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Article

Intoxication of Mesotrione in Corn Inoculated with *Azospirillum brasilense* and with Application of Plant Growth Regulators

*Intoxications Mesotrione in maize inoculated with **Azospirillum brasilense** and with application of Plant Growth Regulator*

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ABSTRACT - The objective of this study was to evaluate the effect of intoxication by mesotrione herbicide on the initial development and yield of maize after seed inoculation or foliar spray application with *Azospirillum brasilense* and application of plant regulators. Therefore, three experiments were performed: one under greenhouse conditions and two under field conditions, in different locations. In all experiments, a randomized block design was used with a 2 x 4 factorial arrangement, representing presence (192 g ha^{-1}) or absence of mesotrione and four treatments corresponding to the control; seed inoculation with *A. brasilense*; foliar spray application of *A. brasilense*; foliar spay application of auxin + gibberellin + cytokine (AX + GA + CK), and foliar spay applications with mesotrione were carried out at the V_3 stage of the crop. The results from the greenhouse experiment, with mesotrione application, were intoxication of 7.12%, which reduced total chlorophyll content by 10.15% and carotenoid content by 75.86%, leading to reductions in gas exchange and chlorophyll *a* fluorescence, and increased activity of antioxidative enzymes. Under field conditions, the treatments did not reduce the effects of intoxication during the initial development of maize crop and did not increase crop yield. It was concluded that the use of *A. brasilense* and plant regulators did not protect maize against mesotrione intoxication in the initial development, nor did it increase yield.

Keywords: *Zea mays*, gas exchange, fluorescence of chlorophyll *a*, peroxidase, catalase.

*RESUMO - Objetivou-se neste estudo avaliar a intoxicação do herbicida Mesotrione no desenvolvimento inicial e na produtividade do milho, inoculado via semente ou foliar com **Azospirillum brasilense** e aplicação de reguladores vegetais. Portanto, foram realizados três experimentos: um em condição de casa de vegetação e dois em condições de campo, em diferentes locais. Em todos eles utilizou-se o delineamento em blocos casualizados no esquema fatorial 2 x 4, representando presença (192 g ha^{-1}) ou ausência de mesotrione e quatro tratamentos, correspondentes à testemunha, inoculação das sementes com **A. brasilense**, aplicação foliar de **A. brasilense** e aplicação foliar de auxina + giberelina + citocina (AX+GA+CK), sendo as aplicações foliares com mesotrione realizadas no estádio V_3 da cultura. Em casa de vegetação, a aplicação de mesotrione originou intoxicação de 7,12%, o que reduziu em 10,15% o conteúdo de clorofila total e em 75,86% o teor de carotenoides, resultando em reduções nas trocas gasosas, na fluorescência da clorofila a e no incremento da atividade das enzimas antioxidativas. Em condições de campo, os tratamentos não reduziram os efeitos da intoxicação provocados durante o desenvolvimento inicial da cultura do milho*

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e não incrementaram a produtividade da cultura. Concluiu-se que o uso de ***A. brasilense*** *e de regulador vegetal não protegeu o milho da intoxicação do mesotrione no desenvolvimento inicial, bem como não incrementou a produtividade.*

Palavras-chave: *Zea mays*, trocas gasosas, fluorescência da clorofila *a*, peroxidase, catalase.

INTRODUCTION

Maize is one of the world's most important commodities, especially because it is the basis for animal protein production, as well as a source of energy and an important component of the human diet. In Brazil, maize production is particularly important; production is estimated at 92.8 million tonnes (Conab, 2017).

Maize crop yield is affected by several factors; weeds, for example, may cause losses up to 87%. Therefore, chemical management is required, especially in the critical period of weed interference, between emergence to stage V_7 (Kozlowski, 2002), which corresponds to approximately 42 days after sowing (Balbinot et al., 2016).

Mesotrione is an HPPD herbicide often used for chemical weed management in maize crops. It inhibits the enzyme 4-hydroxyphenylpyruvate dioxygenase during carotenoid synthesis. This causes plant death as a result of photooxidation of chlorophyll molecules, because excess light energy is not dissipated by carotenoids during photosynthesis (Mitchell et al., 2001). Maize is tolerant to mesotrione as a consequence of compartmentation of the herbicide molecules, whose herbicidal activity is lost (Beaudegnies et al., 2009).

However, although mesotrione is a selective herbicide for use in maize, it can cause up to 35% intoxication (Bulegon et al., 2017), which is characterized by white spots in the new leaves of maize plants. Such spots become chlorotic and there is subsequent necrosis of the leaf tissue in the most extreme conditions (Barchanska et al., 2014), thereby interfering with crop plant metabolism (O'Sullivan et al., 2002; Ogliari et al., 2009).

In this sense, application of herbicides, even when they are selective for the crop, can reduce gas exchange and increase the activity of enzymes that help eliminate reactive oxygen species (ROS) (Agostinetto et al., 2016; Langaro et al., 2017), which are produced by O_2 left after degradation of chlorophyll molecules (Streit et al., 2005). Thus, maize plants that received mesotrione application showed reduction of 78% in net CO_2 assimilation rate and of 52% in the Fv/Fm ratio (Ogliari et al., 2009).

To maximize mesotrione selectivity in maize, the visual effects of intoxication (chlorosis) on new plant leaves have to be reduced, because they are negatively correlated with carotenoid content (Barchanska et al., 2014). Therefore, reducing carotenoid degradation is crucial to reduce phytotoxic effects. Thus, it has been shown that, in plants treated with HPPD-inhibiting herbicides, there was increased activity of antioxidative enzymes (Grigoryuk et al., 2016), degradation of chlorophyll molecules (Vítek et al., 2017), as well as inhibition of carotenoid synthesis, which results in albino phytoene (Beaudegnies et al., 2009).

Therefore, using strategies that increase antioxidant system activity, increase chlorophyll synthesis and help maintain carotenoid content may help plants to maintain their physiological and biochemical activity in the event of mesotrione-induced intoxication.

Thus, the use of plant growth-promoting bacteria (PGPB) of the species *Azospirillum brasilense* is promising, because they increase the activity of the antioxidant system of plants in stressful conditions (Vurukonda et al., 2016), as found for brachiaria plants under water stress (Bulegon et al., 2016) and under saline stress (Zawoznik and Ameneiros, 2011). PGPB also increase chlorophyll synthesis (Bashan et al., 2006) and help maintain the levels of carotenoids after incidence of environmental stress (Bulegon et al., 2016). Also, they may increase initial plant growth (Inagaki et al., 2015), and more developed plants are more tolerant to phytotoxicity (Godar et al., 2015).

