Revista Ciência Agronômica, v. 55, e20238715, 2024
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Phytotoxicity of mixtures of phytosanitary products recommended for use in maize[1]

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ABSTRACT - The use of tank mixtures has long been a practice in the phytosanitary management of agricultural crops. However, when different molecules are mixed in a tank, interactions may occur that reduce the efficiency of the products and cause a loss of selectivity in the crops. The aim of this study was to evaluate selectivity in maize, with a tank mixture of different phytosanitary products recommended for use with the crop. The following products were used: herbicides – Sanson®, Soberan®, Callisto®, Zapp Pro®; fungicides – Priori Xtra®, Nativo®, Tebufort®, Aproach Prima®; insecticides – Connect®, Lorsban® 480 BR, Karate Zeon®; and nutrition – Kellus Manganese®, Kellus Blindex®. The experimental units consisted of 5-litre pots, containing two plants per pot, with the products applied at phenological stage V_6. Assessments were made of phytotoxicity, transient chlorophyll *a* fluorescence, chlorophyll content and shoot dry weight. The data obtained in the experiment were submitted to ANOVA ($p \leq 0.05$), and when significant, to the Scott-Knott test ($p \leq 0.05$). The maize crop suffered phytotoxicity of up to 30%, with the plants recovering from 28 DAA. The phytotoxicity was caused by mixing the different products in the spraying solution, mainly due to the synergism caused by the mixture of the herbicides nicosulfuron and tembotrione with the insecticide chlorpyrifos and the fertiliser Kellus Manganese®, which aggravated the incompatibility of some treatments. The results of this study defined which phytosanitary products recommended for use with maize should not be mixed in the same spraying solution due to the possibility of damaging the crop.

Key words: Tank mix incompatibility. Herbicide selectivity. *Zea mays*.

DOI: 10.5935/1806-6690.20240045
Editor-in-Chief: Prof. Alek Sandro Dutra - alekdutra@ufc.br
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Received for publication 13/02/2023; approved on 09/10/2023
[1]This research was extracted from the master's dissertation of the lead author presented to the Post-Graduate program in Agricultural and Environmental Engineering (PGEAAmb - UFRRJ). Funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001
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INTRODUCTION

The last grain harvest in the country (2021/2022) produced 271.2 million tons, with maize accounting for 41.7% of all production, reaching 113.3 million tons, including the first, second and third harvests (CONAB, 2022). Maize cultivation is of great economic interest in Brazil, but can undergo a loss in productivity due to various factors classified as biotic and abiotic, which directly or indirectly affect the crop, particularly reducing the quality and final yield of the grain (VITORINO *et al*., 2017).

Phytosanitary problems occur simultaneously in any one agricultural area, and the products used for control do not have a spectrum of action that is capable of controlling all the targets at once. For this reason, one strategy used to reduce operating costs is to mix the products in tanks (TREZZI *et al*., 2005).

Mixing different phytosanitary products in a tank may result in various problems, including the physicochemical incompatibility of the products in the spray solution (CLOYD, 2011). Incompatibility can result in such negative effects as changing the stability, efficiency and degradation of molecules, inhibiting the action of one product in the mixture on the target, and possibly also stimulating or inhibiting the processes of metabolic detoxification found in various target biotypes (VECHIA; FERREIRA; ANDRADE, 2018).

From various studies carried out to test a mixture of spraying solutions, it is known that some mixtures cannot be recommended due to their incompatibility when mixed in solution, which in some cases may cause phytotoxicity to crops when applied. Silva *et al*. (2005) found that a mixture of the insecticide chlorpyrifos with the herbicide nicosulfuron causes a reduction in the selectivity of the herbicide for maize, resulting in phytotoxicity, with a subsequent reduction in height and dry weight.

Some interactions that occur in mixtures that contain herbicides can be harmful to agricultural crops as they result in the loss of selectivity for a given herbicide, which can result in injury and reduced productivity (SILVA *et al*., 2005). Despite constant advances in the technological level of chemical industries in developing herbicides with a high level of efficiency and selectivity, herbicides can still cause injury and effect changes during crop development (MACIEL, 2004). For this reason, the selectivity of a herbicide for a given crop plays a fundamental role in applying these molecules, as the products, when used incorrectly, can cause irreversible damage to plants.

In general, selectivity occurs due to the differences between species in intercepting and absorbing herbicides, the different metabolic pathways, sensitivity of the site of action, and tolerance to the applied product (CATANEO *et al*., 2003). It is also based on the characteristics of the product, such as the method of application, the use of safeners, and genetic engineering (transgenic plants) (GALON *et al*., 2011). Selectivity should therefore be seen as a set of factors that afford phytosanitary protection to the crops without causing phytotoxicity (CARVALHO *et al*., 2009).

Given all the problems related to incompatibility caused by the mixture of phytosanitary products in the spraying solution, and the possible loss of selectivity of certain herbicides for maize, the aim of this study was to evaluate whether mixing the principal fungicides, insecticides, herbicides and fertilisers recommended for use in maize causes a loss of selectivity for the crop.

MATERIAL AND METHODS

All the treatments shown had been selected in earlier tests to evaluate the physicochemical compatibility of the products in the spraying solution based on the Brazilian ABNT regulation NBR 13875:2014 (Pesticides and similar – Assessment of physicochemical compatibility). This method consists in evaluating phytosanitary products (pesticides) individually and as a mixture, visually analysing whether any interaction occurs between the molecules (ABNT, 2014). As per the regulation, the evaluation was split into static and dynamic testing.

The tests were carried out to identify which phytosanitary products (fertilisers, fungicides, herbicides and insecticides) recommended for maize cultivation could be mixed in the spray tank without causing any physicochemical incompatibility in the solution, and show whether these were compatible or not when mixed. Only the treatments that were compatible in solution were selected to test for selectivity for maize.

The experiments for selectivity were conducted in a greenhouse located in the district of Seropédica, Rio de Janeiro, Brazil. The maize seeds (*Zea mays*) used in the experiments contained RR® technology (seeds with tolerance to the herbicide glyphosate), since all the mixtures included this active ingredient. The seeds came from the Comigo cooperative. The plants were grown according to their individual needs, with frequent irrigation and the application of nutrients (NPK) to the soil during the initial stages of development (vegetative) whenever necessary. The experimental design was completely randomised, with four replications. Each experimental unit consisted of one 5.0-L pot filled with soil classified as a Eutrophic Haplic Planosol (SANTOS *et al*., 2018) and containing two plants. Soil fertilisation was carried out as per the Liming and Fertilisation Manual of the State of Rio de Janeiro (EMBRAPA, 2013).

