Environmentally realistic concentrations of eprinomectin induce phytotoxic and genotoxic effects in *Allium cepa*

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**Abstract**

Eprinomectin, a veterinary drug within the family of avermectins, is widely used in the agricultural sector to combat a variety of parasites, mainly nematodes. However, only 10% of the drug is metabolized in the organism, so large quantities of the drug are released into the environment through urine and/or feces. Soil is the first and main environmental compartment to be contaminated by it, and nontargeted organisms can be affected. Thus, the present study aims to evaluate the phytotoxicity (through the evaluation of germination, root development, and germination speed) and genotoxicity (through an assessment of the induction of micronuclei and chromosomal aberrations) of eprinomectin. For the analyses, *Allium cepa* seeds were germinated in soil contaminated with a range of concentrations of eprinomectin: from 0.5 to 62.5 μg/g for the genotoxicity test and from 0.5 to 128.0 μg/g for the phytotoxicity test. The results showed that seed germination was not affected, but root development was affected at concentrations of 0.5 μg/g, 1.0 μg/g, 4.0 μg/g, 8.0 μg/g, 64.0 μg/g, and 128.0 μg/g, and germination speed was significantly changed at concentrations of 1.0 μg/g, 4.0 μg/g, 16.0 μg/g, 32.0 μg/g, and 64.0 μg/g. Significant differences in the mitotic index and genotoxicity index were observed only at concentrations of 2.5 μg/g and 12.5 μg/g, respectively. Only the 0.5 μg/g concentration did not show significant induction of micronuclei in the meristematic cells, but the damage observed at other concentrations did not persist in F1 cells. According to the results, eprinomectin is both phytotoxic and genotoxic, so the release of eprinomectin into the environment should be minimized.

**Keywords** Avermectin · Mutagenicity · Micronucleus · Cytotoxicity · Chromosomal aberrations · Germination · Root development

**Introduction**

Antiparasitic drugs such as avermectin have been widely used in veterinary medicine to combat both ecto- and endo-parasites (Seelanan et al. 2006), and their antiviral activity against a broad range of viruses, including dengue (Tay et al. 2013) and SARS-CoV-2 (Caly et al., 2012), has been studied. The avermectin family comprises a complex of chemically related agents produced by the actinomycete *Streptomyces avermitilis* and includes a variety of drugs, such as abamectin, doramectin, ivermectin, emamectin, and eprinomectin (Burg et al. 1979). Among these, abamectin and ivermectin have been used the longest. The most recently developed drug, eprinomectin, has shown satisfactory results at low doses (Giannetti et al., 2011).

Eprinomectin (4″-epi-acetylamino-4″-deoxy-avermectin B1) was developed to reduce the risk to human health in the mid-1990s by Shoop et al. (1996). It can be used to treat livestock at any stage of life and has much lower levels of milk residues than other avermectins. However, similar to other medicines, only a small percentage of the eprinomectin administered is metabolized by animals. Approximately, 90% is released into the environment through feces or urine without modification (Merck 1996). As a result, the drug has been found in soil, the initial environmental compartment affected, and in surface and groundwater (Jensen et al. 2003; Kolar et al. 2008).

Pharmaceutical drugs are an important group of chemical contaminants since they are developed to produce biological
effects at low doses. Due to the high level of conservation of features within vertebrate taxa, both human and veterinary medicines may produce effects in nontargeted organisms (Celander et al. 2011). Therefore, it is important to evaluate the effects of these substances on the environment to determine if their presence will affect organisms living within that ecosystem. Eprinomectin, as a recently developed drug, has not been thoroughly studied in regard to its possible effects on nontargeted organisms.

The concentration of avermectins in environmental compartments initially depends on the method of application, the dose applied, the frequency of dosing, and the number of treated animals in a given area (Kovecses and Marcogliese 2005). According to Oliveira-Ferreira et al. (2016), these drugs can be found in the soil in the μg/g range. When a single animal is treated with a recommended dose of topically formulated avermectin, concentrations of 18.5 μg/g dry weight can be detected 2 days posttreatment on a single dung pile, and even after 28 days, avermectin is still being excreted (Hernd, 1996). Weather conditions are one of the key driving factors involved in avermectin degradation (Erzen et al., 2005), and consequently, the half-life of drugs in the avermectin family varies significantly under different field conditions (Bai and Ogbourne 2016). According to Kovecses and Marcogliese (2005), the half-life of avermectins in soil can vary from 91–217 days outdoors in winter to 7–14 days outdoors in summer. Regarding eprinomectin, its half-life in soil is approximately 64 days, according to the Merck Company (1996). Avermectins can remain in the environment for a considerable period of time, and their high binding affinity for soils and other organic matter might result in their environmental accumulation (Halley et al. 1989), so it is important to assess a wide concentration range of avermectin that could be present in soil.

