Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Phytotoxic and cytogenotoxic assessment of glyphosate on *Lactuca sativa* L.

Avaliação fitotóxica e citogenotóxica do glifosato em *Lactuca sativa* L.

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Abstract

The active ingredient glyphosate is the most commercialized herbicide on the world market due to its capability in eliminating weeds. However, it can harm the development of non-target organisms and threaten environmental quality. This study analyzed the effects of potentially toxic concentrations of glyphosate on germination, growth, cell cycle and genomic stability of *Lactuca sativa* L., and identified the most sensitive variables for assessing the toxicity of this herbicide to this biomonitor. Seeds of *L. sativa* were germinated in Petri dishes containing a sheet of filter paper moistened with 5 mL of a concentration of glyphosate (1.34, 3.35, 6.70, 10.05, 13.40 mg L^{-1}). Controls consisted of distilled water (negative) and 3 mg L^{-1} $CuSO_4$ (positive). Macroscopic and microscopic variables were analyzed. The germination of *L. sativa* was not affected by the concentrations of glyphosate. Root length and shoot height of the plants and the mitotic index decreased from the lowest concentration tested on. The chromosomal anomaly index and frequency of micronuclei increased by 3.2 and 22 times, respectively, with the presence of the lowest concentration of glyphosate compared to the negative control. The observed phytotoxic and cytogenotoxic effects demonstrate the negative influence that glyphosate has on the development of *L. sativa*. Root length and microscopic variables showed the highest sensitivity. This study warns of the possible harmful effects that glyphosate can have on non-target organisms and suggests greater control over the use of this herbicide to mitigate its environmental impact.

Keywords: biomonitor, herbicide, non-target organism, environmental risk, toxicity.

Resumo

O ingrediente ativo glifosato é o herbicida mais comercializado do mercado mundial, pela sua capacidade de eliminar as plantas daninhas. No entanto, ele pode prejudicar o desenvolvimento dos organismos não-alvo e ameaçar a qualidade do ambiente. O estudo teve como objetivo analisar os efeitos de concentrações potencialmente tóxicas de glifosato sobre a germinação, o crescimento, o ciclo celular e a estabilidade genômica de *Lactuca sativa* L., e identificar as variáveis mais sensíveis para avaliar a toxicidade deste herbicida ao biomonitor. Sementes de *L. sativa* foram germinadas em placas de Petri contendo uma folha de papel-filtro umedecida com 5 mL das concentrações de glifosato (1,34, 3,35, 6,70, 10,05, 13,40 mg L^{-1}). Os controles consistiram em água destilada (negativo) e 3 mg L^{-1} de $CuSO_4$ (positivo). Variáveis macroscópicas e microscópicas foram analisadas. A germinação de *L. sativa* não foi afetada pelas concentrações de glifosato. O comprimento da raiz e a altura da parte aérea das plantas e o índice mitótico reduziram desde a menor concentração testada. O índice de anomalias cromossômicas e a frequência de micronúcleos aumentaram, respectivamente, 3,2 e 22 vezes na presença da menor concentração de glifosato em comparação ao controle negativo. Os efeitos fitotóxicos e citogenotóxicos observados demonstram a interferência negativa do herbicida no desenvolvimento de *L. sativa*. O comprimento da raiz e as variáveis microscópicas foram as que apresentaram maior sensibilidade. Este estudo alerta sobre os possíveis efeitos prejudiciais que o glifosato pode provocar nos organismos não-alvo, sugerindo um maior controle quanto à utilização deste herbicida, a fim de mitigar o seu impacto ambiental.

Palavras-chave: biomonitor, herbicida, organismo não-alvo, risco ambiental, toxicidade.