Twenty-five mixtures and eight individual products were evaluated (Table 1) in the experiments, plus the control with no application. The mixtures and individual products were applied when the maize plants were at phenological stage V_6. The application was carried out using a CO_2 pressurised backpack sprayer at a pressure of 40 psi, with a 1.0 m application bar equipped with two XR 110.020 nozzles spaced 0.5 m apart, to give a spray volume of 80 L ha^{-1}.

The following response variables were evaluated:

Phytotoxicity

Phytotoxicity was evaluated 7, 14 and 28 days after applying the treatments (DAA), using a visual phytotoxicity scale, the grades being assigned based on the symptoms, where 0% represents the absence of damage and 100% represents the death of the plants (FRANS; CROWLEY, 1986).

Transient fluorescence of chlorophyll *a*

The transient fluorescence of chlorophyll *a* was analysed 3, 14 and 28 days after applying the treatments (DAA), using the portable Handy PEA fluorometer (Plant Efficiency Analyser - Hansatech Instruments, Norfolk, UK) by means of a saturating light pulse on the leaf with an intensity of 3,000 µmol m^{-2} s^{-1} (GONÇALVES *et al*., 2010). The analyses were carried out on fully expanded apical leaves in the early hours of the morning (05:00 to 08:00), with the leaves adapted to darkness for 20 minutes using reading clips placed over the middle third of the leaves; two replications were carried out per plant, for a total of eight readings per treatment. From the transient fluorescence emission curve obtained following the saturating pulse, the parameters established for the JIP Test were calculated, as proposed by Strasser and Strasser (1995).

SPAD index and quantification of the chlorophyll content

The SPAD index was assessed 7, 14 and 28 days after application using the Falker CFL1030 ClorofiLOG electronic chlorophyll content meter.

To extrapolate the data and calibrate the ClorofiLOG, a curve was constructed relating the chlorophyll content to the values of the SPAD index. The curve was constructed based on the method proposed by Wellburn (1994), where small discs measuring 1.0 cm in diameter were removed from the leaves, immediately packed in aluminium foil bags and placed in a Styrofoam container with ice to preserve the chlorophyll content.

Table 1 - Combinations of the phytosanitary products used on maize (herbicides, fungicides, insecticides and fertilisers) selected in the tests of compatibility

Individual products	Insecticide + Herbicide + Fertiliser	Insecticide + Herbicide
Sanson®	CBSaZ	CSaZ
Soberan®	CBSoZ	CSoZ
Zapp PRO®	CBZ	CZ
Connect®	CMSaZ	LSoZ
Lorsban®	CMSoZ	LZ
Karate Zeon®	CMZ	KSoZ
Kellus Manganese®	LBSaZ	KZ
Kellus Blindex®	LBZ	
	LMSaZ	
Herbicide mixture	LMSoZ	
	LMZ	
	KBSaZ	
	KBSoZ	
Soberan® + Zapp QI 620®	KBZ	
	KMSaZ	
	KMSoZ	
	KMZ	

C = Connect®; B = Kellus Blindex®; Sa = Sanson®; Z = Zapp PRO®; So = Soberan®; M = Kellus Manganese®; L = Lorsban® e K = Karate Zeon®

In the laboratory, the discs were weighed and then placed in 15 mL polypropylene falcon tubes with a conical base, which contained a DMSO solution (neutralised with 5% $CaCO_3$). The solution was prepared using calcium carbonate at 5% of the total amount of DMSO. $CaCO_3$ was added to the DMSO solution which was then stirred for 30 minutes. At the end of this period, the solution was vacuum filtered using a Büchner funnel and qualitative filter paper (80 g) until total transparency was achieved with the removal of the $CaCO_3$. A volume of 7.0 mL of the DMSO solution was added to each tube. The tube was then closed and placed in a water bath at ±90 ºC for 40 minutes to 1 hour, until the discs were transparent. After extraction, the discs were removed and left in the dark to prevent the chlorophyll from degrading until they cooled to room temperature.

The chlorophyll *a* and *b* content of the extracted solution was determined using a BEL® Engineering model V-M5 spectrophotometer with a spectral bandwidth of 4 nm using quartz cuvettes with a beam of 10 mm in the 480, 649 and 665 nm bands. The formulas used to calculate the total chlorophyll content are shown below.

$$Chlorophyll\ \alpha = ((12.19 \times A_{665}) - (3.45 \times A_{649})) \quad (1)$$

$$Chlorophyll\ b = ((21.99 \times A_{649})(5.32 \times A_{665})) \quad (2)$$

$$Total\ chlorophyll = Chlorophyll\,\alpha + Chlorophyll\ b \quad (3)$$

where A665 and A649 are the values measured during the evaluation using the spectrophotometer. Following these calculations, the results were expressed in µg mL^{-1}, so it was necessary to convert to mg g^{-1} FM:

$$content\ (\mu g\ mL^{-1}) \Rightarrow 1mL \quad (4)$$

$$X \Rightarrow 7\ mL\ DMSO \quad (5)$$

$$X\ (\mu g) \rightarrow weight\ of\ the\ leaf\ disk(g) \quad (6)$$

$$X \rightarrow 1.0\ g\ \ fresh\ weight \quad (7)$$

$$X(\mu g\ g^{-1}\ MF) \div 1.000 \quad (8)$$

From the results of the chlorophyll content and the SPAD index measured using the ClorofiLOG, the instrument calibration curve was generated for the maize (Figure 1).

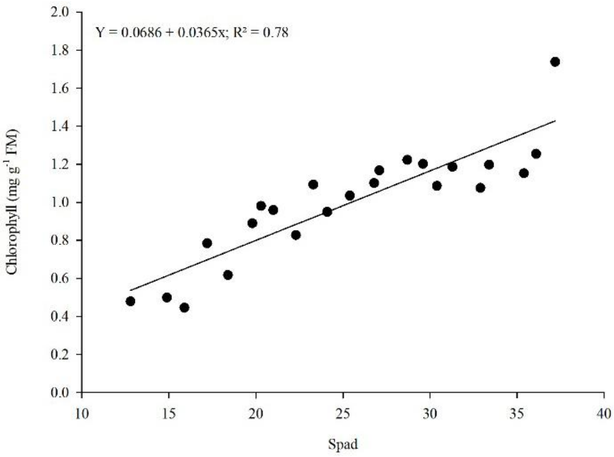
Shoot dry weight (SDW)

Shoot dry weight was determined 28 DAA. The plants were collected close to the ground, placed in paper bags, and then left in a forced air circulation oven at 65 ± 5 ºC to constant weight, when the shoot dry weight was determined using an analytical balance.