Similarly, the plant hormones auxin, gibberellin and cytokinin can help strengthen the plant defense system by increasing the antioxidant system (Xia et al., 2015), the levels of photosynthetic pigments and initial plant growth (Rademacher, 2015).

Based on the above-mentioned facts, the aim of this study was to evaluate the effect of mesotrione intoxication on initial growth and yield of maize after leaf or seed inoculation with *A. brasilense*, or after application of a plant growth regulator.

MATERIAL AND METHODS

Three experiments - one greenhouse and two field experiments - were conducted. All of them used a randomized block design in a 4 x 2 factorial arrangement, with four replications. The treatments consisted of presence (192 g ha^{-1}) or absence of Mesotrione application. The second factor was represented by the following treatments: control; seed inoculation with *A. brasilense* strains AbV5 And AbV6; leaf inoculation with *A. brasilense* strains AbV5 And AbV6; and foliar application of auxin + gibberellin + cytokine (AX+GB+CK).

For maize seed inoculation, 100 mL of liquid inoculant was used for 60 thousand seeds, containing $2x10^8$ CFU per mL of *A. brasilense* strains AbV5 and AbV6 (commercial product), which were homogenized and kept in shade for approximately 30 minutes and then sown.

Foliar application of the treatments was performed when the plants reached the third phenological stage (V_3), which corresponds to approximately 14 days after sowing (DAS). The applications were performed with a CO_2-pressurized backpack sprayer, fitted with a 3 m wide bar and six flat fan nozzles (Magno 11002 ADGA), working pressure of 2.2 bar, flow rate of 200 L ha^{-1} and application height of 0.5 m above the apex of the plants. Application in the greenhouse was performed at 28 ^oC, relative humidity of 61% and wind speed of 5.4 km h^{-1}. Field application in Marechal Cândido Rondon was performed at 23.3 ^oC, 61% and 8.8 km h^{-1}, and Entre Rios do Oeste, at 21.8 ^oC, 65% and 5.4 km h^{-1} for temperature, relative humidity and wind speed, respectively.

Application of *A. brasilense* and the plant growth regulator was tank-mixed with Mesotrione, according to a preliminary trial, which showed that the combined application showed no limitations on gas exchanges, intoxication and development of maize (data not shown). The rates of Mesotrione for all treatments were 192 g ha^{-1} (commercial formulation: Callisto® 480 g L^{-1}), and the adjuvant Nimbus was added at 0.5% v v^{-1}.

Foliar application of *A. brasilense* used the rate of 400 mL ha^{-1} of inoculant containing $2x10^8$ CFU per mL, strains AbV5 and AbV6 (commercial product). The treatment with AX+GB+CK used the rates of 0.025 g ha^{-1} of 4-indol-3-butyric acid, 0.025 g ha^{-1} of gibberellic acid and 0.045 g ha^{-1} of kinetin.

The experiment was conducted in an arch greenhouse, covered with low-density polyethylene film (150 micron thickness), located at - 24°558'S and - 54°045'O. Five seeds of simple hybrid 30F53 VYH were sown per pot (12 dm^3). After emergence, the seeds were thinned to two plants per pot. The substrate consisted of soil from the A horizon of a clayey Eutroferric Red Latosol (578 g kg^{-1} clay; 348.58 g kg^{-1} silt; 3.42 g kg^{-1} sand), with organic matter content: 5.47 g dm^3; P content: 2.07 mg dm^{-3}; H+Al: 3.01 $cmol_c$ dm^{-3}; Al^{+3}: 0.0 $cmol_c$ dm^{-3}; K^+: 0.11 $cmol_c$ dm^{-3}; Ca^{+2}: 3.92 cmolc dm^{-3}; Mg^{+2}: 1.03 $cmol_c$ dm^{-3}; cation exchange capacity (CEC): 8.08 $cmol_c$ dm^{-3}; V(%): 62.68, and a diazotrophic bacterial population of $7.5x10^4$ CFU g^{-1} soil.

Fertilization was performed as recommended by Novais et al. (1991), by adding 300 mg dm^{-3} of P_2O_5 in the form of simple superphosphate, 150 mg dm^3 of K_2O in the form of potassium chloride and 50 mg dm^3 of N in the form of urea. After three days of application (DAA) of Mesotrione, at stage V_4, N fertilization was supplemented with 100 mg dm^3 of N in the form of urea. After sowing, the pots were irrigated daily.

Assessments in the greenhouse were made at 1, 7 and 14 DAA. The following parameters were determined in the maize plants: intoxication percentage, SPAD index, leaf pigment content; antioxidant enzyme activity, gas exchange and chlorophyll *a* fluorescence.

The field experiments were carried out in the towns of Marechal Cândido Rondon, located at coordinates -24°53'S and -54°01'O, and Entre Rios do Oeste, located at coordinates -24°68'S and -54°28'O.

Both experiments were set up in a clayey Eutroferric Red Latosol. In Marechal Cândido Rondon, the soil had 831.20 g kg^{-1} clay; 114.80 g kg^{-1} silt; 54.00 g kg^{-1} sand; organic matter content: 2.90 g dm^3; P content: 13.7 mg dm^{-3}; H+Al: 6.30 $cmol_c$ dm^{-3}; Al^{+3}: 0.05 $cmol_c$ dm^{-3}; K^+ 0.50 $cmol_c$ dm^{-3}; Ca^{+2}: 4.90 $cmol_c$ dm^{-3}; Mg^{+2}: 2.2 cmol C dm^{-3}; cation exchange capacity (CEC): 13.09 $cmol_c$ dm^{-3}; V(%): 54.70, and a diazotrophic bacterial population of 7.5x10^4 CFU g^{-1}. In Entre Rios do Oeste, the soil had 587.29 g kg^{-1} clay; 351.00 g kg^{-1} silt; 61.71 g kg^{-1} sand; organic matter content: 25.97 g dm^{-3}; P content: 8.99 mg dm^{-3}; H+Al: 4.53 $cmol_c$ dm^{-3}; Al^{+3}: 0.00 $cmol_c$ dm^{-3}; K^+: 1.96 $cmol_c$ dm^{-3}; Ca^{+2}: 5.59 $cmol_c$ dm^{-3}; Mg^{+2}: 1.19 $cmol_C$ dm^{-3}; cation exchange capacity (CEC): 13.28 $cmol_c$ dm^{-3}; V(%): 65.88, and a diazotrophic bacterial population of 7.5x10^4 CFU g^{-1}.