1. Introduction

Herbicides are used to control the growth of unwanted plants and are applied in agriculture to eliminate weeds (Roman et al., 2007). The use of glyphosate as an herbicide was launched in 1974 by the Monsanto company's Roundup® trademark, and today it is the most commercialized active herbicidal ingredient on the world market (Duke and Powles, 2008). Glyphosate, an organophosphate compound, is classified as a non-selective herbicide, with systemic

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Received: October 6, 2021 – Accepted: January 31, 2022

action and a broad spectrum, making it highly efficient in controlling weeds and the first choice of most agricultural producers (Galli and Montezuma, 2005). It is applied in a wide range of fruits, vegetables, nuts, and glyphosate-resistant field crops (US-EPA, 2022). The mode of action is related to interference in the activity of the enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), causing the shikimic acid metabolic pathway to be deregulated. This, in turn, reduces the levels of the aromatic amino acids tryptophan, tyrosine and phenylalanine, which are necessary for the synthesis of proteins involved in plant growth. This process halts development and degrades several tissues due to the lack of proteins, resulting in cellular disorder and, finally, plant death (Yamada and Castro, 2007).

A total of 217,000 tons of the active ingredient glyphosate were sold in Brazil in 2019, four times more than the second most commercialized pesticide, 2,4-dichlorophenoxyacetic acid (2,4-D) (IBAMA, 2019). This dominance is related to the increase in areas sown with genetically modified crops, such as soybeans, corn and cotton, that are resistant to the application of this herbicide (Dill et al., 2008; Duke and Powles, 2008). However, non-target organisms located in the vicinity of plantations are subject to exposure risks due to the dispersion of droplets by drift, since spray application of pesticides is still the most common practice (Lucadamo et al., 2018; Van Bruggen et al., 2018). Pollution from agricultural practices is considered a diffuse source due to the complexity involved in its identification, monitoring and control (Hatfield, 1993).

After a long review process, started in 2008 and concluded in 2019, the Agência Nacional de Vigilância Sanitária (ANVISA) decided to continue to allow the active ingredient glyphosate on the Brazilian market. A series of studies found that the herbicide did not meet the prohibitive criteria established by the country's legislation, as it is not classified as mutagenic, carcinogenic, or toxic for reproduction or the cause of fetal malformation. In addition, the toxicological classification of most commercial glyphosate-based products was reduced, such that many products that were considered as "extremely toxic" were now classified as "products unlikely to cause acute harm" (ANVISA, 2019).

In contrast, several countries, such as Germany, Austria, France, Mexico, Sri Lanka and Vietnam, have restricted or even prohibited the use of glyphosate-based products (Malkanthi et al., 2019; Beckie et al., 2020; Alcántara-de la Cruz et al., 2021), based on evidence already available on the toxic potential of glyphosate for water resources, soil, the atmosphere and human health, and its classification as a probable carcinogen for humans by the International Agency for Research on Cancer (IARC, 2017; Silva et al., 2018; Fernandes et al., 2019; Sousa et al., 2019; ATSDR, 2020). It is estimated that less than 0.1% of pesticides applied on plantations actually reach the target organism, with the remaining 99.9% moving into the ecosystem (Pimentel and Levitan, 1986). Different classifications and risk assessments have raised uncertainties and concerns about the safety of glyphosate, as well as its potential toxicological effects for the environment and carcinogenicity for humans (Agostini et al., 2020).

Ecotoxicological tests can be performed to assess the environmental risks of these effects for non-target organisms (Rodrigues et al., 2017), based on the assessment of different variables at visual, anatomical, cytogenetic, molecular, biochemical and physiological levels in a test organism (Freitas-Silva et al., 2020). Phytotoxicity, characterized by the toxic action of environmental pollutants on the appearance and development of a biomonitor organism (OECD, 2006), can be measured by macroscopic analysis with metric and visual observations of the germination process and plant growth (Monteiro et al., 2010; Moraes et al., 2015; Vieira and Droste, 2019). Such assessment is considered simple, fast, reliable and inexpensive (Charles et al., 2011). Cytogenotoxicity demonstrates the toxicity of a given substance on the mitotic cell cycle, chromosomal behavior during cell division and the formation of micronuclei and can be evaluated by microscopic analysis of root meristem cells of a biomonitor plant (Vieira and Silveira, 2018). The combination of macro and microscopic analyses helps to identifying possible environmental risks by contributing to a better understanding of the toxic effects of environmental pollutants and the mechanism of action of potentially toxic substances that are released into the environment (Vieira and Silveira, 2018).

