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ANTIPROLIFERATIVE AND GENOTOXIC POTENTIAL OF AQUEOUS EXTRACTS OF *STEVIA REBAUDIANA* BERTONI (BERTONI) (ASTERACEAE) USING THE *ALLIUM CEPA* L. TEST.

Potencial antiproliferativo e genotóxico de extratos aquosos de Stevia rebaudiana Bertoni (Bertoni) (Asteraceae) pelo teste Allium cepa L.

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Abstract: *Stevia rebaudiana* Bertoni (Bertoni) (stevia), a species of the Asteraceae family, has antibacterial, antiseptic, anti-inflammatory, hypotensive, diuretic, cardiotonic, antioxidant and contraceptive properties. The aim of this study was to analyze the genotoxic and antiproliferative potential of aqueous extracts of fresh and dried *S. rebaudiana* leaves treated with different concentrations of glyphosate on the plant bioindicator *Allium cepa* L. Specimens of plants *S. rebaudiana* were grown in a greenhouse and the following treatments were carried out: control (plants receiving only distilled water) and plants treated with glyphosate at concentrations of 0.25, 0.5, 1.0 and 2.0 mL L^{-1}, which were collected 96 hours after application. The aqueous extracts were prepared from the fresh and dried leaves of *S. rebaudiana* at concentrations of 1.5 and 3.0 g L^{-1}, with distilled water and glyphosate 31.25 mL L^{-1} (1.5%) as negative and positive controls, respectively. All treatments involving aqueous extracts of *S. rebaudiana* plants, whether fresh or dried, regardless of glyphosate application, exhibited antiproliferative activity, resulting in a significant reduction in mitotic index compared to the negative control, except for treatments T3 and T4 (aqueous extracts of 1.5 g L^{-1} of fresh *S. rebaudiana* plants). Genotoxicity displayed an opposite trend to cytotoxicity, as the treatments that least inhibited cell division also exhibited the most chromosomal changes. It was observed that *S. rebaudiana* plants receiving only distilled water showed the highest genotoxic index; however, considering the total number of cells analyzed, this value is less than 1% and is therefore considered to be of low genotoxicity.
Keywords: Glyphosate, Antiproliferative potential, Genotoxicity, Medicinal plants.

Resumo: A *Stevia rebaudiana* Bertoni (Bertoni) (estévia), espécie da família Asteraceae, possui propriedades antibacteriana, antisséptica, anti-inflamatória, hipotensora, diurética, cardiotônica, antioxidante e contraceptiva. O objetivo deste estudo foi analisar o potencial genotóxico e antiproliferativo de extratos aquosos das folhas frescas e secas de *S. rebaudiana* tratadas com diferentes concentrações de glifosato sobre o bioindicador vegetal *Allium cepa* L. Plantas de *S. rebaudiana* foram cultivadas em casa de vegetação, sendo realizados os seguintes tratamentos: controle (plantas que receberam apenas água destilada) e plantas tratadas com glifosato nas concentrações de 0.25; 0.5; 1.0 e 2.0 mL L^{-1}, sendo que, após 96 horas da aplicação, foram coletadas. Os extratos aquosos foram preparados a partir das folhas frescas e secas de *S. rebaudiana* nas concentrações de 1.5 e 3.0 g L^{-1} sendo que a água destilada e o glifosato 31.25 mL L^{-1} (1.5%) representaram os controles negativo e positivo, respectivamente. Todos os tratamentos envolvendo extratos aquosos de plantas de *S. rebaudiana*, frescos ou secos, independente da aplicação de glifosato, exibiram atividade antiproliferativa, resultando em redução significativa do índice mitótico em relação ao controle negativo, exceto os tratamentos T3 e T4 (extratos aquosos de 1,5 g L^{-1} de folhas frescas de *S. rebaudiana*). A genotoxicidade apresentou tendência oposta à citotoxicidade, pois os tratamentos que menos inibiram a divisão celular também exibiram mais alterações cromossômicas. Foi observado que as plantas de *S. rebaudina* que receberam apenas água destilada apresentaram maior índice genotóxico, porém, considerando o total de células analisadas, esse valor é menor que 1%, portanto, considerado de baixa genotoxicidade.
Palavras-chave: Glifosato, Potencial antiproliferativo, Genotoxicidade, Plantas medicinais.