Single-cross hybrid maize 30F53 VYH was sown, spaced 0.7 m apart, with density of 4.6 plants per meter, aiming at a population of 65,717 plants ha^{-1}, and using fertilization of 320 kg ha^{-1} of 10-15-15 (NPK) formulation.

The experimental plot in Marechal Cândido Rondon consisted of eight rows, 5 m in length, with a total area of 28 m^2. In Entre Rios do Oeste, the plot was composed of six rows, 5 m in length, with a total area of 21 m^2. In both places, the usable area of the plot was represented by four central rows, 3 m length (8.4 m^2).

Topdressing nitrogen fertilization was performed at stage V_4 of the maize crop, providing 140 kg ha^{-1} of N, using urea as a source (45% N). Also, 30 kg ha^{-1} of potassium was provided in the form of potassium chloride (60% of K_2O). The maize crop was grown in the absence of weed interference throughout the total interference prevention period, thus avoiding crop-weed competition.

The evaluations were performed at 10 DAA to determine the following parameters in the maize plants: intoxication percentage, SPAD index, total chlorophyll and carotenoid contents, and yield (at the end of the crop cycle).

Visual assessment of intoxication in both experiments was performed using a 0 - 100% scale, where 0% corresponds to absence of intoxication symptoms and 100%, to plant death, as proposed by the Brazilian Weed Science Society (SBCPD, 1995). At the same time, the SPAD index was determined with the aid of a Minolta SPAD-502 chlorophyll meter by measuring ten points in all fully-expanded leaves of the plants in the greenhouse. Under field conditions, every leaf was measured of five plants randomly chosen within the usable plot.

In the greenhouse, the assessments used the shoots of the plants in the pots, while the assessments in the field were made of five plants randomly selected in the usable area. After the plants were collected, samples were prepared with leaf segments with fresh weight of 0.5 g. The samples were stored in sealed bottles which were covered with aluminum foil to prevent light leaks; 20 mL of 80% acetone solution was added to the samples, and the bottles were stored under refrigeration at 4 °C and in the absence of light for 72 hours. After that, spectrophotometer reading was performed using the following values: 665 nm for chlorophyll *a*, 649 nm for chlorophyll *b*, and 480 nm for carotenoids; total chlorophyll content was determined by the sum of the chlorophyll *a* and chlorophyll *b* values (Lichtenthaler and Buschmann, 2001).

In greenhouse conditions, fresh samples of maize leaves with fresh mass of 0.5 g were collected at 1, 7 and 14 DAA of mesotrione and macerated in 0.01 M sodium phosphate buffer (pH 6.0). Polyvinylpyrrolidone (PVP) was added, and then the samples were centrifuged at 20,000 g for 20 min for homogenization; the supernatant was collected and stored at a temperature of -80 °C.

After that, peroxidase (POD; EC 1.11.1.7) activity was determined through direct spectrophotometry, based on guaiacol oxidation at 30 °C (Hammerschmidt et al., 1982), with readings at 470 nm for two minutes, with 15 second scanning intervals. Catalase (CAT; EC 1.11.1.6) was determined by monitoring the variation in hydrogen peroxide absorption (Havir and Mchale, 1987) with reading at 240 nm.

Gas exchanges were determined in the greenhouse with an LI-6400XT Liquor Inc. (Lincoln, NE) infrared gas analyzer, with CO_2 content of 400 µmol mol^{-1} and 1,500 µmol m^{-2} s^{-1} of photons in the last completely expanded leaf of the plant, in the central portion of the leaf. Net CO_2 assimilation rate (*A*), leaf transpiration rate (*E*) and stomatal conductance (*gs*) were determined. The photosynthetic response curve, as a function of luminosity levels, was determined at the photosynthetic densities of 2,500; 2,000; 1,500; 1,000; 800; 500; 200; 100; 50; 25; and 0.0 µmol m^{-2} s^{-1}, and maximum photosynthesis was determined by fitting a rectangular hyperbolic function (Machado et al., 2005).

Analysis of chlorophyll *a* fluorescence was based on the saturation pulse method (Schreiber et al., 1994) in leaves in the dark. Readings were performed during the early morning hours to determine initial fluorescence (Fo), maximum fluorescence (FM) and potential quantum efficiency of photosystem II (Fv/Fm). In the presence of solar radiation, effective quantum efficiency of PSII (SPSII), maximum efficiency of PSII (Fv'/Fm') and photochemical extinction coefficient (qP) were determined.

When the maize crop reached maturity, the spikes in the usable area of the plot were harvested to determine the maize crop yield, which was corrected to 13% moisture content on a wet basis, expressed in kg ha^{-1}.

The data were evaluated in the experiments and subjected to analysis of variance by Fisher's F test at 5% probability of error; where appropriate, the means were compared by the Student-Newman-Keuls (SNK) test at 5% probability of error, using the SISVAR statistical software.

RESULTS AND DISCUSSION

In the greenhouse, the intoxication percentage values of the herbicide mesotrione in maize plants showed differences only for the presence and absence of the herbicide. There was no significant effect on the treatments applied and the interaction between the factors, except for the assessment at seven days after application (DAA), in which the SPAD index was influenced by the treatments; foliar application of *A. brasilense* resulted in the highest mean (Table 1).

Mesotrione intoxication is due to inhibition of carotenoid synthesis, which leads to chlorophyll degradation of membranes which were broken down by free radicals formed through photooxidation (Mitchell et al., 2001; Streit et al., 2005; Beaudegnies et al., 2009). The action of mesotrione on carotenoid synthesis metabolism is quick (Beaudegnies et al., 2009). It has been reported that

Table 1 **-** Intoxication (Into - %) and SPAD index in maize plants after application of Mesotrione, and plant growth-promoting bacteria and plant growth regulator, under greenhouse conditions. Marechal Cândido Rondon, 2016