The use of higher plants, also known as vascular plants (Akeroyd and Syringe 1992), in the assessment of environmental impact is widely recommended by international institutions such as the United Nations Environmental Program (UNEP), the World Health Organization (WHO), the US Environmental Protection Agency (USEPA), and the International Organization for Standardization (ISO 18763:2016). *Allium cepa* represents one of the main plants used in ecotoxicological tests since it can demonstrate the ability of different substances to alter DNA (Leme and Marin-Morales, 2009), germination, and the initial development of an organism (Fiskejö 1985). Notably, it has been used for the detection of contamination caused by pesticides (Souza et al. 2017; Verma and Srivastava 2018; Fiorese et al. 2020; Mota et al. 2022), medicinal drugs (Gupta et al. 2020; Das et al. 2021; Eckert et al. 2022), wastewater (Yadav et al. 2019; Furtado et al. 2020; Sharma et al. 2021), and sewage sludge (Sommaggio et al. 2018; Abreu-Junior et al. 2019; Moraes Cunha Gonçalves et al., 2020).

Ecotoxicological studies testing the effects of veterinary drugs on plants are scarce, and the few studies that have been reported described experiments that were performed in vitro. Furthermore, they did not involve the exposure of plants to contaminated soil and used concentrations unlikely to be found in the environment. In addition, studies conducted in vitro need to be verified, as bioavailability in soil can greatly differ from in vitro conditions (Jjemba 2002). According to these studies, avermectins do not negatively affect plants (Vokřál et al. 2019). Fully understanding the phytotoxicity of these compounds is important since plant sensitivity to drugs may reduce biodiversity in certain areas (Bártiková et al., 2016).

Thus, this study aimed to evaluate the phytotoxic and genotoxic effects of eprinomectin in vivo using the bioindicator organism *A. cepa* in the concentration range from 0.5 to 62.5 μg/g for the genotoxicity test and from 0.5 to 128.0 μg/g for the phytotoxicity test. Phytotoxic analyses were carried out using parameters such as the germination index, initial root development, and germination speed index. Genotoxic analyses were performed by analyzing chromosomal and nuclear aberrations and detecting the presence of micronuclei (MN) in cells within the meristematic region of plants and the presence of MN in F₁ cells.

### Materials and methods

#### Materials

Seeds of *A. cepa* from the same batch, variety (Baia perform), without the addition of chemical agents, were acquired from the Isla Sementes Company. Eprinomectin (CAS 123997-26-2) at 96% purity was purchased from Sigma-Aldrich. The soil used in the present study was collected in the region of Campinas, São Paulo state, Brazil (22° 24′ 36″ S/47° 33′ 36″ W) and was characterized as oxisol. The soil physicochemical properties and the results of the granulometry analysis performed according to NBR7181 (2016) are summarized in Tables 1 and 2. The soil was dried at room temperature and sieved (mesh = 5 mm). The water-holding capacity (WHC) of the soil was calculated based on ISO 16387 (2013) and OECD 232 (2016); a tube with filter paper was filled with soil to a depth of 5 cm and placed on a rack in a water bath. The tube was gradually submerged until the water level rose above the top of the soil but below the upper edge of the tube. The soil sample was left in the water for approximately 3 h. Afterward, the tube containing the soil sample was placed for a period of 2 h on wet, finely ground quartz sand for draining. The soil sample was then weighed, dried at 105 °C, and reweighed. The following formula was used to calculate the WHC:

\[
\text{WHC} = \frac{\text{Dried weight} - \text{Initial weight}}{\text{Initial weight}} \times 100
\]
Where

\[ WHC = \frac{m_S - m_T - m_D}{m_D} \times 100 \]

is the water-holding capacity as a percentage of dry mass, %.

\[ m_S \] is the combined mass of the water-saturated substrate, the tube, and the filter paper.

\[ m_T \] is the tare (mass of tube plus mass of filter paper).

\[ m_D \] is the dry mass of the substrate.