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INTRODUCTION

Stevia rebaudiana Bertoni (Bertoni), also known as stevia, belongs to the Asteraceae family and is the only cultivated species of the *Stevia* genus (Ahmad et al., 2020; Caponio et al., 2019). It is a perennial species with a sub-shrubby habit that occurs spontaneously in the Serra do Amambai region, between Brazil and Paraguay and in Argentina (Gorzi et al., 2020; Martins et al., 2017), and can reach up to 90 centimeters (cm) in height in its natural habitat (Cruz, 2015) and up to 100 cm in cultivation (García et al., 2017). In Brazil, *S. rebaudiana* occurs naturally in the Central-West region and in Mato Grosso do Sul (Nakajima & Gutiérrez, 2020).

The species produces a variety of natural sweeteners (Gorzi et al., 2020; Khiraoui et al., 2018), including steviol and rebaudioside, glycosides with sweetening power 300 times greater than sucrose (Ascrizzi et al., 2022; Lemus-Mondaca et al., 2012), which are used to replace sugar, and it has been reported that the concentration of these metabolites in this species depends on the genotype, the environmental growth conditions and the stage of development of the plant (Gorzi et al., 2020; Uçar et al., 2018). These substances can be used without restrictions, as they are not caloric or toxic or metabolized by the body (Fronza & Folegatti, 2003). In addition to the glycosides, this species also contains proteins, fibers, carbohydrates, phosphorus, iron, calcium, potassium, flavonoids (rutin), zinc, vitamin A and vitamin C, with the glycoside being the main one (Chowdhury et al., 2022; Gorzi et al., 2020; Uçar et al., 2018). In addition, the species has antibacterial, antiseptic, anti-inflammatory, hypotensive, diuretic and cardiotonic properties (Chowdhury et al., 2022; Gorzi et al., 2020; Hossain et al., 2017) and, according to Kim et al. (2011) and Khiraoui et al. (2018), the aqueous extracts of *S. rebaudiana* leaves have a high capacity as a natural antioxidant.

The production of *S. rebaudiana* can be hindered by unwanted plants that compete for available resources; manual weeding with a chemical complement, glyphosate, applied in a localized manner, is indicated (Taiariol & Molina, 2010).

Glyphosate, N-(phosphonomethyl)glycine, is a non-selective post-emergence herbicide (Samanta et al., 2018) that acts by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a catalyst for amino acids essential for plant growth, leading to plant death (Galli & Montezuma, 2005; Giaquinto et al., 2017). This chemical is highly effective in eliminating unwanted plants and is typically sold at a concentration of 48%, but its application can result in residues both on the crop and in contaminating herbivores (Amarante Junior et al., 2002).

The use of plant biological assays to monitor the bioactivity of extracts, fractions and compounds isolated from plants has often been incorporated in the identification and monitoring of potentially toxic substances, and cytotoxicity tests using plant systems have been validated by several researchers who have carried them out with different plant species and using *Allium cepa* L. (Costa et al., 2022; Rodrigues et al., 2020; Ubessi et al., 2019).

The *A. cepa* root analysis test, since the roots are in direct contact with the tested substance (Luz et al., 2012), is a widely used method to assess chromosomal alterations in *A. cepa* roots, and has been validated by the International Program on Chemical Safety (IPCS, WHO) and the United Nations Environment Programme (UNEP) as an efficient test for in situ analysis and monitoring of the genotoxicity of environmental substances (Costa et al., 2022; Lessa et al., 2017).

In view of the above, the aim of this study was to analyze the genotoxic and antiproliferative potential of aqueous extracts of fresh and dried *Stevia rebaudiana* leaves treated with different dilutions of glyphosate, and the analysis of genotoxicity was carried out using the *in vivo Allium cepa* bioassay to determine chromosomal alterations and mitotic index.

MATERIAL AND METHODS

Cultivation of Stevia rebaudiana Bertoni (Bertoni) and application of treatments

Stevia rebaudiana plants, approximately thirty days old and thirty centimeters (cm) tall, were purchased from local stores and transplanted into 3.5 L pots containing organic compost. The plants were acclimatized and kept in

a protected environment (greenhouse) at the Laboratory of Plant Cytogenetics and Genotoxicity (Labcitogen) of the Federal University of Santa Maria (UFSM), for six days, receiving only distilled water. On the seventh day, the plants were divided into five treatments with six plants each and received glyphosate dilutions on their leaves, with the help of a spray bottle. Glyphosate Shadow was used, at commercial concentration of 48%, diluted in 1 L of distilled water for subsequent application in the treatments, where T1: the control received only distilled water (negative control) and the other treatments received 20 mL of glyphosate dilutions: T2 received a dilution of 0.25 mL L^{-1} (0,012%); T3 received 0.5 mL L^{-1} (0,024%); T4 received 1.0 mL L^{-1} (0,048%) and T5 received 2.0 mL L^{-1} (0,096%).