Treatment	1 DAA		7 DAA		14 DAA	
	Into (%)	SPAD	Into (%)	SPAD	Into (%)	SPAD
Mesotrione						
With Mesotrione	1.93 a	40.46	7.21 a	42.49 b	0.68 a	50.00 b
Without Mesotrione	0.00 b	41.95	0.00 b	48.08 a	0.00 b	52.95 a
Treatment						
Control	1.25	40.45	3.87	44.51 b	0.50	52.62
Foliar application of *A. brasilense*	1.00	40.97	3.31	49.35 a	0.00	50.25
Seed inoculation of *A. brasilense*	0.87	39.98	3.75	42.36 b	0.37	49.93
AX+GB+CK	0.75	43.41	3.50	44.86 b	0.50	53.07
Calculated F values						
Treatment	1.244^{ns}	1.250^{ns}	0.256^{ns}	7.073**	4.013^{ns}	1.342^{ns}
Mesotrione	102.442**	1.192^{ns}	210.122**	25.300**	33.800**	4.504*
Interaction	1.244^{ns}	2.280^{ns}	0.256^{ns}	2.107^{ns}	4.013^{ns}	1.439^{ns}
CV (%)	55.89	9.37	39.02	6.90	97.19	7.61

^{ns} non-significant by the F-test at 5% probability; **; * significant at 1% and 5% probability, respectively, by the F test. Similar uppercase letters similar in the column do not differ by the Student-Newman-Keuls (SNK) test at 5% probability.

70% and 40% of herbicides can be absorbed and translocated, respectively, eight hours after application (Godar et al., 2015), inhibiting the synthesis of new carotenoids, and hence leaving plants predisposed to photooxidation effects.

Tolerance of plants is due to their ability to metabolize herbicide molecules to inactive molecules, which in the case of maize is stimulated by the action of H^+ pumps (Ogliari et al., 2009). These authors reported that the phytotoxic effects lasted for 12 DAA, which corroborates the findings of the present study. Importantly, the symptoms of leaf whitening remain in old leaves, which results in a lower SPAD index even at 14 DAA. However, the physiological and biochemical mechanism is no longer influenced.

At 7 DAA, total chlorophyll and carotenoid contents were influenced by the interaction between mesotrione application and the treatments (Table 2). The plants that received Mesotrione showed lower total chlorophyll content, with a 10.15% reduction in comparison to the plants without the herbicide.

Table 2 - Leaf pigment content of maize at 7 days after application (DAA) of Mesotrione, submitted to different treatments with plant hormones and plant growth promoting bacteria under greenhouse conditions. Marechal Cândido Rondon, 2016

Treatment	Total chlorophyll (μg g^{-1})			Carotenoids (μg g^{-1})		
	With Mesotrione	Without Mesotrione	Mean	With Mesotrione	Without Mesotrione	Mean
Control	51.68 aA	47.93 bA	48.81	0.57 abA	0.65 bA	0.61
Foliar application of *A. brasilense*	49.61 aA	51.47 abA	50.54	0.25 bB	1.44 aA	0.84
Seed inoculation of *A. brasilense*	39.32 bB	57.06 aA	48.19	0.61 abA	0.90 bA	0.76
AX+GB+CK	47.64 aA	50.89 abA	49.27	0.90 aA	1.10 abA	1.00
Mean	47.06 B	51.84 A		0.58 B	1.02 A	
Calculated F values						
Treatment	0.540^{ns}			2.651^{ns}		
Mesotrione	12.541**			19.319**		
Interaction	167.59**			6.441**		
CV (%)	7.71			35.08		

^{Ns} non-significant by the F test at 5% probability; **; * significant at 1% and 5% probability, respectively, by the F test. Similar lowercase letters in the column and uppercase letters in the row do not differ by the Student-Newman-Keuls (SNK) test at 5% of probability.

An analysis of the treatments without Mesotrione showed that the lowest mean occurred in the control, without any difference from AX+GB+CK. For plants that received Mesotrione application, total chlorophyll content was lower for seed inoculation with *A. brasilense*. When comparing plants with and without Mesotrione application, there were differences only when the seeds were inoculated with *A. brasilense*, with a 45.11% reduction in chlorophyll content in plants with Mesotrione (Table 2).

Carotenoid content was reduced by 75.86% in plants with Mesotrione application, in comparison to plants without the herbicide. Foliar application of *A. brasilense* led to higher carotenoid content in the absence of Mesotrione, while the same treatment in plants with Mesotrione showed a reduction of 576% on carotenoid content, which was the only one to be reduced (Table 2).

Reduction of pigment content at 7 DAA resulted from the occurrence of an intoxication peak at that moment. Thus, these results corroborate those found for application of mesotrione in *Amaranthus palmeri* (105 g a.i. ha^{-1}) (Godar et al., 2015), *Lolium perenne* (280 g a.i. ha^{-1}) (Mccurdy et al., 2008) and maize (192 g a.i. ha^{-1}) (Ogliari et al., 2009). It can also be inferred that the greatest concentration of active herbicide molecules in the plant was found at that moment, because there was a negative correlation between chlorophyll content and herbicide molecules in eight plant species (Barchanska et al., 2014).

To overcome the effects of mesotrione intoxication resulting from chlorophyll photooxidation and consequent formation of free radicals, plants use a series of enzymes, e.g., catalase (CAT) and peroxidase (POD). CAT activity was increased at 1 DAA in plants that received Mesotrione application. It was 44.3% higher than in the plants which did not receive the herbicide (Table 3). There were no differences among treatments for the plants without mesotrione application. However, in the plants that received mesotrione, the highest CAT activity was found in AX+GB+CK. In the control, they also exceeded their patterns without mesotrione by 108.7% for AX+GB+CK and 238.5% for the control.

Table 3 - Activity of catalase (CAT) and peroxidase (POD) in maize leaves at 1 and 7 days after application (DAA) of Mesotrione, submitted to different treatments with plant hormones and plant growth promoting bacteria under greenhouse conditions. Marechal Cândido Rondon, 2016

Treatment	CAT 1 DAA UE min^{-1} g fresh weight^{-1}			POD 1 DAA UE min^{-1} g fresh weight^{-1}		
	With Mesotrione	Without Mesotrione	Mean	With Mesotrione	Without Mesotrione	Mean
Control	9.48 aA	2.80 aB	6.14	107.09 cA	142.36 abA	124.73 c
Foliar application of *A. brasilense*	3.51 bA	5.51 aA	4.51	217.49 bA	119.61 bB	168.55 b
A. brasilense seed	3.02 bA	4.59 aA	3.81	143.10 cA	97.57 bA	120.34 c
AX+GB+CK	8.60 aA	4.12 aB	6.36	277.79 aA	197.06 aB	237.43 a
Mean	6.15A	4.26 B		186.37 A	139.15 B	
Calculated F values						
Treatment	2.837^{ns}			16.518**		
Mesotrione	6.582**			12.478**		
Interaction	8.661**			4.895**		
CV (%)	40.11			23.23		