Lactuca sativa L. is commonly used in bioassays because it is sensitive to potentially toxic substances (Aragão et al., 2019; Lyu et al., 2018; Vieira and Droste, 2019). The species is considered a biomonitor organism due to its ability to provide quantitative information about the quality of the environment (Markert, 2007), which is why international organizations recommend it for tests that assess the toxicity of environmental pollutants (US-EPA, 1996; OECD, 2006; ISO, 2012). As *L. sativa* has a low chromosome number (2n = 18), a karyotypic characteristic that facilitates the microscopic visualization of chromosomes (Silveira et al., 2017), cytogenetic alterations can be clearly observed and evaluated.

The toxicity of glyphosate can vary and be influenced by the concentration applied and the time of exposure, the species exposed, as well as environmental conditions (Duke, 2020). The interference of this herbicide in the development of non-target organisms has already been reported for angiosperms (Batista et al., 2018; Cruz et al., 2021) and ferns (Droste et al., 2010; Aguilar-Dorantes et al., 2015), as in biomonitor plant species (Khan et al., 2020; Mercado and Caleño, 2020). However, the cytogenotoxic effects on non-target plants are not widely known. Nor is it known about which plant groups or weed species this herbicide interferes with, altering the control of their development. Despite studies on the toxic potential of glyphosate showing controversial results in recent decades (Santos et al., 2023), it is currently the most commercialized herbicide worldwide (Zyoud et al., 2017).

Thus, this study aimed to analyze the effects of environmentally of potentially toxic concentrations of glyphosate on the germination, growth, cell cycle and genomic stability of *L. sativa*, and to identify the most sensitive variables for assessing the toxicity of this herbicide to this biomonitor.

2. Material and Methods

2.1. Bioassay

The bioassay with *L. sativa* was carried out in a laminar flow chamber. The following concentrations of glyphosate were prepared: 1.34, 3.35, 6.70, 10.05 and 13.40 mg L^{-1}. The lowest concentration (1.34 mg L^{-1}) is based on the commercial product Roundup Original®DI (Monsanto, USA), an aqueous solution containing 445 g L^{-1} of the active ingredient glyphosate. The recommended amount of glyphosate varies depending on the target organism. For example, the manufacturer recommends the application of 2 to 4 L per cultivated hectare to control the weed *Oryza sativa* L. (red rice) in irrigated rice crops. In the present study, we used 3 L ha^{-1} as a reference, which represents 1.335 g ha^{-1} of the herbicide. As a negative control distilled water was used, and a solution with 3 mg L^{-1} of $CuSO_4$ was used as a positive control, equivalent to the lowest concentration of the metal capable of inhibiting the root growth of this species (Di Salvatore et al., 2008). Seeds of the "baba de verão" cultivar of *L. sativa* (N. 123352-001-52, ISLA Ltda, Brazil) were germinated in 9-cm diameter Petri dishes containing a sheet of sterilized quantitative filter paper moistened with 5 mL of one of the different treatments. The process was completely randomized, with three Petri dishes for each concentration of glyphosate and controls, containing 20 seeds each (Vieira and Droste, 2019). The material remained at 25±1°C under a photoperiod of 12 h light in a growth room at the Laboratório de Biotecnologia Vegetal of the Universidade Feevale.

2.2. Macroscopic analyses

Phytotoxicity analyses were conducted with *L. sativa* in accordance with the OPPTS 850.4200 ecological effect testing guidelines (US-EPA, 1996). Macroscopic variables were evaluated after six days of exposure (Carvalho et al., 2014; Vieira and Droste, 2019) to treatments by counting the germinated seeds and measuring the root and leaves of 15 individuals per Petri dish for a total of 45 individuals per treatment. The criterion for considering a seed as germinated was the visible protrusion of the radicle, observed without the use of instruments (Curiel and Moraes, 2011). Root length and shoot height were measured with a millimeter ruler, considering the distance from the collar of the plant to the meristematic apex of the root system and the distance from collar of the plant to its apex, respectively (Gatti et al., 2004).