Statistical analysis

The data generated in the experiments were submitted to analysis of variance - ANOVA ($p \leq 0.05$), and if statistically significant, the mean values were compared by the Scott-Knott test at a level of 5% ($p \leq 0.05$) using the Sisvar statistical software. The graphs were generated using the SigmaPlot 12.5 software.

Figure 1 - Instrument calibration curve for the ClorofiLOG using the Spad indices generated in the evaluations and extrapolation of the chlorophyll content, based on maize



RESULTS AND DISCUSSION

Phytotoxicity

The selectivity of plants to the application of herbicides is characterised by the interaction of different mechanisms that protect the crop from the intoxication caused by the herbicides, where the principal mechanism is the different metabolism of these products for weeds and cultivated plants (CARVALHO *et al*., 2009) originating from the metabolisation of non-toxic plant compounds (KARAM *et al*., 2009). As such, characteristics relating to the sensitivity of a plant to a given herbicide molecule can vary between maize hybrids (KARAM *et al*., 2009). Synergistic interactions between the products can reduce the safety margin for the crops, causing symptoms such as phytotoxicity, a reduction in height, decreased shoot and root dry weight, a loss of productivity, and even plant death (ZANATTA *et al*., 2007).

When evaluating phytotoxicity in the maize seven days after application (DAA), Sanson®, Soberan®, Lorsban®, Karate Zeon®, Kellus Manganese®, Kellus Blindex® as individual treatments, and Sob+Zapp, CBSaZ, KBSaZ, KBSoZ and KSoZ as mixtures caused no percentage phytotoxicity and did not differ from the control (Table 2). The Connect®, CBSoZ, CBZ, LBSaZ, LBZ, CSaZ, CSoZ, CZ, LSoZ, LZ and KZ treatments caused only 5% phytotoxicity and did not differ from each other (Table 2). The Zapp® PRO, CMSaZ, CMSoZ, LMSaZ, KBZ and KMSaZ treatments caused 10% phytotoxicity in relation to the control, without differing from each other (Table 2). The LMSoZ, KMSoZ and KMZ treatments resulted in 15% phytotoxicity in relation to the control, again not differing from each other, and the CMZ and LMZ treatments caused 30% and 20% phytotoxicity, respectively, in relation to the control, these being the most severe of the treatments under evaluation (Table 2).

Fourteen days after application (DAA), the individual treatments Sanson®, Soberan®, Connect®, Lorsban®, Karate Zeon®, Kellus Blindex®, and the mixtures Sob+Zapp, CBSaZ, CBSoZ and KBSoZ caused no percentage phytotoxicity, and did not differ from the control (Table 2). The Zapp® PRO, Kellus Manganese®, CBZ, LBSaZ, LBZ, KBSaZ, CSaZ, CSoZ, LSoZ and KSoZ treatments caused 5% phytotoxicity in relation to the control (Table 2), whereas the CMSaZ, CMSoZ, KBZ, KMSaZ, CZ, LZ and KZ treatments caused 10% phytotoxicity in relation to the control. The LMSaZ, LMSoZ, KMSoZ and KMZ treatments caused 15% phytotoxicity in relation to the control, and did not differ from each other (Table 2). The two treatments that caused the highest rates of phytotoxicity, without differing from each other, were CMZ, whose phytotoxicity of approximately 30% remained the same as in the evaluation at 7 DAA, and LMZ, which also caused 30% phytotoxicity in relation to the control (Table 2).

Various bad interactions have caused damage to maize crops, with reports of problems related to a mixture of herbicides from the sulfonylurea chemical group and organophosphate insecticides (STECKEL; STEWART; STECKEL, 2015). Silva *et al*. (2005) found that a mixed application of the herbicide nicosulfuron and the insecticide chlorpyrifos, regardless of the phenological stage of the maize, reduced the components of plant height and shoot dry weight, making a five-day interval between applying the products necessary to reduce the effects of toxicity in the maize caused by the nicosulfuron, as can be seen in the present research with the LBSaZ and LMSaZ treatments, which have these active ingredients in their mixtures.

Table 2 - Percentage phytotoxicity, chlorophyll content and shoot dry weight (SDW) in the individual and mixed treatments 7, 14 and 28 days after application (DAA)

Treatment	Phytotoxicity (%)		Chlorophyll (mg g^{-1} FM)			SDW (g)
	7 DAA	14 DAA	7 DAA	14 DAA	28 DAA	28 DAA
Control	0.00 a	0.00 a	1.19 a	0.96^{ns}	0.99 a	21.37 a
Sanson®	0.00 a	0.00 a	1.06 a	0.92	1.06 a	19.69 b
Soberan®	0.00 a	0.00 a	1.01 b	0.84	0.97 a	18.50 b
Zapp PRO®	7.50 c	5.00 c	0.96 b	0.89	1.01 a	18.11 b
Connect®	6.25 b	0.00 a	1.07 a	0.85	0.87 b	20.03 b
Lorsban®	0.00 a	0.00 a	1.07 a	0.88	0.82 b	19.87 b
Karate Zeon®	0.00 a	0.00 a	1.09 a	0.86	0.91 b	20.44 a
Kellus Manganese®	2.50 a	2.50 b	1.18 a	0.93	0.92 a	18.94 b
Kellus Blindex®	0.00 a	0.00 a	1.09 a	0.82	0.89 b	18.91 b
Sob+Zapp	0.00 a	0.00 a	1.13 a	0.89	0.83 b	21.52 a
CBSaZ	0.00 a	0.00 a	1.02 a	0.91	0.88 b	18.17 b
CBSoZ	5.00 b	0.00 a	1.04 a	0.86	0.92 a	20.58 a
CBZ	5.00 b	5.00 c	0.98 b	0.8	0.86 b	19.02 b
CMSaZ	8.75 c	10.00 e	0.99 b	0.79	0.96 a	20.71 a
CMSoZ	10.00 c	10.00 e	0.96 b	0.81	0.94 a	23.81 a
CMZ	32.50 f	32.50 g	0.77 b	0.75	0.91 b	22.31 a
LBSaZ	5.00 b	5.00 c	1.04 a	0.83	0.97 a	20.37 a
LBZ	5.00 b	5.00 c	0.9 b	0.85	0.92 a	17.24 b
LMSaZ	10.00 c	15.00 f	0.95 b	0.82	0.91 b	23.76 a
LMSoZ	15.00 d	16.25 f	0.96 b	0.86	0.9 b	19.42 b