Treatment	CAT 7 DAA (UE min^{-1} g fresh weight^{-1})		
	With Mesotrione	Without Mesotrione	Mean
Control	6.66 aA	8.10 aA	7.38 a
Foliar application of *A. brasilense*	8.14 aA	4.00 bB	6.07 a
Seed inoculation of *A. brasilense*	2.90 bA	4.83 bA	3.86 b
AX+GB+CK	6.01 aA	8.43 aA	7.22 a
Mean	5.93	6.34	
Calculated F values			
Treatment	6.874**		
Mesotrione	0.440^{ns}		
Interaction	6.121**		
CV (%)	28.50		

^{ns} non-significant by the F-test at 5% probability; **; * significant at 1% and 5% probability, respectively, by the F test. Similar lowercase letters in the column and uppercase letters in the row do not differ by the Student-Newman-Keuls (SNK) test at 5% probability.

Similarly, POD activity at 1 DAA in plants treated with Mesotrione was 33.9% higher than in plants without application. When the treatments were assessed for presence and absence of mesotrione, there was higher POD activity in AX+GB+CK and foliar inoculation of *A. brasilense* (40.9% and 81.8%, respectively) when the plants received mesotrione (Table 3).

Foliar inoculation of *A. brasilense* and AX+GB+CK increased POD activity at 1 DAA, which may suggest that they assist the process of ROS removal, an important mechanism for mitigating the effects of Mesotrione (Agostinetto et al., 2016; Langaro et al., 2017). There was an increase in the antioxidant system of *Urochloa ruziziensis* leaves sprayed with *A. brasilense* under stress caused by water deficit (Bulegon et al., 2016). The use of plant growth regulators stimulates the development of plants under saline stress (Oliveira et al., 2016).

The increase in antioxidant activity after herbicide application in plants, as found in the present study, corroborates the findings for wheat crop, in which the application of the herbicides clodinafop-propargyl (0.25 L ha^{-1} p.c.) increased CAT activity at 1 DAA (Agostinetto et al., 2016). In a study on rice crops, it was also found that the herbicide oxyfluorfen (960 g a.i. ha^{-1}) caused intoxication and increased the activity of ROS-eliminating enzymes (Langaro et al., 2017). In maize, it was found that acetochlor (2.5 L ha^{-1} p.c.), dimethenamid-P (1.5 L ha^{-1} p.c.) and indaziflam + isoxaflutole (130 g ha^{-1} p.c.), which are preemergent herbicides, cause changes in the antioxidant system of plants (Grigoryuk et al., 2016), and this action occurs synchronously with alterations in gas exchange and chlorophyll fluorescence, as also found in the present study.

At 7 DAA, only in treatment with foliar inoculation of *A. brasilense*, it was found that the CAT activity remained higher at 103.5% in plants with mesotrione, in comparison to plants that received no application (Table 3).

In the evaluation performed at 7 DAA, there was a statistical effect of mesotrione for all variables of gas exchange and chlorophyll fluorescence, with the exception of initial fluorescence (Fo). For all other sources of variation, there were no statistical effects (p>0.05) (Table 4). Net CO_2 assimilation rate (*A*), transpiration (*E*) and stomatal conductance (*gs*) in plants that received application of mesotrione had lower means in comparison to those without herbicide application*; there were* reductions of 32.9%, 30.9% and 46.3%, respectively (Table 4).

Table 4 - Gas exchange and chlorophyll *a* fluorescence in maize leaves at 7 days after application (DAA) of Mesotrione, submitted to different treatments with plant hormones and plant growth promoting bacteria under greenhouse conditions. Marechal Cândido Rondon, 2016

Treatment	*A* (µmol CO_2 m^{-2} s^{-1})	*E* (mmol H_2O m^{-2} s^{-1})	*gs* (µmol m^{-2} s^{-1})
With Mesotrione	34.48 b	3.55 b	0.203 b
Without Mesotrione	51.16 a	4.64 a	0.297 a
Calculated F values			
Treatment	0.285^{ns}	0.516^{ns}	0.703^{ns}
Mesotrione	30.775**	20.809**	16.221**
Interaction	0.776^{ns}	0.583^{ns}	0.808^{ns}
CV (%)	14.42	16.64	26.28

Treatment	Fo	Fm	Fv/Fm	ΦPSII	Fv'/Fm'	qP
With Mesotrione	117.36	556.55 b	0.788 b	0.284 b	0.464 b	0.612 b
Without Mesotrione	118.47	584.57 a	0.797 a	0.334 a	0.499 a	0.669 a
Calculated F values						
Treatment	0.016^{ns}	2.076^{ns}	1.559^{ns}	0.286^{ns}	0.121^{ns}	2.343^{ns}
Mesotrione	0.807^{ns}	10.034**	4.396*	38.205**	11.476**	42.540**
Interaction	0.360^{ns}	0.466^{ns}	0.559^{ns}	0.224^{ns}	0.071^{ns}	0.732^{ns}
CV (%)	2.96	4.38	1.48	7.38	6.12	3.89

^{Ns} non-significant by the F-test at 5% probability; ** ;* significant at 1% and 5% probability, respectively, by the F test. Similar lowercase letters in the column do not differ by the Student-Newman-Keuls test (SNK) at 5% probability.

Similarly to gas exchange, for chlorophyll *a* fluorescence there was a difference between plants with and without application of Mesotrione in maximum fluorescence (Fm), maximum quantum efficiency of photosystem (Fv/Fm), effective quantum efficiency of PSII (SPSII), maximum quantum efficiency of the photosystem in the presence of light (Fv'/Fm') and photochemical extinction coefficient (qP); the plants with mesotrione showed a reduction of 4.79%, 1.12%, 14.97%, 7.01% and 8.52%, respectively (Table 4).

The results of this study corroborate the findings at 1 DAA of mesotrione in maize (Ogliari et al., 2009), in which the phytotoxic effect of this herbicide interfered in net CO_2 assimilation rate and chlorophyll *a* fluorescence, and this condition is related to a reduction of the PS II antenna complex, as a consequence of lower carotenoid content(Ye et al., 2013).