After eleven days of cultivation (four days after glyphosate dilutions applications), the plants were removed with the roots and three plants from each treatment were allowed to naturally dry at room temperature, stored dry and fresh (frozen) for approximately 60 days.

Analysis of cell proliferation and genotoxicity by in vivo test on Allium cepa L.

The experiment was carried out at Labcitogen (UFSM) and the extracts were prepared from 1.5 (Martins et al., 2003) and 3.0 g L^{-1} of fresh and dried *Stevia rebaudiana* leaves by infusion in boiling water (100 °C) for 10 minutes. The extracts were then filtered and cooled to room temperature and then used as a treatment on *A. cepa* bulbs.

The apical root meristem of *A. cepa* was used to assess chromosomal aberrations and determine mitotic indices; for this purpose, 88 *A. cepa* bulbs were scraped at the roots and then rooted in distilled water for seven days. After rooting, they were divided into 22 treatments (Table 1), each treatment with four bulbs (replicates) and subjected to the treatments for a period of 24 hours. Distilled water was used as the negative control of the experiment and 31.25 mL L^{-1} (1.5%) of glyphosate as the positive control, according to Souza et al. (2010).

Table 1
Treatments used in the evaluation of the genotoxic and antiproliferative potential of aqueous extracts of Stevia rebaudiana Bertoni (Bertoni), by the Allium cepa test.

Treatments
T1: Negative control (distilled water) – NC
T2: Positive control (31.25 mL L^{-1} glyphosate (1.5%)) – PC
T3: Extract of 1.5 g L^{-1} fresh stevia leaves from the control group (distilled water)
T4: Extract of 1.5 g L^{-1} fresh stevia leaves treated with glyphosate 0.25 mL L^{-1} (0.012%)
T5: Extract of 1.5 g L^{-1} fresh stevia leaves treated with glyphosate 0.5 mL L^{-1} (0.024%)
T6: Extract of 1.5 g L^{-1} fresh stevia leaves treated with glyphosate 1.0 mL L^{-1} (0.048%)
T7: Extract of 1.5 g L^{-1} fresh stevia leaves treated with glyphosate 2.0 mL L^{-1} (0.096%)
T8: Extract of 3. 0 g L^{-1} fresh stevia leaves from the control group (distilled water)
T9: extract of 3.0 g L^{-1} fresh stevia leaves treated with glyphosate 0.25 mL L^{-1} (0.012%)
T10: extract of 3. 0 g L^{-1} fresh stevia leaves treated with glyphosate 0.5 mL L^{-1} (0.024%)
T11: extract of 3.0 g L^{-1} fresh stevia leaves treated with glyphosate 1.0 mL L^{-1} (0.048%)
T12: extract of 3.0 g L^{-1} fresh stevia leaves treated with glyphosate 2. 0 mL L^{-1} (0.096%)
T13: extract of 1.5 g L^{-1} dried stevia leaves from the control group (distilled water)
T14: extract of 1.5 g L^{-1} dried stevia leaves treated with glyphosate 0.25 mL L^{-1} (0.012%)
T15: extract of 1.5 g L^{-1} dried stevia leaves treated with glyphosate 0. 5 mL L^{-1} (0.024%)
T16: extract of 1.5 g L^{-1} dried stevia leaves treated with glyphosate 1.0 mL L^{-1} (0.048%)
T17: extract of 1.5 g L^{-1} dried stevia leaves treated with glyphosate 2.0 mL L^{-1} (0.096%)
T18: extract of 3. 0 g L^{-1} dried stevia leaves from the control group (distilled water)
T19: extract of 3.0 g L^{-1} dried stevia leaves treated with glyphosate 0.25 mL L^{-1} (0.012%)
T20: extract of 3.0 g L^{-1} dried stevia leaves treated with glyphosate 0.5 mL L^{-1} (0.024%)
T21: extract of 3. 0 g L^{-1} dried stevia leaves treated with glyphosate 1.0 mL L^{-1} (0.048%)
T22: extract of 3.0 g L^{-1} dried stevia leaves treated with glyphosate 2.0 mL L^{-1} (0.096%)

After 24 h in the treatments, the roots were collected and fixed in ethanol: acetic acid (3:1) for 24 h and then stored under refrigeration in 70% ethanol until the slides were prepared.

