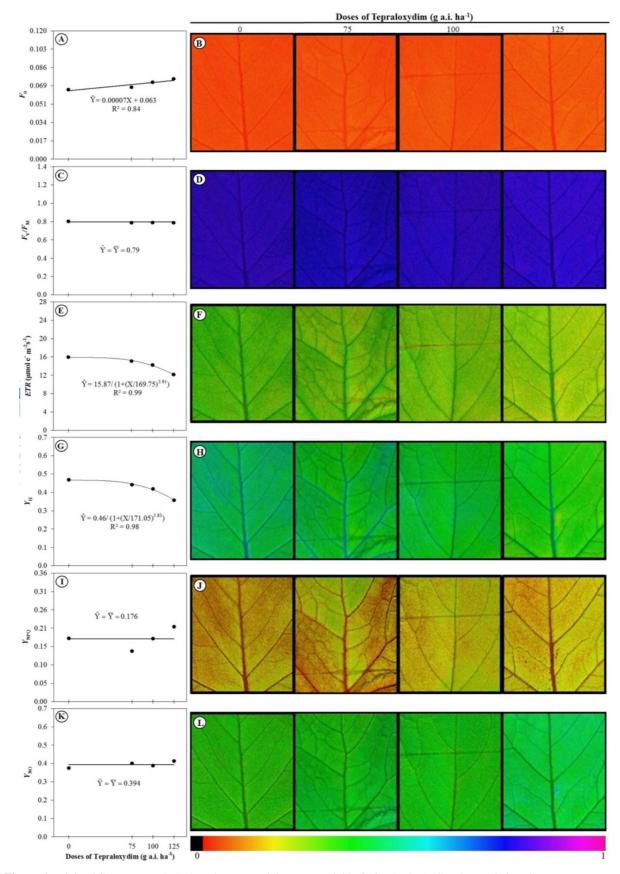


Figure 6: Minimal fluorescence (F_0) (A and B), potential quantum yield of PSII (F_{V}/F_{M}) (C and D), relative electron transport rate (ETR) (E and F), effective quantum yield of PSII (Y_{II}) (G and H), quantum yield of regulated energy dissipation of PSII (Y_{NPQ}) (I and J), and quantum yield of non-regulated energy dissipation of PSII (Y_{NQ}) (K and L) of *Raphanus sativus* plants submitted to different doses of the herbicide tepraloxydim. Data are means of (n=6).

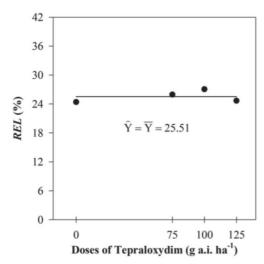


Figure 7: Rate of electrolyte leakage (*REL*) of *Raphanus sativus* plants submitted to different doses of the herbicide tepraloxydim. Data are means of (n = 6).

The doses of 125 g a.i. ha⁻¹ of the herbicide tepraloxydim mainly inhibited the growth and formation of leaves, branches and flowers of *R. sativus* at the development stage analyzed in this study. However, doses of 75 and 100 g a.i. ha⁻¹ do not compromise the photosynthetic apparatus and the stability of cell membranes.

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