Thus, the reductions found in chlorophyll *a* fluorescence are due to the reduction of carotenoid concentration, which may be short-lasting (momentary effects) and long-lasting (prolonged effects), as at 7 DAA (Vítek et al., 2017). The reduction of carotenoid values leads to a reduction of the antenna complex and light capture in the photosystems (McCurdy et al., 2008), which interferes with chlorophyll *a* fluorescence, showing that the electron transport rate was negatively affected. Another condition is the degradation of membranes of chlorophyll molecules and, consequently, photooxidation-induced degradation.

Therefore, the treatments with plant growth-promoting bacteria and plant growth regulators cannot reduce the effects of mesotrione intoxication on gas exchange of maize plants; also, they do not protect the photosystem II against the harmful effects caused by inhibition of carotenoid synthesis, as a result of mesotrione intoxication.

Because mesotrione intoxication results in momentary reduction of gas exchange and chlorophyll *a* fluorescence, the photosynthetic response curve of maize was determined at 7 and 14 DAA to provide further insights into the reduction of photosynthetic capacity. At 7 DAA, the plants that had not received mesotrione presented maximum net CO_2 assimilation rate (A_{max}) of 43.47 µmol CO_2 m^{-2} s^{-1}, while the plants with mesotrione presented 41.92 µmol CO_2 m^{-2} s^{-1}, i.e., 3.56% less (Figure 2A). However, at 14 DAA, the plants without mesotrione presented A_{max} of 39.57 µmol CO_2 $m^{-2}s^{-1}$, while those with mesotrione presented 29.21 µmol CO_2 m^{-2} s^{-1} (Figure 1B), indicating that the photosynthetic potential of the plants was resumed at similar rates in plants treated with mesotrione, in comparison to the control group.

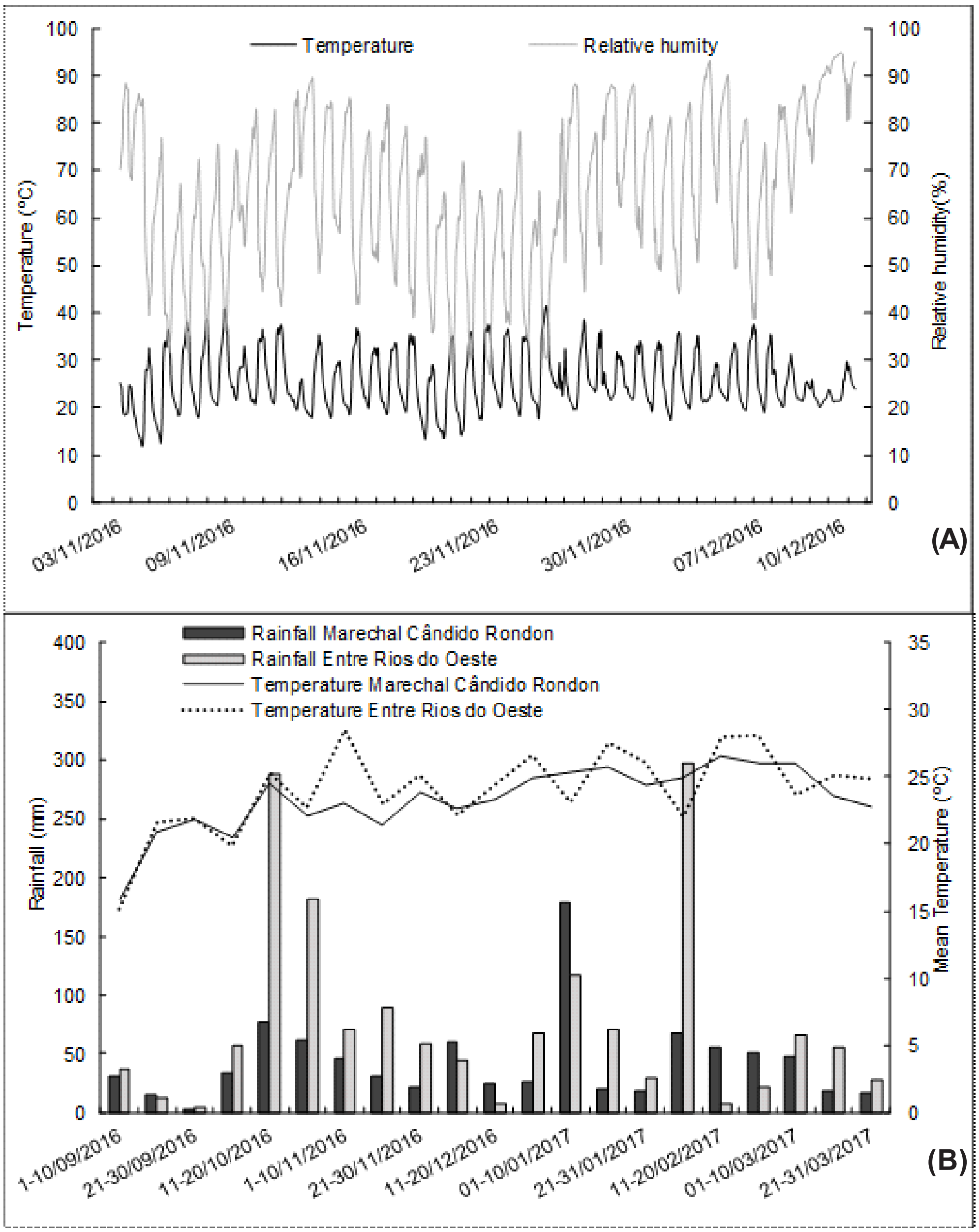
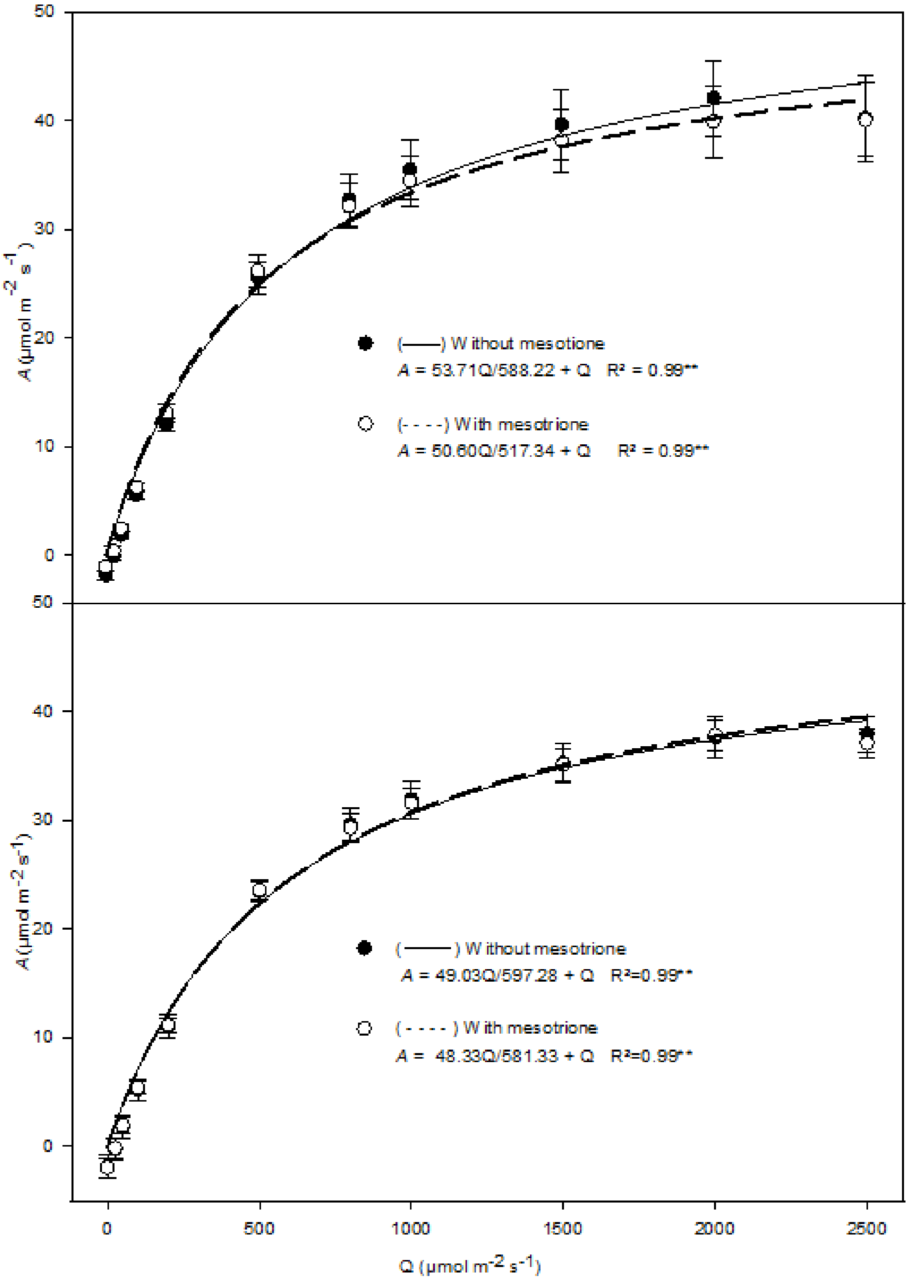