2.3. Microscopic analyses

Cytogenotoxicity analysis involved removing root tips of five seedlings of each Petri dish after 48 h of exposure for a total of 15 roots per treatment. The tips were fixed in ethanol:acetic acid (3:1, v/v) for 24 h at room temperature with subsequent transfer to 70% ethyl alcohol in refrigeration. Of the 15 removed root tips, only 10 chosen at random had their meristematic region analyzed, while the others were kept as reserve material in case of need for analysis. One root tip was used per slide, which was, in sequence, treated for 2 min in distilled water, hydrolyzed for 6 min in 1N HCl, washed again for 2 min in distilled water and then stained with 2% acetic orcein. Five-hundred cells per root were counted by the scanning technique (Guerra and Souza, 2002) using an optical microscope (Nikon Eclipse E200) at 400x magnification. The mitotic index (MI) was calculated by the formula MI = [(number of mitotic cells/total cells)x100] (Vieira and Droste, 2019). The chromosomal anomaly index (CAI) was calculated as CAI = [(number of cells with anomalies/total dividing cells)x100] (Vieira and Droste, 2019). The frequency of micronuclei was expressed in MCN/100 cells (Thewes et al., 2011).

2.4. Statistical analysis

Data were submitted to the Shapiro-Wilk normality test. The mitotic index and the chromosomal anomaly index, as well as shoot height of *L. sativa*, were normal. Means for micronuclei frequencies (MCN) were square root (x) + 1 transformed for normalization. Normal data were submitted to ANOVA followed by Tukey's test for microscopic data and Duncan's test for shoot height. Seed germination and root length were analyzed using the Kruskal-Wallis test followed by the Mann-Whitney test with Bonferroni correction. The tests were performed using the SPSS 25 statistical program with the significance set at 5%.

3. Results

3.1. Macroscopic analyses

The germination percentage for *L. sativa* seeds was not affected by glyphosate concentration, nor by the positive control compared to the negative control (Figure 1A), ranging from 93 to 100% among treatments ($H = 7.5556$; $P = 0.2725$). The other macroscopic variables were sensitive to herbicide concentration, and glyphosate interfered in plant development, both of root ($H = 214.6$; $P < 0.001$) (Figure 1B) and shoot ($Z = 5.451$; $P < 0.001$) (Figure 1C). The lowest herbicide concentration (1.34 mg L^{-1}) caused a 58% reduction in root length while the highest (13.40 mg L^{-1}) caused a reduction of 81%. The inhibition of shoot height was less expressive, with approximately 30% and 40% for the lowest and highest concentrations, respectively, compared to the negative control.

3.2. Microscopic analyses

Glyphosate caused a significant reduction in cell divisions and an increase in the rate of chromosomal anomalies and the frequency of micronuclei in meristematic cells of *L. sativa* (Table 1). The lowest concentration used (1.34 mg L^{-1}), had already been shown to be harmful to the analyzed cytogenotoxic variables, and reduced mitotic divisions by around 32%, and increased the number of anomalies and the frequency of micronuclei by 69 and 95%, respectively, compared to the negative control. The highest concentration (13.40 mg L^{-1}) reduced mitotic divisions by around 68% compared to the negative control and by 52% compared to the positive control, thus demonstrating its high cytotoxic potential. This same concentration also demonstrated