Continuation Table 2

LMZ	20.00 e	31.25 g	0.91 b	0.85	0.88 b	22.82 a
KBSaZ	0.00 a	5.00 c	1.06 a	0.79	0.83 b	17.91 b
KBSoZ	0.00 a	0.00 a	1.16 a	0.8	0.94 a	19.06 b
KBZ	10.00 c	10.00 e	1.07 a	0.87	0.81 b	19.59 b
KMSaZ	10.00 c	10.00 e	0.95 b	0.82	0.95 a	18.70 b
KMSoZ	16.25 d	16.25 f	0.98 b	0.91	0.87 b	20.84 a
KMZ	15.00 d	15.00 f	0.94 b	0.83	0.95 a	18.76 b
CSaZ	5.00 b	2.50 b	0.96 b	0.78	0.96 a	18.96 b
CSoZ	5.00 b	5.00 c	0.92 b	0.85	0.96 a	20.53 a
CZ	5.00 b	7.50 d	1.17 a	0.83	0.96 a	20.74 a
LSoZ	6.25 b	6.25 c	1.09 a	0.91	0.94 a	21.12 a
LZ	5.00 b	8.75 d	1.13 a	0.96	0.93 a	21.01 a
KSoZ	0.00 a	5.00 c	1.05 a	0.83	0.86 b	20.38 a
KZ	6.25 b	10.00 e	0.94 b	0.85	0.84 b	18.63 b
CV (%)	23.26	19.43	12.67	11.20	8.74	11.60

Mean values with the same letters in a column do not differ by Scott-Knott test at 5% probability. C = Connect®, L = Lorsban®, K = Karate Zeon®, B = Kellus Blindex®, M = Kellus Manganese®, Sa = Sanson®, So = Soberan®, Z = Zapp PRO®. ns = not significant

In maize, herbicides that inhibit the ALS enzyme (acetolactate synthase), as well as the organophosphate insecticides, are degraded by the same enzyme system; however, in the presence of the insecticide, the rate of herbicide metabolisation is reduced, causing the herbicide to accumulate at levels that could cause toxicity in the crop (KREUZ; FONN-PFISTER, 1992; MACIEL *et al*., 2018). It is believed that the concentration of enzymes responsible for metabolising molecules in the differential metabolism of the plants, the CytP450 enzymes (cytochrome P450), are strongly modified by the organophosphate insecticides (MATZENBACHER *et al*., 2015). According to Silva *et al.* (2005), the symptoms caused in plants by intoxication resulting from the interaction of these products in maize are chlorosis, death of the apical bud and reduced tillering.

Increasing phytotoxicity was seen when assessing the maize. One possible explanation for the 5% and 15% phytotoxicity seen, respectively, in the LBSaZ and LMSaZ treatments, which contain in their solutions a mixture of the active ingredients nicosulfuron (Sanson®) and chlorpyrifos (Lorsban®) in addition to glyphosate, the zinc, manganese, and copper-based fertiliser Kellus Blindex®, and the manganese-based fertiliser Kellus Manganese®, would be the negative biological interaction of both molecules (nicosulfuron and chlorpyrifos) in the plant, causing the maize to lose its natural selectivity for the herbicide molecule. However, it can be seen that only by changing the fertiliser in the LBSaZ treatment was the phytotoxicity reduced. This was also seen in the other treatments: when the Kellus Manganese® fertiliser was replaced by the Kellus Blindex® fertiliser, phytotoxicity was at most 5% or was not seen at all (0%).

It can also be seen that the CMZ and LMZ treatments presented a maximum phytotoxicity of 30% (the highest values observed) and that when the above fertilisers were changed, the CBZ and LBZ treatments presented a minimum phytotoxicity of 5%, and later, when the fertilisers were removed, the CZ and LZ treatments showed minimal phytotoxicity, confirming that the possible cause of phytotoxicity in some of the treatments that have a mixture of insecticide + fertiliser + herbicide is probably heightened by the Kellus Manganese® fertiliser.

Another relevant point was the mixture of the active ingredients tembotrione (Soberan®) and chlorpyrifos (Lorsban®) together with the herbicide glyphosate and the fertiliser Kellus Manganese® in the LMSoZ treatment, where a phytotoxicity of 16.25% was seen, indicating possible interaction between the active ingredients tembotrione and chlorpyrifos. A study by Maciel *et al.* (2018) showed that a mixture of the active ingredients mesotrione and chlorpyrifos (144 g ha^{-1} and 240 g ha^{-1}, respectively) was among the mixtures that caused the most damage to the 30F35 maize hybrid when applied at stage V_6 of the crop. The herbicides tembotrione and mesotrione belong to the chemical group of triketones, and act on weeds by inhibiting the biosynthesis of carotenoids, thereby interfering in the activity of the HPPD enzyme (4-hydroxyphenyl-pyruvate-dioxygenase) in the chloroplasts (KARAM *et al*., 2009). One possible explanation for the phytotoxicity caused by the LMSoZ treatment, therefore, may be a loss of selectivity of the maize for the herbicide tembotrione, also caused by interaction with the organophosphate insecticide chlorpyrifos.

In the evaluations that followed 14 DAA, phytotoxicity in the maize caused by the treatments began to fall. In the evaluation 28 days after application, none of the treatments, whether applied individually or as a mixture, caused any percentage phytotoxicity, all the treatments showing 0% phytotoxicity in the maize plants recovering from damage, indicating that the plants were progressively reestablishing the rate of metabolisation of the products that were causing phytotoxicity.

Spad Index and Chlorophyll Content

The chlorophylls present in plants are directly related to their photosynthetic efficiency, and consequently, to their growth and adaptation to different environments. The pigments are responsible for capturing the light that is used in photosynthesis, and are essential in the conversion of light energy into chemical energy, resulting in the formation of ATP and NADPH (FONSECA *et al*., 2012; JESUS; MARENCO, 2008). Quantifying the chlorophyll content is necessary in studies that seek to achieve better cropping and management practices, with the aim of increasing the photosynthetic potential of the plants and increasing crop production (FONSECA *et al*., 2012).

Seven days after application (DAA), the treatments CBZ, CMSaZ, CMSoZ, CMZ, LBZ, LMSaZ, LMSoZ, LMZ, KMSaZ, KMSoZ, KMZ, CSaZ, CSoZ, KZ, Soberan® and Zapp PRO® presented a value for the chlorophyll index less than that of the control, of between 17% and 35%, and did not differ from each other. The other treatments did not differ from the control (Table 2).