To prepare the slides, the roots were hydrolyzed in 1N HCl for 5 min and then washed in distilled water. The meristematic region was stained with a drop of 2% acetic acid orcein (Guerra & Souza, 2002), crushed using a small glass rod, and a glass coverslip was applied to the material. The slides were observed and analyzed under a light microscope at 40X magnification. Under the microscope, the total number of dividing and interphase cells was counted and the mitotic index (MI) was calculated based on the percentage of dividing cells according to the methodology of Pires & Oliveira (2001). Genotoxicity was also evaluated through the analysis of chromosomal irregularities, such as anaphase bridges, lost chromosomes and micronuclei. The genotoxic index was calculated using the formula GI = number of cells with chromosomal aberrations/ total number of cells observed x 100 (Böck et al., 2023; Cruz et al., 2019; Souza et al., 2017).

To analyze the slides, two slides were prepared per onion bulb, analyzing 1.000 cells per onion (repetition), for a total of 4.000 cells per treatment, with 22 treatments, for a total of 88.000 cells analyzed.

Statistical analysis

The experimental design was completely randomized (DIC) with four repetitions. For the analysis of cell cycle and chromosomal alterations, each bulb was considered to represent a repetition.

The results of cell division obtained (MI) were evaluated using the chi-square test ($\chi 2$), using BioEstat software version 5.3 (Ayres et al., 2007), with a margin of error of 5%. The data related to chromosomal alterations (GI) was analyzed by analysis of variance and the Scott-Knott mean comparison test at a 5% probability of error level, using the Sisvar statistical program (Ferreira, 2014).

RESULTS AND DISCUSSION

The cell cycle was analyzed in the *Allium cepa in vivo* system and the results obtained regarding the total number of cells analyzed, interphase, cell division and the mitotic indices (MI) of each treatment are shown in Table 2.

Table 2
Number of meristematic cells of *Allium cepa* L. analyzed in interphase, in cellular division and the mitotic indices (MI) of the negative and positive controls and treatments with aqueous extracts of *Stevia rebaudiana* Bertoni (Bertoni) plants with or without glyphosate applications.

Treatments	Total cells analyzed	Cells in interphase	Cells in division	Mitotic index (%)
T1	4000	3816	184	4.6 b*
T2	4000	3936	64	1.6 f
T3	4000	3753	247	6.2 a
T4	4000	3808	186	4.6 b
T5	4000	3877	123	3.0 d
T6	4000	3844	156	3.9 c
T7	4000	3844	159	3.97 c
T8	4000	3867	133	3.3 d
T9	4000	3862	138	3.45 d
T10	4000	3753	147	3.8 c
T11	4000	3897	103	2.5 e
T12	4000	3837	163	4.0 c
T13	4000	3836	164	4.1 c
T14	4000	3851	149	3.7 c
T15	4000	3881	119	2.9 d
T16	4000	3885	115	2.8 d
T17	4000	3878	122	3.0 d
T18	4000	3939	61	1.5 f
T19	4000	3939	61	1.5 f
T20	4000	3905	95	2.3 d
T21	4000	3929	71	1.7 f
T22	4000	3935	65	1.6 f

Where: T1: Negative control (distilled water); T2: Positive control (31.25 mL L-1 of glyphosate (1.5%)); T3: Extract of 1.5 g L-1 of fresh stevia leaves from the control group (water); T4: Extract of 1.5 g L-1 of fresh stevia leaves treated with glyphosate 0.25 mL L-1; T5: Extract of 1.5 g L-1 of fresh stevia leaves treated with glyphosate 1.0 mL L-1; T7: Extract of 1.5 g L-1 of fresh stevia leaves treated with glyphosate 2.0 mL L g L-1 of fresh stevia leaves treated with glyphosate 0.5 mL L-1; T6: Extract of 1.5 g L-1 of fresh stevia leaves -1; T8: Extract of 3.0 g L-1 of fresh stevia leaves from the control group (water); T9: Extract of 3.0 g L-1 of fresh stevia leaves treated with glyphosate 0.25 mL L-1; T10: Extract of 3.0 g L-1 of fresh stevia leaves treated with glyphosate 0.5 mL -1; T11: Extract of 3.0 g L-1 of fresh stevia leaves treated with glyphosate 1.0 mL L-1; T12: Extract of 3.0 g L-1 of fresh stevia leaves treated with glyphosate 2.0 mL L-1; T13: Extract of 1.5 g L-1 of dried stevia leaves from the control group (water); T14: Extract of 1.5 g L-1 of dried stevia leaves treated with glyphosate 0.25 mL L-1; T15: Extract of 1.5 g L-1 of dried stevia leaves treated with glyphosate 0.5 mL L-1; T16: Extract of 1.5 g L-1 of dried stevia leaves treated with glyphosate 1.0 mL L-1; T17: Extract of 1.5 g L-1 of dried stevia leaves treated with glyphosate 2.0 mL L-1; T18: Extract of 3 g L-1 of dried stevia leaves from the control group (water); T19: Extract of 3.0 g L-1 of dried stevia leaves treated with glyphosate 0.25 mL L-1; T20: Extract of 3.0 g L-1 of dried stevia leaves treated with glyphosate 0.5 mL L-1; T21: Extract of 3.0 g L-1 of dried stevia leaves treated with glyphosate 1.0 mL L-1; T22: Extract of 3.0 g L-1 of dried stevia leaves treated with glyphosate 2.0 mL L-1.
*Numbers followed by the same letter in the column do not differ statistically by the $\chi 2$ test at a 5% probability of error.