Table 1 Physicochemical analysis of soil sample used in the experiments

| Parameters          | Results | Error \(^1\) | MAV \(^2\) (mg/kg) | Method            | Reference                        |
|---------------------|---------|--------------|-------------------|-------------------|----------------------------------|
| Arsenic mg/kg       | 6.850   | 0.097        | 15.0              | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Barium mg/kg        | 19.98   | 0.18         | 120               | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Cadmium mg/kg       | < 0.153 | 0.0023       | 1.3               | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Calcium mg/kg       | 857     | 40           | -                 | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Lead mg/kg          | 17.70   | 0.5          | 72                | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Copper mg/kg        | 23.52   | 0.26         | 60                | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Chromium mg/kg      | 24.94   | 0.29         | 75                | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Magnesium mg/kg     | 377     | 19           | -                 | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Mercury mg/kg       | < 0.010 | 0.00033      | 0.5               | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Molybdenum mg/kg    | 2.500   | 0.043        | 5                 | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Nickel mg/kg        | 5.820   | 0.074        | 30                | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Organic matter (g/dm\(^3\)) | 20    | -            | -                 | Ashing at 440 °C | NBR 13600: 1996 |
| pH                  | 5.4     | 0.76         | -                 | Potentiometric | USEPA 9045D (2004) |
| Selenium mg/kg      | < 0.255 | 0.016        | 1.2               | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Sodium mg/kg        | 131.0   | 5.1          | -                 | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Zinc mg/kg          | 24.87   | 0.48         | 86                | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Nitric nitrogen mg/kg | 225.2 | 2.7          | -                 | Ion chromatography | USEPA 300.1 (1993) |
| Sulfur mg/kg        | 8.02    | 0.18         | -                 | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Potassium mg/kg     | 308     | 12           | -                 | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Ammoniacal nitrogen mg | 62.3 | 1.4          | -                 | Titulometric | - |
| Kjeldahl nitrogen mg NH\(_3\)N/kg | 186 | 4             | -                 | Titulometric | Baird et al. (2017) |
| Phosphorus mg/kg    | 871     | 42           | -                 | Plasma spectrometry | USEPA 3050B (1996)/USEPA 6010C (2007) |
| Nitrite mg/kg       | < 0.030 | 0.00059      | -                 | Ion chromatography | USEPA 300.1 (1993) |
| Total organic carbon % p/p | 7.82 | -            | -                 | -                  | - |
| Electrical conductivity dS/m | 0.623 | -            | -                 | -                  | - |

\(^1\)Confidence level of 95%. \(^2\)MAV, maximum allowed values according to CETESB 045/2014/E/C/I (2014)
The soil was then contaminated with a stock solution containing eprinomectin diluted in ethanol.

**Experimental procedures**

The soil was contaminated with increasing doses of eprinomectin that corresponded to concentrations in which the drug has been found within the environment. Soil contamination occurred 1 day prior to the experiments so that the solvent used to create the stock solution could completely evaporate. After being contaminated, the soil was homogenized and dried for 24 h in a fume hood.

For all of the experiments, a control treatment containing soil and solvent (SolvC) was carried out to compare it with the negative control (NC), which contained soil mixed with ultrapure water, to statistically analyze any significant differences. If no difference was observed, we concluded that the solvent did not affect the parameters tested. On the day of each experiment, soil moisture was adjusted using ultrapure water to maintain 70% of the maximum WHC, as recommended by the USEPA (1996).

**Assessment of genotoxicity**

Genotoxicity was analyzed using four different concentrations of eprinomectin ($C_1_{Gen} = 0.5 \mu g/g$, $C_2_{Gen} = 2.5 \mu g/g$, $C_3_{Gen} = 12.5 \mu g/g$, and $C_4_{Gen} = 62.5 \mu g/g$). NC and SolvC conditions were also included, as was a positive control (PC) which consisted of soil mixed with the herbicide trifluralin (Fernandes et al., 2007). Each treatment consisted of 90 g of dry soil distributed between 3 Petri dishes (30 g per dish), so 45 μg of eprinomectin was added to treatment C1, 225 μg of eprinomectin to treatment C2, 1124 μg to treatment C3, and 5625 μg to treatment C4. To achieve the desired concentration for each treatment, a stock solution containing 37.5 mL of solvent and 7020 μg (7.02 mg) of eprinomectin was made, and the stock solution (quantities listed below) was added to 90 g of soil. Each quantity of stock solution was diluted in solvent to allow for the homogenization of the soil and, consequently, an effective dispersal of the eprinomectin into the soil sample.