In the evaluation made 14 days after application, none of the mixed treatments Sob+Zapp, CBSaZ, CBSoZ, CBZ, CMSaZ, CMSoZ, CMZ, LBSaZ, LBZ, LMSaZ, LMSoZ, LMZ, KBSaZ, KBSoZ, KBZ, KMSaZ, KMSoZ, KMZ, CSaZ, CSoZ, CZ, LSoZ, LZ, KSoZ and KZ, or the individual treatments Sanson®, Soberan®, Zapp PRO®, Connect®, Lorsban®, Karate Zeon®, Kellus Manganese® and Kellus Blindex® differed from the control. (Table 2).

In the evaluation made 28 days after application, the treatments Sob+Zapp, CBSaZ, CBZ, CMZ, LMSaZ, LMSoZ, LMZ, KBSaZ, KBZ, KMSoZ, KSoZ, KZ, Connect®, Lorsban®, Karate Zeon® and Kellus Blindex® presented a value for the chlorophyll index below that of the control, of between 8% and 18%, and did not differ from each other. The other treatments did not differ from the control (Table 2).

Various biotic and abiotic factors can directly influence the chlorophyll content of the leaves, and may be responsible for their degradation since, as mentioned above, chlorophylls are directly related to the potential of a plant for photosynthetic activity (TAIZ; ZEIGER, 2002). Degradation of the chlorophylls can be caused by several factors, one of them being oxidative stress, which results in the loss of the green colour of the leaves (PAVANI, 2013). The reduced level of green seen in the leaf blade reflects the phytotoxicity induced by herbicides that may compromise the photosynthetic capacity of the plant, resulting in a reduction in biomass and grain production (GONÇALVES *et al*., 2018).

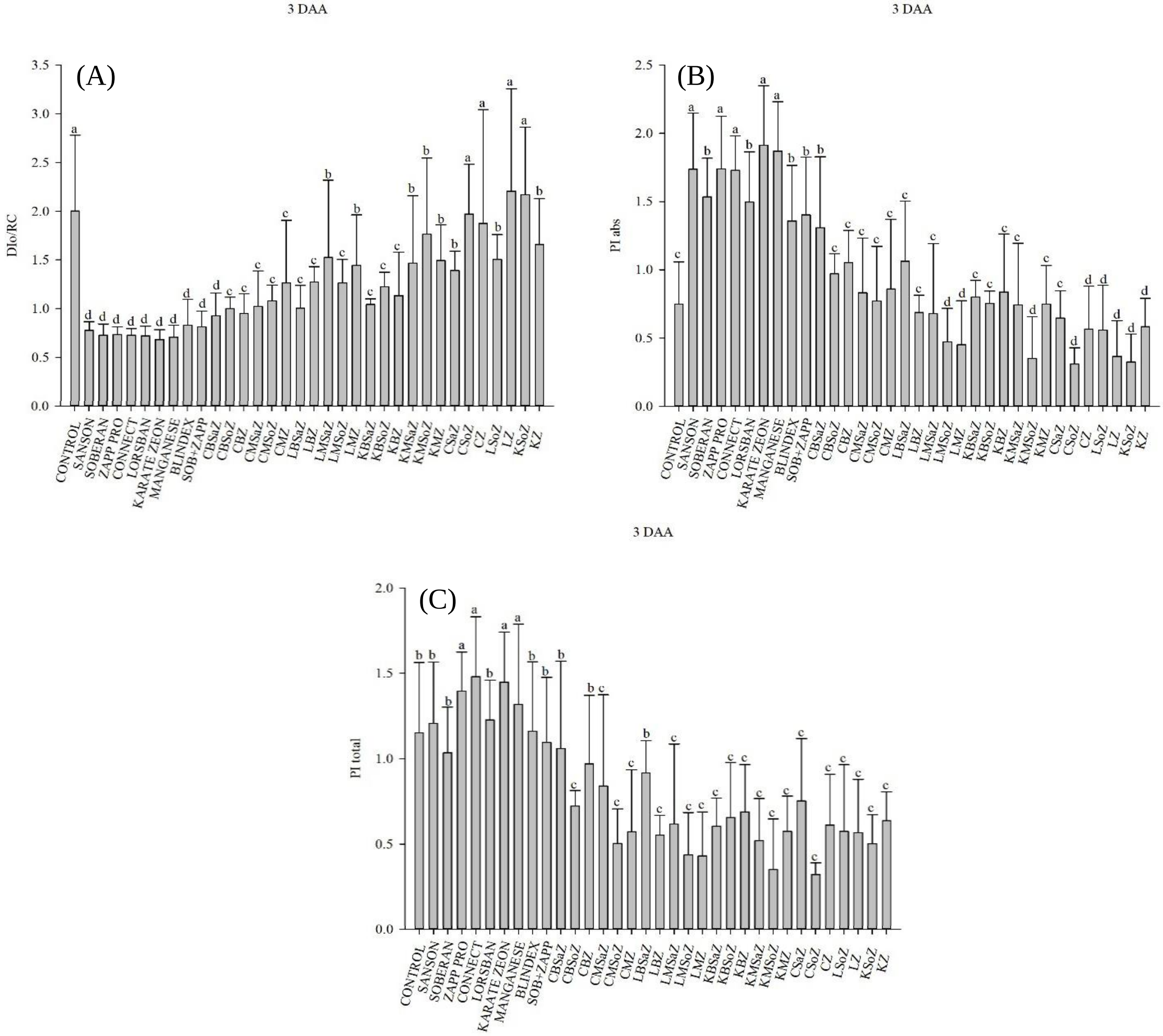
The maize showed differences in relation to the chlorophyll content of the leaves for various treatments, both mixtures and individually. The reduction in the SPAD index and chlorophyll content is possibly related to damage to the chloroplast, with a subsequent reduction in the rate of photosynthesis (ANDRADE *et al*., 2018; REDDY; RIMANDO; DUKE, 2004), and linked to the yellowing (chlorosis) that occurs in the leaves. It may also be due to the immobilisation of such cations as Mg^{2+}, which is used in forming chlorophyll, and Mn^{2+} which is used during the steps of photosynthesis, both influenced by the herbicide glyphosate (ANDRADE *et al*., 2018; TAIZ *et al*., 2017). The synthesis of metabolites and the activity of enzymes of the Calvin-Benson cycle can be affected by any chemical substance that induces changes in the metabolism of the leaves (ALBRECHT *et al*., 2011).

According to the study carried out by Torres Netto *et al.* (2005), the reduction in chlorophyll content begins when the SPAD readings present values of less than 40, which can then affect photosynthetic activity. This pattern of behaviour was also seen in the present study in maize. Therefore, the lower the SPAD value, the lower the chlorophyll content of the leaves, and the greater the yellowing.