Based on the mitotic index (MI) values, it was observed that T4 (extract of 1.5 g L^{-1} of fresh stevia leaves treated with glyphosate 0.25 mL L^{-1}; MI = 4.6%) was the only treatment that did not differ from the negative control NC (T1: distilled water), but differed significantly from all the other treatments. The NC, with MI = 4.6%, differed significantly from the positive control PC (T2: glyphosate 1.5%), with MI = 1.6%, showing a strong decrease in cell divisions.

The concentrations of 1.5 g L^{-1} of fresh *S. rebaudiana* leaves in the control group (T3) differed significantly from all the other treatments, and it was the only treatment that increased the number of cell divisions. With the exception of treatment T3 (MI = 6.2%) and T4 (IM = 4.6%), all the other treatments with aqueous extracts of plants of *S. rebaudiana* in which, the fresh or dried leaves, received glyphosate applications showed a significant decrease in cell divisions compared to the NC.

Among the extracts prepared from control plants (those not subjected to glyphosate application – T3, T8, T13 and T18), T3, with an aqueous extract of 1.5 g L^{-1} from fresh plants, notably increased the MI compared to CN, whereas T8 (MI = 3.3%), T13 (MI = 4.1%) and T18 (MI = 1.5%) significantly reduced MI. Regarding the T3 treatment specifically, it is concerning that the aqueous stevia extract at the concentration recommended for use in folk medicine (1.5 g L^{-1}) caused more pronounced cell proliferation than distilled water. In this case, caution is advised when consuming it.

A comparison with fresh plants from the control group reveals significant differences in MI between T3 and T8, indicating that as the concentration of fresh leaf plant extracts increased, MI decreased. Similarly, with dry plant extracts, both T13 and T18 differed from each other, resulting in a reduction in MI. Notably, comparing the negative control (CN: distilled water) with treatments involving fresh and dried plant extracts, it becomes apparent that stevia plants induced an inhibition of cell division, where the higher concentration of the dry plant aqueous extract led to lower MI. When comparing only the treatments with aqueous extracts to each other, the groups of onions treated with dried leaves extracts of 1.5 g L^{-1} of stevia were statistically similar to the groups treated with fresh leaves at the two concentrations tested.

Studies by Pereira et al. (2019), evaluating aqueous extracts of *Handroanthus chrysotrichus* (Mart. Ex DC.) Mattos bark using the *Allium cepa* test, also found an antiproliferative effect in the 1.5% positive control (glyphosate). In similar studies with glyphosate at concentrations of 1.0, 2.0, 2.5 and 3.0%, Dornelles et al. (2017), Frescura et al. (2013), Rodrigues et al. (2020) and Trapp et al. (2015), found a reduction in cell division and chromosomal irregularities caused by glyphosate, regardless of the concentration tested.

Other studies have also shown that aqueous extracts have an antiproliferative effect, as demonstrated with extracts of dried and fresh leaves of *Rubus* sp. (Hister et al., 2019), with extracts of dried leaves of *Helianthus annuus* L. (Borillo et al., 2017) and with aqueous extracts of *Phyllanthus tenellus* Roxb. under different light conditions (Pereira et al., 2022).

According to Rosa et al. (2023), the mitotic index is a parameter indicative of the cytotoxicity of extracts, since values lower than the control may indicate cytotoxicity caused by the treatment, while values higher than the control may indicate a proliferative effect. According to Table 2, it can be seen that with increasing concentrations of aqueous extracts of dried and fresh leaves, there was a decrease in the mitotic index. These results are supported by the studies of Pasqualli et al. (2015) with aqueous extracts of fresh and dried leaves of *Allophylus edulis* (St.– Hil. Hil.) Radlk at a concentration of 16 g L^{-1}, and the same was found by Coelho et al. (2017), where the highest concentration, 24 g L^{-1}, of the aqueous extract of *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli inhibited proliferation and stimulated cell division at a concentration of 6 g L^{-1}.