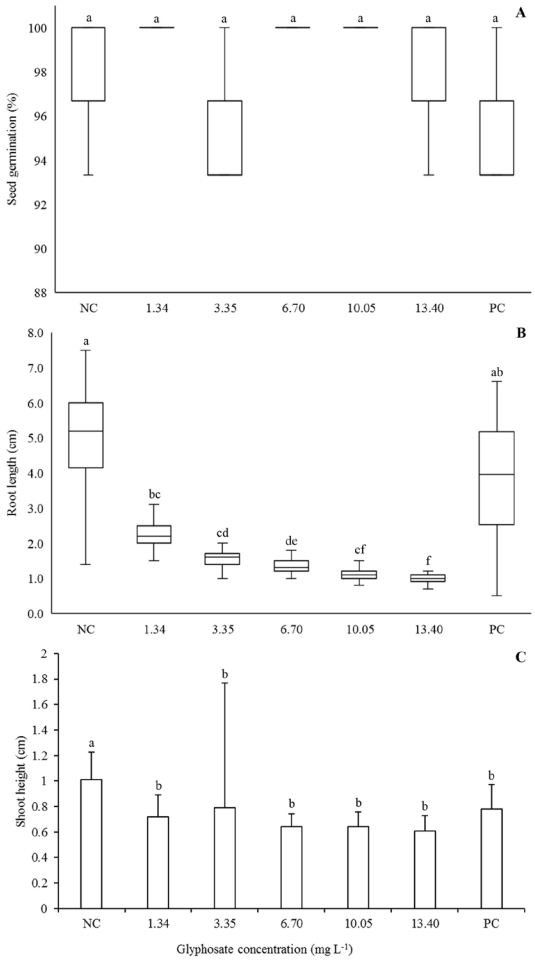

Figure 1. Phytotoxic effects of glyphosate on *Lactuca sativa* after six days of exposure to treatments. **(A)** germinated seeds (germination percentage boxplot, Kruskal-Wallis + Mann-Whitney with Bonferroni correction); **(B)** root length (boxplot, Kruskal-Wallis + Mann-Whitney with Bonferroni correction) and **(C)** shoot height (mean ± standard deviation, ANOVA + Duncan). Different letters represent statistical differences at 5% probability. NC = negative control. PC = positive control.

its genotoxic effects by promoting a 78% increase in chromosomal anomalies and a 97% increase in the frequency of micronuclei in relation to the negative control.

Different chromosomal anomalies were observed during mitotic divisions, such as chromosome loss and sticky chromosome, anaphase and telophase with bridges, multipolar anaphase and C-metaphase, in addition to the formation of micronuclei (Figure 2). The mean sum of chromosomal anomalies in the herbicide treatments was approximately twice that of the negative control. Increasing herbicide concentration reduced mitotic divisions and, consequently, the number of cells with chromosomal anomalies was also reduced (Table 2). C-metaphase was the most recurrent anomaly, representing 44% of the total, while telophase with bridge was the least representative, corresponding to less than 2% of the observed anomalies.

4. Discussion

No effect was observed on the germination of *L. sativa* seeds, indicating that this variable is not the most suitable for evaluating glyphosate toxicity. Previous studies have reported the same finding, with different concentrations of the herbicide not altering the germination of *Lepidium sativum* L., *Sinapis alba* L., *Sorghum saccharatum* (L.) Moench, *Brassica napus* L., *Lupinus luteus* L., *Avena sativa* L. (Piotrowicz-Cieslak et al., 2010), and *Zea mays* L. (Gomes et al., 2019). Reductions in the germination rates of *L. sativa* (Rodrigues et al., 2017) and *Lycopersicon esculentum* L. (Khan et al., 2020) were reported only for seeds exposed to high concentrations of glyphosate (360 mg L^{-1} and 30 mg L^{-1}, respectively), 27 and twice as high as the highest concentration used in the present study.

The other macroscopic variables evaluated in this study proved to be effective as indicators of phytotoxicity. The root, being the first contact organ and the main system of entry and accumulation of substances in the plant (Pourrut et al., 2011), was more sensitive to glyphosate than the shoot part. Greater root sensitivity was also reported for *Medicago sativa* L. exposed to concentrations of 12 to

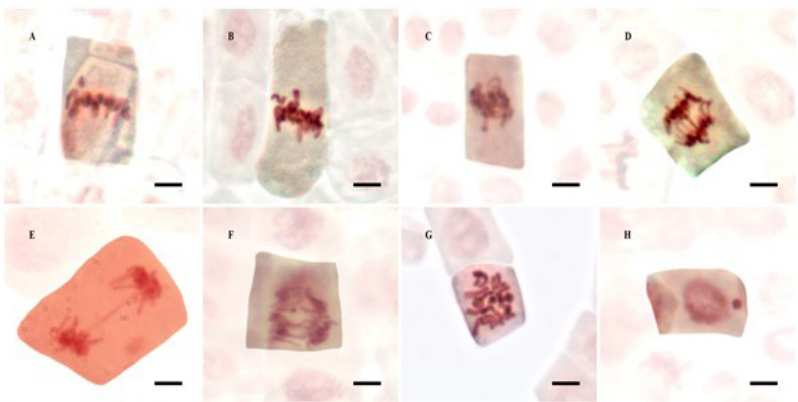

Figure 2. Types of chromosomal anomalies and micronucleus formation observed in meristematic cells of *Lactuca sativa* (400x magnification). **(A-B)** Chromosome loss. **(C)** Sticky chromosome. **(D)** Anaphase with bridge. **(E)** Telophase with bridge. **(F)** Multipolar anaphase. **(G)** C-metaphase. **(H)** Micronucleus. **Bars:** 10 µm.