Transient fluorescence of chlorophyll *a*

The maize presented variations in the parameter related to the specific fluxes of each reaction centre (DIo/RC) and in the indices of photosynthetic performance (PI_{ABS} and PI_{Total}). In evaluating fluorescence three days after application (DAA), the mixed treatments Sob+Zapp, CBSaZ, CBSoZ, CBZ, CMSaZ, CMSoZ, CMZ, LBSaZ, LBZ, LMSaZ, LMSoZ, LMZ, KBSaZ, KBSoZ, KBZ, KMSaZ, KMSoZ, KMZ, CSaZ, LSoZ and KZ showed a significant reduction in the values of the DIo/RC parameter (energy lost by the plant in the form of heat) of 12% to 60% in relation to the control. The only treatments that as a mixture did not differ from the control for this parameter were CSoZ, CZ, LZ and KSoZ (Figure 2A). Each of the individually applied treatments (Sanson®, Soberan®, Zapp® PRO, Connect®, Lorsban®, Karate Zeon®, Kellus Manganese® and Kellus Blindex®) showed a reduction in DIo/RC ranging from 59% to 66% in relation to the control, and did not differ from each other (Figure 2A).

Figure 2 - Evaluation of the transient fluorescence of chlorophyll *a* in maize for the individual and mixed treatments three days after application (DAA), with evaluation of the parameters (a) DIo/RC, (b) PI abs, and (c) PI total. Mean values with the same letters on the graph do not differ by the Scott-Knott test at 5% probability. C = Connect®, L = Lorsban®, K = Karate Zeon®, B = Kellus Blindex®, M = Kellus Manganese®, Sa = Sanson®, So = Soberan®, Z = Zapp PRO®



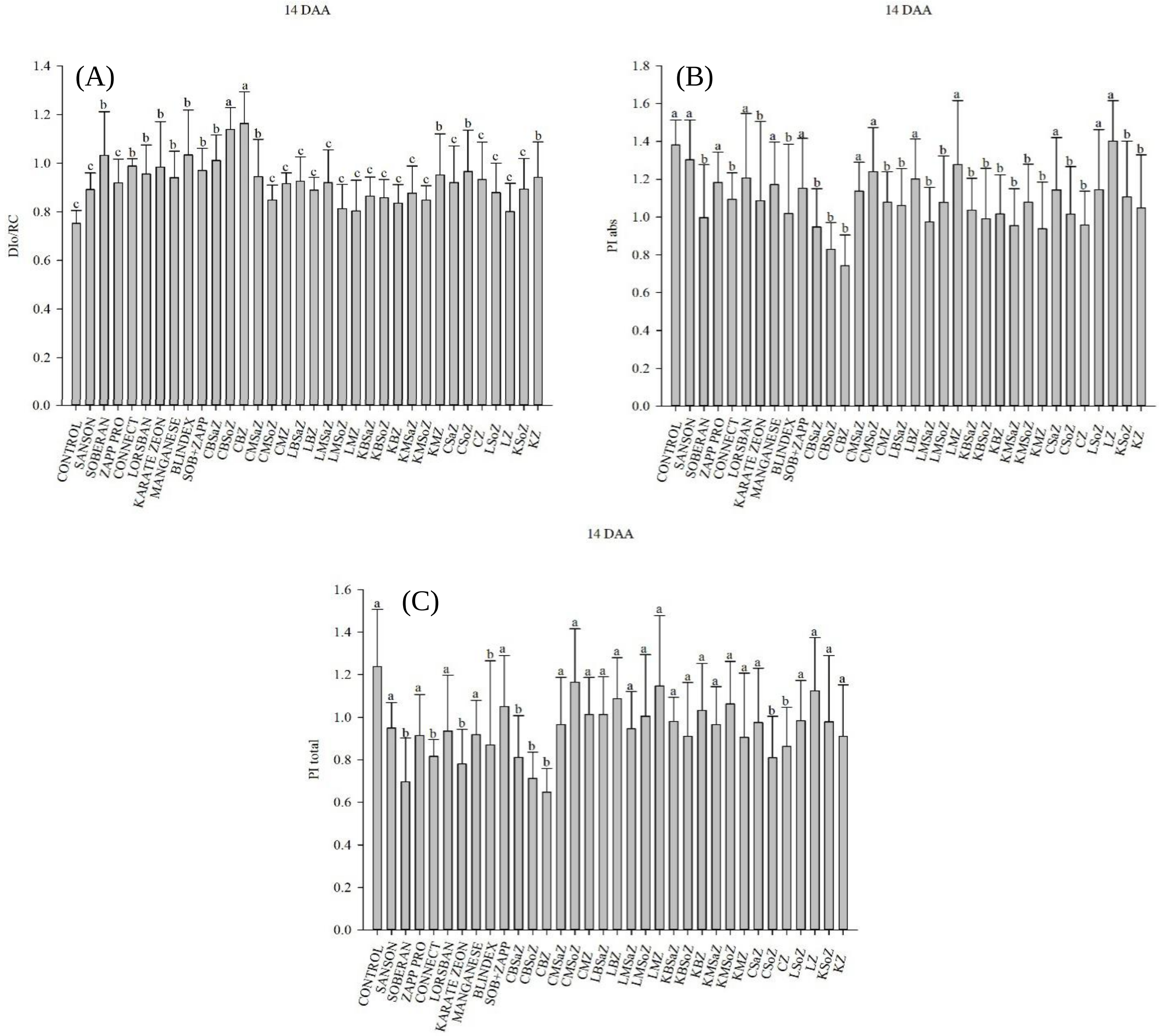
For the PI_{ABS} parameter (photosynthetic performance index), treatments LMSoZ, LMZ, KMSoZ, CSoZ, CZ, LSoZ, LZ, KSoZ and KZ suffered a reduction in relation to the control of between 22% and 59%, and did not differ from each other. Treatments CBSoZ, CBZ, CMSaZ, CMSoZ, CMZ, LBSaZ, LBZ, LMSaZ, KBSaZ, KBSoZ, KBZ, KMSaZ, KMZ and CSaZ did not differ from the control. The only treatments that as a mixture showed an increase in relation to the control for PI_{ABS} were Sob+Zapp and CBSaZ, equivalent to 89% and 77% respectively, again not differing from each other (Figure 2B). Each of the individual treatments caused an increase of greater than 100% in relation to the control in the PI_{ABS} parameter (Figure 2B).