We also mention the works of Frescura et al. (2013) with aqueous extracts of dried leaves of *Psychotria brachypoda* (Müll. Arg.) Britton, at concentrations of 5 g L^{-1} and 20 g L^{-1}, Frescura et al. (2012) with aqueous extracts of dried leaves (6 g L^{-1} and 30 g L^{-1}) and bark (32 g L^{-1} and 160 g L^{-1}) of *Luehea divaricata*, and Rosa et al. (2023), who worked with ethanolic and aqueous extracts of leaves and flowers of *Schinus terebinthifolia* Raddi on the plant bioindicator lettuce (*Lactuca sativa* L.), observed cytotoxic activity at the highest concentrations (20 and 40 mg mL^{-1}).

It is emphasized that some compounds may be responsible for the inhibition of cell division. *S. rebaudiana* has the compounds pyrogallol, 4-methoxybenzoic acid, p-coumaric acid, 4-methylcatechol, sinapic acids and cinnamic acid and the sweeteners stevioside and rebaudioside

A (Ascrizzi et al., 2022; Chowdhury et al., 2022; Gorzi et al., 2020). These compounds have antibacterial, antiseptic, anti-inflammatory (Ascrizzi et al., 2022; Chowdhury et al., 2022; Hossain et al., 2017), antioxidant (Kim et al., 2011) and, as some studies have shown, these compounds present in this species, in high concentrations, may be responsible for antiproliferative effects.

Multiple studies involving human cancer cells suggest that stevia has anti-cancer potential. According to López et al. (2016), stevia ethanolic extract exhibited stronger antitumor activity compared to stevioside alone, as it induced cell death in cervical, pancreatic, and colon cancer cells at lower concentrations than stevioside. Additionally, other studies have demonstrated the antiproliferative effects of stevioside against ovarian cancer cell lines, potentially through a combination of apoptosis induction and cell cycle arrest (Li et al., 2017), as well as its antiproliferative action against breast cancer cells through the induction of apoptosis and inhibition of DNA synthesis (Khare & Chandra, 2019; Paul et al., 2012). Because it has chemotherapy potential, experts recommend adding steviol to chemotherapy for cancer patients (Jawad et al., 2022).

The extracts treated with dilutions of glyphosate showed lower MI values than the NC, demonstrating a cytotoxic effect. Studies of herbicides used in agriculture in the *A. cepa* test have been reported by Verri et al. (2017), where he tested concentrations of glyphosate, fention, betaciflutrin and mancozeb with reductions in cell division, including glyphosate at concentrations of 5, 10, 15 and 20 µL L^{-1} and lower concentrations (1, 2, 3 and 4 µL L^{-1}), with cytotoxic effects occurring at all concentrations.

However, an interesting point to be discussed are the treatments that presented the lowest MI (T18, T19, T21 and T22), not differing from the MI found in CP. These treatments are aqueous extracts prepared from dried stevia plants.

While most treatments led to a decrease in cell division in *A. cepa* roots, it is not accurate to attribute this reduction solely to glyphosate applications on stevia leaves, as the decrease in MI did not correlate with the increase in glyphosate doses applied. Hence, it is more accurate to assert that it was the aqueous extracts, comprising a complex blend of chemical compounds, that induced this antiproliferative effect, notably evidenced by the greater reduction in MI observed in treatments with the highest concentration of aqueous extract (3 g L^{-1}), particularly those prepared with dried plants.

In addition to analyzing the proliferative potential of aqueous extracts of *S. rebaudiana*, this study also evaluated the genotoxic potential of these extracts in the different treatments, as shown in Table 3, where the chromosomal alterations are presented with the total value found in each treatment evaluated and genotoxic index (GI) of each treatment.

Table 3
Number of meristematic cells of Allium cepa L. analyzed, in cellular division, with chromosomal alterations and genotoxic index of the controls and treatments with aqueous extracts of Stevia rebaudiana Bertoni (Bertoni) plants with or without glyphosate applications.