Table 1. Values (mean ± standard deviation) for the mitotic index (MI), chromosomal anomaly index (CAI) and micronuclei frequency (MCN) in *Lactuca sativa* exposed to glyphosate herbicide concentrations and to control treatments. Means followed by the same letter in a column do not differ significantly according to Tukey's test at 5% probability.

Treatments	Citogenotoxic parameters		
	MI	CAI	MCN
Negative Control	4.74 ± 0.54 a	11.86 ± 4.42 a	0.02 ± 0.06 a
1.34 mg L^{-1}	3.20 ± 0.46 b	37.91 ± 8.09 bc	0.44 ± 0.60 b
3.35 mg L^{-1}	2.70 ± 0.59 b	36.69 ± 6.46 b	0.30 ± 0.25 b
6.7 mg L^{-1}	2.42 ± 0.73 bc	40.59 ± 15.68 bc	0.46 ± 0.28 b
10.05 mg L^{-1}	1.82 ± 0.89 cd	45.90 ± 18.19 bcd	0.48 ± 0.27 b
13.40 mg L^{-1}	1.54 ± 0.60 d	52.99 ± 12.65 cd	0.64 ± 0.29 b
Positive Control	3.22 ± 0.50 b	54.36 ± 6.69 d	0.36 ± 0.29 b
Z	28.460	15.633	6.606
P	<0.001	<0.001	< 0.001

Table 2. Types of chromosomal anomalies in *Lactuca sativa* meristematic cells exposed to concentrations of the herbicide glyphosate and to control treatments.

Treatments	Chromosomal Anomalies								
	CL	SC	AB	TB	MPA	CM	MCN	Total	Number of cells in division
Negative Control	3	6	9	0	0	10	1	28	237
1.34 mg L^{-1}	7	17	15	4	1	18	22	62	161
3.35 mg L^{-1}	5	9	6	1	0	29	15	50	135
6.7 mg L^{-1}	4	18	10	0	1	16	23	49	121
10.05 mg L^{-1}	3	2	10	0	1	28	24	44	90
13.40 mg L^{-1}	1	8	5	0	2	24	32	40	77
Positive Control	6	27	15	0	7	33	18	88	160
Total	29	87	70	5	12	158	135	361	

(CL) Chromosome loss; (SC) Sticky chromosome; (AB) Anaphase with bridge; (TB) Telophase with bridge; (MPA) Multipolar anaphase; (CM) C-metaphase; (MCN) micronucleus.

40 mg L^{-1} (Fernandes et al., 2020). For *Pisum sativum* L., however, root and shoot part growth were proportionally affected by the tested glyphosate concentrations (1 to 4 mg L^{-1}) (Mondal et al., 2017). Glyphosate also reduced root length in *Z. mays* (Gomes et al., 2019). Leaf and root length of the aquatic fern *Regnellidium diphyllum* L. were strongly affected, respectively, by 0.32 and 0.64 mg L^{-1} of glyphosate after 35 days of exposure *in vitro* (Droste et al., 2010). Limitations in root and shoot development may be associated with reduced production of auxin, a hormone that regulates plant growth, as products of the shikimic acid pathway, such as the aromatic amino acid tryptophan, are used in the synthesis of this hormone (Gomes et al., 2014). Therefore, by interfering with this metabolic pathway, glyphosate alters auxin levels in plant tissues.