Three days after application, a reduction was also seen in relation to the control in PI_{Total} (index of total photosynthetic performance) for treatments CBSoZ, CMSaZ, CMSoZ, CMZ, LBZ, LMSaZ, LMSoZ, LMZ, KBSaZ, KBSoZ, KBZ, KMSaZ, KMSoZ, KMZ, CSaZ, CSoZ, CZ, LSoZ, LZ, KSoZ and KZ, varying between 28% and 70%, and not differing from each other. The Sob+Zapp, CBSaZ, CBZ and LBSaZ mixed treatments, and the Sanson®, Soberan®, Lorsban® and Kellus Blindex® individual treatments did not differ from the control for this parameter (Figure 2C). The individual treatments Zapp PRO®, Connect®, Karate Zeon® and Kellus Manganese® caused an increase of up to 29% in PI_{Total} in relation to the control, without differing from each other (Figure 2C).

A reduction in the performance indices may indicate severe damage to the photosynthetic apparatus, showing a loss of photochemical efficiency by the plant (THACH *et al*., 2007). In contrast, the individual treatments and only the Soberan+Zapp mixture showed an increase in performance parameters. According to Oukarroum *et al.* (2007), an increase in the PI_{ABS} parameter at the start of flooding stress, for example, may indicate the plant's compensating for its low photosynthetic capacity in an attempt to adapt to the initial stress (MARTINAZZO *et al*., 2013).

In the evaluation made 14 days after application (DAA), some of the mixed treatments (Sob+Zapp, CBSaZ, CBSoZ, CBZ, CMSaZ, KMZ, CSoZ and KZ) showed an increase in relation to the control for DIo/RC, varying between 25% and 55%. The other mixed treatments did not differ from the control (Figure 3A). The treatments Soberan®, Connect®, Lorsban®, Karate Zeon®, Kellus Manganese® and Kellus Blindex® showed an increase of between 25% and 37% in relation to the control, and did not differ from each other. Only the Sanson® and Zapp PRO® treatments did not differ from the control (Figure 3A).

Figure 3 - Evaluation of the transient fluorescence of chlorophyll *a* in maize for the individual and mixed treatments 14 days after application (DAA), with evaluation of the parameters (a) DIo/RC, (b) PI abs and (c) PI total. Mean values with the same letters on the graph do not differ by the Scott-Knott test at 5% probability. C = Connect®, L = Lorsban®, K = Karate Zeon®, B = Kellus Blindex®, M = Kellus Manganese®, Sa = Sanson®, So = Soberan®, Z = Zapp PRO®



For the PI_{ABS}, the mixed treatments CBSaZ, CBSoZ, CBZ, CMZ, LBSaZ, LMSaZ, LMSoZ, KBSaZ, KBSoZ, KBZ, KMSaZ, KMSoZ, KMZ, CSoZ, CZ, KSoZ and KZ caused a reduction in relation to the control of up to 46%, and did not differ from each other; the remaining treatments did not differ from the control for this parameter (Figure 3B). The individual treatments Soberan®, Connect®, Karate Zeon® and Kellus Blindex® also showed a reduction in relation to the control of between 21% and 28%, and also did not differ from each other. The remaining individual treatments did not differ from the control (Figure 3B).

Only the CBSaZ, CBSoZ, CBZ, CSoZ and CZ treatments showed a reduction in relation to the control for PI_{Total}, ranging from 30% to 48%, and did not differ from each other; the other treatments did not differ from the control (Figure 3C). The Soberan®, Connect®, Karate Zeon® and Kellus Blindex® treatments showed a reduction in relation to the control ranging from 30% to 44%, again not differing from each other. The remaining individual treatments did not differ from the control for this parameter (Figure 3C).

An increase in DIo/RC may be related to an attempt by the plants to avoid excess unused energy accumulating in the reaction centre so that it does not result in the formation of reactive oxygen species (SZABÓ; BERGANTINO; GIACOMETTI, 2005). Associated with this, maize plants showed a marked reduction in performance indices, which may indicate that the absorbed energy was not being used efficiently, represented by a reduction in photosynthetic activity and increase in energy dissipated in the form of heat (LAWLOR; TEZARA, 2009).

Twenty-eight days after application (DAA), the mixed treatments Sob+Zapp, CBSoZ, CBZ, CMSoZ, CMZ, LBSaZ, LBZ, LMSaZ, LMSoZ, KBSaZ, KBSoZ, KMSaZ, KMZ, CSoZ, CZ and LZ showed a reduction in relation to the control for DIo/RC ranging from 19% to 39%, without differing from each other. The LMZ and KMSoZ mixed treatments showed an increase in relation to the control, of 18% and 20% respectively, again not differing from each other; the other treatments did not differ from the control (Figure 4A). All the individual treatments (Sanson®, Soberan®, Zapp® PRO, Connect®, Lorsban®, Karate Zeon®, Kellus Manganese® and Kellus Blindex®) showed a reduction in relation to the control of up to 49%, not differing from each other. (Figure 4A).

An increase in the PI_{ABS} parameter was also seen in relation to the control for the treatments Sob+Zapp, CBSaZ, CBSoZ, CBZ, CMSaZ, CMSoZ, CMZ, LBSaZ, LBZ, LMSaZ, LMSoZ, KBSaZ, KBSoZ, KBZ, KMSaZ, KMZ, CSaZ, CSoZ, CZ, LSoZ and LZ, ranging from 23% to over 100%; the other treatments did not differ from the control for this parameter (Figure 4B). The Sanson®, Soberan®, Zapp® PRO, Lorsban® and Kellus Manganese® treatments showed an increase in relation to the control of more than 100%, while the Connect®, Karate Zeon® and Kellus Blindex® treatments showed an increase in relation to the control of approximately 92%, 76% and 92% respectively, without differing from each other (Figure 4B).

The PI_{Total} parameter also showed an increase in relation to the control for the KBSoZ, KMSaZ, KMZ, CSaZ, CSoZ, CZ, LSoZ and LZ treatments of up to 40%, not differing from each other; the other mixed treatments did not differ from the control (Figure 4C). The Sanson®, Soberan®, Zapp® PRO and Kellus Manganese® treatments showed a respective increase in relation to the control of 38%, 47%, 24% and 14%, and did not differ from each other. The remaining treatments did not differ from the control for this parameter (Figure 4C).