Treatments	Total number of cells analyzed	Cells in dividing	Total of chromosomal irregularities	Genotoxic Index (%)
T1	4000	184	0	0.00 b*
T2	4000	64	13	0.32 b
T3	4000	247	34	0.85 a
T4	4000	186	20	0.50 a
T5	4000	123	15	0.37 b
T6	4000	156	22	0.55 a
T7	4000	159	25	0.62 a
T8	4000	133	27	0.67 a
T9	4000	138	19	0.47 a
T10	4000	147	26	0.65 a
T11	4000	103	20	0.50 a
T12	4000	163	24	0.60 a
T13	4000	164	21	0.52 a
T14	4000	149	28	0.70 a
T15	4000	119	13	0.32 b
T16	4000	115	20	0.50 a
T17	4000	122	19	0.47 a
T18	4000	61	11	0.27 b
T19	4000	61	3	0.07 b
T20	4000	95	14	0.35 b
T21	4000	71	10	0.25 b
T22	4000	65	9	0.22 b

Where: T1: Negative control (distilled water); T2: Positive control (31.25 mL L-1 of glyphosate (1.5%)); T3: Extract of 1.5 g L-1 of fresh stevia leaves from the control group (water); T4: Extract of 1.5 g L-1 of fresh stevia leaves treated with glyphosate 0.25 mL L-1; T5: Extract of 1.5 g L-1 of fresh stevia leaves treated with glyphosate 0.5 mL L-1; T6: Extract of 1.5 g L-1 of fresh stevia leaves treated with glyphosate 1.0 mL L-1; T7: Extract of 1.5 g L-1 of fresh stevia leaves treated with glyphosate 2 mL L-1; T8: Extract of 3 g L-1 of fresh stevia leaves from the control group (water); T9: Extract of 3.0 g L-1 of fresh stevia leaves treated with glyphosate 0.25 mL L-1; T10: Extract of 3.0 g L-1 of fresh stevia leaves treated with glyphosate 0.5 mL L-1; T11: Extract of 3.0 g L-1 of fresh stevia leaves treated with glyphosate 1.0 mL L-1; T12: Extract of 3.0 g L-1 of fresh stevia leaves treated with glyphosate 2.0 mL L-1; T13: Extract of 1.5 g L-1 of dried stevia leaves from the control group (water); T14: Extract of 1.5 g L-1 of dried stevia leaves treated with glyphosate 0.25 mL L-1; T15: Extract of 1.5 g L-1 of dried stevia leaves treated with glyphosate 0.5 mL L-1; T16: Extract of 1.5 g L-1 of dried stevia leaves treated with glyphosate 1.0 mL L-1; T17: Extract of 1.5 g L-1 of dried stevia leaves treated with glyphosate 2.0 mL L-1; T18: Extract of 3.0 g L-1 of dried stevia leaves from the control group (water); T19: Extract of 3 g L-1 of dried stevia leaves treated with glyphosate 0.25 mL L-1; T20: Extract of 3.0 g L-1 of dried stevia leaves treated with glyphosate 0.5 mL L-1; T21: Extract of 3.0 g L-1 of dried stevia leaves treated with glyphosate 1.0 mL L-1; T22: Extract of 3.0 g L-1 of dried stevia leaves treated with glyphosate 2.0 mL L-1
*Numbers followed by the same letter in the column do not differ by the Scott & Knott test at a 5% probability of error.

Taking into account the negative control group (NC) treated with distilled water where no chromosomal alteration occurred, all other treatments that significantly differed from the NC are considered genotoxic. Despite PC ((31.25 mL L^{-1} of glyphosate (1.5%)) not being statistically different from NC in this case, it serves as a parameter to illustrate the irregularities that may arise during cell division. Since glyphosate, due to its known toxicity, also reduces cell division, it is understandable that fewer alterations appear given the drastic reduction in cell division observed.

Among the extracts prepared from control plants (those not subjected to glyphosate application – T3, T8, T13 and T18 – treatment T3 had the highest number of chromosomal changes (34) of all treatments (GI = 0.85%), but was not different from T4, T6, T7 (aqueous extract of 1.5 g L^{-1} of fresh stevia plants), T8, T9, T10, T11, T12 (aqueous extract of 3 g L^{-1} of fresh stevia plants), T13, T14, T16 and T17 (aqueous extract of 1.5 g L^{-1} of dry stevia plants). All these treatments had the highest number of chromosomal alterations, differing from NC, and for this reason being considered genotoxic. Among the alterations observed were disorganized cells, chromosome breaks, delayed chromosomes and chromosome bridges. The other treatments (T5, T15, T18, T19, T20, T21 and T22) did not differ statistically from NC, showing no genotoxic potential.

Similarly to cytotoxicity, glyphosate applications do not appear to have been decisive for the change in the genotoxicity pattern, as there was no significant difference in GI between the aqueous extracts prepared with plants with or without glyphosate applications on their leaves. Nevertheless, it is emphasized that treatments T5 and T15 (with glyphosate applications) differ significantly from the other treatments, with behavior similar to T18 and T22, presenting low GI. However, when observing treatments T18 to T22, caution is needed as it is possible that the decrease in the number of dividing cells has resulted in fewer visualizations of chromosome irregularities.