Figure 1 - Temperature and relative humidity inside the greenhouse while the experiment was conducted in Marechal Cândido Rondon - PR (A); rainfall and temperature under field conditions (B) in Marechal Cândido Rondon and Entre Rios do Oeste. 2016/2017.



** regression and regression coefficients were significant at 1% probability by the t test; error bars indicate standard error of the mean for n = 16.

Figure 2 **-** Photosynthetic response curve (*A*) of maize plants at 7 DAA (A) and 14 DAA (B) of mesotrione on the basis of different levels of luminosity (Q), under greenhouse conditions. Marechal Cândido Rondon, 2016.

The lowest values of A_{max} at 7 DAA are a result of no dissipation of excess light, represented by the increase of luminosity in the chamber of the IRGA, while considering the effect of photooxidation, associated with a reduction of antenna complex efficiency, which leads to a reduction in the maximum values of *A* (Ye et al., 2013). In turn, at 14 DAA, as a consequence of recovery of the plant, mesotrione intoxication was reduced, and was no longer limiting for plant photosynthesis. Similar results were found for reduction of A_{max} in sugar cane cultivated under water stress (Machado et al., 2013).

Although the use of *A. brasilense* or plant growth regulator has shown potential to aid in elimination of EROs at 1 DAA, these treatments usually did not show potential to reduce mesotrione intoxication in the maize crop, because it helped maintain the gas exchanges, as well as chlorophyll fluorescence, which limited the photosynthetic potential of maize plants at 7 DAA.

Furthermore, the results show that the reduction of carotenoids by mesotrione occurred in about 75% of the maize crop, when comparing the overall means of the treatment with and without mesotrione at 7 DAA, reducing gas exchange and chlorophyll fluorescence, and increasing the enzymatic activity of the plant through intoxication.

The results found in the field experiment in Marechal Cândido Rondon showed that intoxication of the plants had a maximum value of 3%, while the SPAD index did not differ statistically for none of the study sources of variation (Table 5).

Total chlorophyll content was influenced by the interaction of factors, in which the plants that received *A. brasilense* through seeds and leaf inoculation had increases of 19.8% and 20.31%, respectively, in the presence of mesotrione, when compared to the corresponding treatment without mesotrione. For carotenoid content, there was no difference between the factor with and without Mesotrione, nor for the treatments applied. When interaction was analyzed, it was found that plants treated with Mesotrione and *A. brasilense* through the leaves showed lower carotenoid content, while in plants without Mesotrione, there was lower carotenoid content in *A. brasilense* applied through the seeds (Table 5).

Table 5 - Intoxication, SPAD index, total chorophll and carotenoid at 10 DAA content in maize plants under field conditions after application of Mesotrione, submitted to different treatments with plant hormones and plant growth promoting bacteria. Marechal Cândido Rondon, 2016/2017

Treatment	Intoxication (%)			SPAD index		
	With Mesotrione	Without Mesotrione	Mean	With Mesotrione	Without Mesotrione	Mean
Control	3.0	0.0	1.5	34.95	42.45	38.70
Foliar application of *A. brasilense*	2.5	0.0	1.25	37.42	38.42	37.92
Seed inoculation of *A. brasilense*	2.25	0.0	1.125	37.82	38.75	38.28
AX+GB+CK	3.0	0.0	1.5	40.02	36.40	38.21
Mean	2.68 A	0.0 B		37.55	39.00	
Treatment	Total chlorophyll ($\mu g\ g^{-1}$)			Carotenoids ($\mu g\ g^{-1}$)		
	With Mesotrione	Without Mesotrione	Mean	With Mesotrione	Without Mesotrione	Mean
Control	39.16 aA	41.58 aA	40.37	1.07 aA	0.70 abA	0.89
Foliar application of *A. brasilense*	43.11 aA	36.17 aB	39.64	0.37 bB	0.77 abA	0.57
Seed inoculation of *A. brasilense*	44.66 aA	37.12 aB	40.89	0.73 abA	0.45 bA	0.59
AX+GB+CK	37.23 aA	38.72 aA	37.97	0.67 abB	1.03 aA	0.85
Mean	41.04	38.40		0.71	0.74	