Because it significantly reduced cell divisions of *L. sativa*, even at the lowest concentration tested, which was equivalent to that recommended by the manufacturer (1.34 mg L^{-1}), glyphosate can be considered a cytotoxic substance. As in the present study, MI reduction in direct proportion to increasing herbicide concentration has also been reported for root meristematic cells of other plant species, such as *Trigonella foenum-graecum* L., *A. cepa* and *Vigna mungo* (L.) Hepper (Siddiqui et al., 2012; Mercado and Caleño, 2020; Khan et al., 2021). This dose-dependent relationship of glyphosate with MI is probably caused by the harmful action of the herbicide on the protein and enzyme activity of the cell cycle, which hinders the polymerization and synthesis of DNA and the formation of spindle fibers (Khan et al., 2021). The inhibition of mitotic divisions of the root meristem of *L. sativa* observed here, which ranged from 32 to 68%, may explain the significant reduction in root length (58 to 81%), since the growth of a plant organ is directly related to the increase in the number of cells in the tissue that composes it (Vieira and Silveira, 2018).

In addition to interfering with the number of mitotic divisions and root length, the action of glyphosate on meristematic tissues can cause irregularities in the cell division process. The genotoxicity of the herbicide can be evidenced by the different chromosomal anomalies observed

in this study, with the lowest concentration tested (1.34 mg L^{-1}) being able to increase the number of anomalies by 3.2 times, compared to the negative control. C-metaphase, the most frequently observed chromosomal anomaly in *L. sativa*, represents the aneugenic effect of glyphosate, which causes malformation of the mitotic spindle and incorrect attachment of chromosomes to microtubules (Vieira and Silveira, 2018). The results of the present study corroborate Truta et al. (2011), who also reported an increase in metaphase anomalies in *Hordeum vulgare* L., as well as anaphase/telophase bridges and missing chromosomes.

Chromosome stickiness, the second most observed anomaly in the present study, occurs due to changes in the physicochemical structure of DNA as a result of the aneugenic and clastogenic effects of the herbicide (Vieira and Silveira, 2018). Khan et al. (2021) observed sticky chromosome in root meristem cells of *V. mungo*, even at the lowest concentrations of glyphosate, which impaired the optimal movement and segregation of chromosomes. The low occurrence of chromosomal bridges in the present study can be explained by the high incidence of the C-metaphase anomaly, which interrupts cell division in metaphase, not allowing the continuation of cell division. This also reduces the mitotic index, a fact observed in the highest concentrations of glyphosate. The formation of anaphase/telophase bridges evidences the clastogenic effect of the herbicide, being considered the most noticeable abnormality when observing the mitotic cell cycle (Vieira and Silveira, 2018).

The genotoxic potential of glyphosate can also be perceived by the formation of micronuclei, the frequency of which, in this study, increased significantly with the lowest concentration tested (22 times higher than the frequency observed in the negative control). Micronuclei formation results from unrepaired damage to parental cells, and may originate from lost chromosomes, where in daughter cells they are observed as a structure similar to the main nucleus, but smaller in size (Vieira and Silveira, 2018). Previous studies observed an increase in the frequency of micronuclei in *A. cepa* root meristem cells after exposure to the herbicide (Çavuşoğlu et al., 2011; Mercado and Caleño, 2020). Glyphosate-induced micronuclei formation has also been reported in animal cells, such as peripheral white blood cells in humans (Nagy et al., 2021), fin cells of *Misgurnus anguillicaudatus* (Qin et al., 2017), erythrocytes of *Physalaemus* tadpoles (Herek et al., 2021) and larvae cells of the butterfly *Lycaena dipar* (Santovito et al., 2020), demonstrating that the use of this herbicide represents an environmental threat to non-target organisms exposed in different environmental spheres.

5. Conclusion

The results of the present study demonstrate that the concentrations of glyphosate used had phyto- and cytogenotoxic potential for *L. sativa*, with negative effects on the development of this biomonitor plant as observed by changes in macro- and microscopic variables. The bioassay with *L. sativa* proved to be a sensitive and reliable tool that can be used to help identify and monitor the environmental risk arising from the use of this herbicide and we can highlight root length and the analyzed microscopic variables as those having greater sensitivity for assessing glyphosate toxicity. In addition, this study provides a warning of how the indiscriminate and growing use of this herbicide can harm the development of non-target organisms, threatening both the quality of the environment as well as the conservation of species.

Acknowledgements

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting a scholarship to the first author (Finance Code 001). The authors thank Universidade Feevale for the available infrastructure.

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