Similar behavior, where treatments with greater antiproliferative activity also showed lower genotoxicity, was found in aqueous extracts of *Peltodon longipes* Kunth ex Benth. leaves at concentrations of 5 and 15 g L^{-1} (Kuhn et al., 2015a), and aqueous extracts of fresh *Plectranthus barbatus* Andrews leaves (Bezerra & Oliveira, 2016; Frota et al., 2019). In addition, the *S. rebaudiana* extracts showed more chromosomal alterations at the lower concentrations, which was also observed in *Eugenia uniflora* L. at concentrations of 6 and 24 g L^{-1} (Kuhn et al., 2015b), in 5 and 20 g L^{-1} of *Psychotria brachypoda* (Frescura et al., 2013), in 6 and 24 g L^{-1} of two populations of *Echinodorus grandiflorus* (Coelho et al., 2017).

The aqueous extracts of plants that received dilutions of glyphosate showed chromosomal alterations, but not all of them were significantly different from the negative control. A greater number of significant differences were observed in the fresh leaves (Table 3), namely T4, T6, T7, T9, T10, T11 and T12, which showed 20, 22, 25, 19, 26, 20 and 24 alterations, respectively, and were considered genotoxic. In the dried leaves, only three extracts (concentration of 1.5 g L^{-1}) differed significantly and were considered genotoxic: T14, T16 and T17, which showed 28, 20 and 19 changes, respectively.

Although glyphosate is known to be a cytogenotoxic agent, as observed by Krüger, (2009) at concentrations of 3 and 4 µL L^{-1} and by Barzotto et al. (2017) at concentrations 130 and 260 µl L^{-1}, in this work it was not possible to infer that glyphosate is responsible for the antiproliferative and genotoxic activity found in some treatments.

Thus, it can be seen that the responses of extracts and chemical products (herbicides) applied show a very variable response in the parameters of phytotoxicity, cytotoxicity and genotoxicity, depending on the biological material used in the study, plant species, concentrations used and formulation characteristics of the extract (Fonseca et al., 2015; Rosa et al., 2023).

Species rich in bioactive substances that have antiproliferative and genotoxic potential are normally selected for research testing them as bioherbicides. In a study by Costa et al. (2022), α-terpineol, a molecule used in the food industry as a flavoring agent with antioxidant, antitumor, and antiulcer activity, was tested on meristematic cells of *A. cepa* and found that α-terpineol was cytotoxic at concentrations of 34 and 68 µg mL^{-1} for 48 h and genotoxic at the highest concentrations of 34 (72 h) and 68 µg mL^{-1} (48h). This compound, like herbicides, must be used in a controlled manner because of the possibility of environmental contamination and risks to human and animal health. Thus,

it is noteworthy that research on plant extracts with natural herbicidal potential is relevant as chemical compounds are increasingly used, especially in agriculture.

Genetic testing with plant bioassays is an important tool for detecting and classifying the cellular mechanism of action of the substance being evaluated. In addition, researchers and farmers are looking for bioactive products based on plant extracts or essential oils that can reduce the use of herbicides, insecticides and other pesticides, thereby reducing environmental pollution and the risk of toxicity to humans and animals, as a substitute for synthetic agrochemicals, which usually have high toxicity, side effects on humans and animals, and require a long period of time to completely degrade.

CONCLUSION

All treatments involving aqueous extracts of *S. rebaudiana* plants, whether fresh or dried, regardless of glyphosate application, exhibited antiproliferative activity, leading to a significant reduction in cell divisions compared to the negative control, except for treatments T3 and T4 (aqueous extracts of 1.5 g L^{-1} from plants without glyphosate application and with application of 0.25 mL L^{-1} of glyphosate, respectively).

Glyphosate did not account for the decrease in the mitotic index, as this reduction was not linear. The decline in the mitotic index correlates closely with the increase in concentrations of aqueous extracts of stevia plants, where extracts at the highest concentration (3 g L^{-1}), particularly those prepared with dried plants, exhibited a more pronounced antiproliferative activity.

Similarly to cytotoxicity, glyphosate applications did not play a decisive role in altering the level of genotoxicity. The genotoxicity exhibited an opposite trend to cytotoxicity, as the treatments that least inhibited cell division also displayed the most chromosomal changes. In other words, the aqueous extracts of dried plants, at the highest concentration (3 g L^{-1}), which strongly inhibited cell division, were found to be non-genotoxic treatments.

Although aqueous extracts of *S. rebaudiana*, including those treated only with distilled water, exhibit genotoxic activity, when considering the total number of cells analyzed, this value is less than 1%, thus indicating low genotoxicity.

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