Calculated F values				
	Intoxication	SPAD	Total chlorophyll	Carotenoids
Treatment	0.582^{ns}	0.041^{ns}	0.762^{ns}	3.617*
Mesotrione	119.474*	0.845^{ns}	3.297^{ns}	0.112^{ns}
Interaction	0.582^{ns}	2.104^{ns}	3.348*	5.334*
CV (%)	51.76	11.66	10.37	34.04

^{ns} non-significant by the F-test at 5% probability; **; * significant at 1% and 5% probability, respectively, by the F test. Similar lowercase letters in the column and uppercase letters in the row do not differ by the Student-Newman-Keuls (SNK) test at 5% probability.

As with the field experiment, in Entre Rios do Oeste, there was a significant effect of presence or absence of mesotrione on intoxication and SPAD index; in plants that received the herbicide, there was higher values of intoxication and reduction of 14.33% in the SPAD index (Table 6).

Total chlorophyll content was not significantly influenced by the study factors. Carotenoid content showed no differences between the factor with and without Mesotrione, while the highest mean among the treatments occurred in the control and in foliar application of *A. brasilense*. For decomposition of factors in plants treated with mesotrione, the highest means were found in the control and foliar inoculation with *A. brasilense* (Table 6).

Table 6 **-** Intoxication, SPAD index, total chorophll and carotenoid at 10 DAA in maize plants under field conditions after application of Mesotrione, submitted to different treatments with plant hormones and plant growth promoting bacteria. Entre Rios do Oeste, 2016/2017

Treatment	Intoxication (%)			SPAD index		
	With Mesotrione	Without Mesotrione	Mean	With Mesotrione	Without Mesotrione	Mean
Control	15.75	0.0	7.87	34.35	41.25	37.80
Foliar application of *A. brasilense*	8.00	0.0	4.00	32.85	40.60	36.72
Seed inoculation of *A. brasilense*	10.75	0.0	5.37	37.12	41.40	39.26
AX+GB+CK	10.75	0.0	5.37	36.90	38.22	37.56
Mean	11.312 B	0.0 A		35.30 B	40.36 A	
Treatment	Total chlorophyll ($\mu g\ g^{-1}$)			Carotenoids ($\mu g\ g^{-1}$)		
	With Mesotrione	Without Mesotrione	Mean	With Mesotrione	Without Mesotrione	Mean
Control	38.22	39.95	39.08	0.90 aA	1.00 aA	0.95 a
Foliar application of *A. brasilense*	44.59	35.98	40.29	0.52 aB	1.03 aA	0.77 a
Seed inoculation of *A. brasilense*	38.62	39.95	34.54	0.24 bA	0.38 bA	0.31 c
AX+GB+CK	29.39	39.68	34.53	0.18 bB	0.89 aA	0.53 b
Mean	37.71	36.52		0.59	0.70	

	Intoxication	SPAD	Total chlorophyll	Carotenoids
Treatment	2.354^{ns}	0.446^{ns}	1.191^{ns}	18.759**
Mesotrione	115.491*	10.260**	0.184^{ns}	2.892^{ns}
Interaction	2.354^{ns}	0.840^{ns}	2.666^{ns}	15.391**
CV (%)	52.64	11.81	21.03	28.07

^{ns} non-significant by the F-test at 5% probability; **; * significant at 1% and 5% probability, respectively, by the F test. Similar lowercase letters in the column and uppercase letters in the row do not differ by the Student-Newman-Keuls (SNK) test at 5% probability.

As for maize crop yield, for both Marechal Cândido Rondon and Entre Rios do Oeste, there were no significant effects in any of the study sources of variation, nor for their interaction (Table 7). Nicolai et al. (2006) e Procópio et al. (2006) found results similar to those present in maize crops, i.e., the application of mesotrione did not interfere in the development and yield of the crop.

It was believed that inoculations of *A. brasilense* could assist in reducing the phytotoxic effects of mesotrione, because of their mechanisms of action, which act on the reduction of

Table 7 **-** Yield of maize plants after application (DAA) of Mesotrione, submitted to different treatments with plant hormones and plant growth promoting bacteria. 2016/2017

Treatment	Marechal Cândido Rondon			Entre Rios do Oeste		
	Yield (kg ha^{-1})					
	With Mesotrione	Without Mesotrione	Mean	With Mesotrione	Without Mesotrione	Mean
Control	11576.7	11122.3	11345.5	10010.9	9515.7	9778.3
Foliar application of *A. brasilense*	11169.8	10797.3	10983.6	9671.0	9215.4	9443.2
Seed inoculation of *A. brasilense*	10504.2	10860.9	10682.5	9880.9	10612.3	10146.6
AX+GB+CK	11678.5	10545.9	11112.2	9261.6	10400.7	9831.1
Mean	11232.3	10818.1		9713.6	9936.0	
Calculated F values						
Treatment	0.79^{ns}			1.19^{ns}		
Mesotrione	1.65^{ns}			0.54^{ns}		
Interaction	0.95^{ns}			1.94^{ns}		
CV (%)	8.00			8.68		

^{ns} not significant by the F-test at 5% probability; **; * significant at 1% and 5% probability, respectively, by the F test.

environmental stress (Kaushal and Wani, 2016; Vurukonda et al., 2016). One of these mechanisms is the ability to reduce ethylene levels in plants under stress conditions, because of competition against the precursor enzyme in the synthesis (Mayak et al., 2004; Glick, 2014). There were also beneficial effects, e.g., increase in chlorophyll levels during the initial development of plants (Bashan et al., 2006), as well as aid for maintenance of photosynthetic activity by production of abscisic acid (Cohen et al., 2008), which interfere in stomatal opening and closure.

In this way, it can be concluded that the use of seed inoculation with *A. brasilense* or foliar application of *A. brasilense* or plant growth regulator, tank-mixed with mesotrione, does not protect maize against mesotrione intoxication during initial development, nor does it increase crop yield.

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