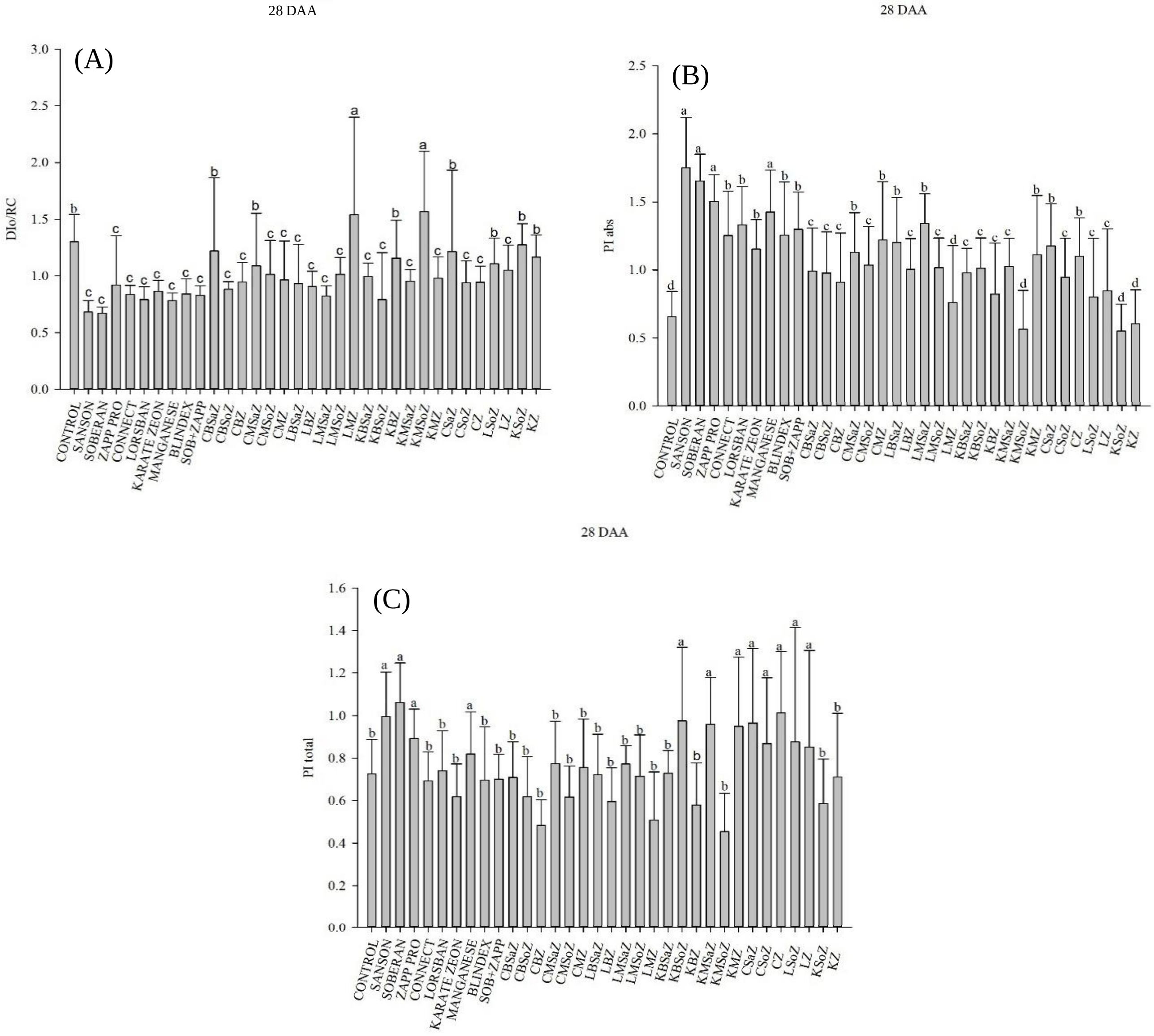
By the end of the stress, at 28 DAA, the maize no longer showed any damage to the photosynthetic apparatus, and the parameters of photosynthetic performance, PI_{ABS} and PI_{Total}, showed an increase, indicating that photosynthetic activity had been reestablished. As the performance indices, PI_{ABS} and PI_{Total}, are parameters that integrate other indicators of fluorescent activity, they are the most representative of energy flow behaviour in the photosynthesis electron transport chain (TSIMILLI-MICHAEL; STRASSER, 2008; YUSUF *et al*., 2010). Changes in these parameters are therefore excellent indicators of photosynthetic activity, or the degree of stress that plants may be suffering, and which can directly or indirectly damage the photosynthetic apparatus (SCHOCK, 2012).

Shoot dry weight (SDW)

When evaluating shoot dry weight, the mixed treatments CBSaZ, CBZ, LMSoZ, LBZ, KBSaZ, KBSoZ, KBZ, KMSaZ, KMZ, CSaZ and KZ, and the individual treatments Sanson®, Soberan®, Zapp® PRO, Connect®, Lorsban®, Kellus Manganese® and Kellus Blindex® presented values between 5% and 14% less than the control without differing from each other. The Sob+Zapp, CBSoZ, CMSaZ, CMSoZ, CMZ, LBSaZ, LMSaZ, LMZ, KMSoZ, CSoZ, CZ, LSoZ, LZ and KSoZ treatments, in addition to Karate Zeon® applied individually, did not differ from the control (Table 2).

As mentioned above, organic compounds are synthesised using the energy from photosynthesis, resulting in the accumulation of plant biomass (TAIZ *et al*., 2017); a reduction in photosynthetic activity results in lower biomass production and lower plant height, resulting in plants with less productive potential (ANDRADE *et al*., 2018),

Figure 4 - Evaluation of the transient fluorescence of chlorophyll *a* in maize for the individual and mixed treatments 28 days after application (DAA), with evaluation of the parameters (a) DIo/RC, (b) PI abs and (c) PI total. Mean values with the same letters on the graph do not differ by the Scott-Knott test at 5% probability. C = Connect®, L = Lorsban®, K = Karate Zeon®, B = Kellus Blindex®, M = Kellus Manganese®, Sa = Sanson®, So = Soberan®, Z = Zapp PRO®



which is one possible explanation for what occurred in the maize when evaluating shoot dry weight (SDW), where some of the mixed and individual treatments showed a slight reduction in plant development and growth.

Although the maize had recovered from all damage by the end of the evaluations, it is important to note that the present study did not evaluate the productivity of the crop. There is, therefore, no information on whether the mixtures that caused damage to the crop might reduce its productivity.

CONCLUSION

The CMZ, LMSaZ, LMSoZ, LMZ, KMSoZ and KMZ treatments tested on maize showed a maximum phytotoxicity of 30%, although the crop was later able to recover from the damage caused by the treatments. However, as already mentioned, this study did not evaluate the components of crop productivity. There is, therefore, no information on whether the mixtures that showed a percentage of phytotoxicity would or would not cause a reduction in productivity. As such, these mixtures are not recommended for use with the crop.

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