- $C_1_{Gen}$: 0.24 mL stock solution + 28.56 mL solvent = 45 μg of eprinomectin in 90 g of soil
- $C_2_{Gen}$: 1.2 mL stock solution + 27.6 mL solvent = 225 μg of eprinomectin in 90 g of soil
- $C_3_{Gen}$: 6.0 mL stock solution + 22.8 mL solvent = 1123 μg of eprinomectin in 90 g of soil
- $C_4_{Gen}$: 28.8 mL stock solution + 0 mL solvent = 5500 μg of eprinomectin in 90 g of soil

After contamination, the soil was dried for 24 h to allow for the complete evaporation of solvent. On the day of the experiment, the soil of each treatment was moistened with 28.8 mL (70% WHC) of ultrapure water, and fifty seeds of A. cepa were randomly placed within each Petri dish (90 mm × 1.5 mm).

Petri dishes were then arranged randomly in a BOD incubator at 22 ± 2 °C with a 12:12 h light:dark photoperiod (Souza et al. 2017).

**Preparation of the slides of A. cepa**

After 5 days of exposure, the roots were collected and fixed in Carnoy 3:1 (ethanol:acetic acid, v/v) for 12 h. To prepare slides, roots were hydrolyzed in 1 M HCl at 60 °C for 10 min and then submitted to a Schiff reagent for 2 h (Mello and Vidal 1978). Both the meristematic region and the F1 region were sectioned and arranged on slides. To enhance staining and improve cell spreading, a drop of 2% acetic carmine acid was added, and then the material was covered by a coverslip and lightly macerated. Coverslips were removed with liquid nitrogen, and the permanent slides were mounted with Entellan®. The slides were analyzed under an optical microscope at a magnification of 1000X. Fifteen slides were prepared per Petri dish, totaling 45 slides per treatment.

**Evaluation of genotoxicity in meristematic cells of A. cepa**

Genotoxicity in meristematic cells was evaluated according to Souza et al. (2017). Approximately, 15,000 meristematic cells were analyzed per treatment. Cytotoxicity was evaluated based on the mitotic index (MI) according to the formula:

\[
MI = \frac{\text{number of cells in division}}{\text{total number of cells observed}} \times 100
\]

Genotoxicity was analyzed based on the number of cells carrying chromosomal and nuclear aberrations, such as nuclear buds, anaphasic and telophasic bridges, and chromosomal losses and breaks. The genotoxicity index (GenI) was calculated using the formula:

| Texture  | Diameter (mm) | (%)   |
|----------|---------------|-------|
| Clay     | < 0.002       | 7.59  |
| Silt     | 0.002–0.06    | 16.4899 |
| Fine sand| 0.06–0.2      | 74.3000 |
| Medium sand| 0.2–0.6      | 1.1400  |
| Coarse sand| 0.6–2.0      | 0.4400  |
| Fine gravel| 2.0–6.0      | 0.0000  |
| Medium gravel| 6–20        | 0.0000  |
| Thick gravel| 20–60        | 0.0000  |

Table 2: Granulometry of the soil sample used in the experiments
The frequency of micronuclei (FMN) was determined by assessing the number of cells containing MN:

\[ \text{FMN} = \frac{\text{number of cells with MN}}{\text{number of cells observed}} \times 100 \]

The presence of MN can be used as a biomarker for genotoxicity and chromosomal instability-related events. When this damage is not repaired, it has the capacity to produce mutagenic effects.

**Evaluation of cells with micronuclei in the F₁ region of A. cepa**

Approximately, 15,000 cells from the F₁ region of each treatment were analyzed to confirm findings indicating that eprinomectin has the capacity to produce mutagenic effects. The number of cells carrying MN was counted, and the FMN was determined.

**Assessment of phytotoxicity**

For the evaluation of phytotoxicity, nine different concentrations of eprinomectin were used: C₁₇ Phyto = 0.5 μg/g, C₂ Phyto = 1.0 μg/g, C₃ Phyto = 2.0 μg/g, C₄ Phyto = 4.0 μg/g, C₅ Phyto = 8.0 μg/g, C₆ Phyto = 16.0 μg/g, C₇ Phyto = 32.0 μg/g, C₈ Phyto = 64.0 μg/g, and C₉ Phyto = 128.0 μg/g.

Each treatment consisted of 150 g of dry soil divided into 5 Petri dishes (30 g per dish). Soil contamination was carried out in the same way as for the genotoxicity test but adjusted to 150 g of soil.

The NC consisted of soil and ultrapure water, and the PC contained a mixture of soil and zinc sulfate heptahydrate (0.05 M). A SolvC condition was also performed. Thirty seeds of A. cepa were randomly placed in each Petri dish (90 mm × 1.5 mm), and then, the dishes were arranged in a BOD incubator at 22 ± 2 °C for 96 h (Palmieri et al. 2014). After periods of 24, 32, 40, 48, 56, 64, 72, 80, 88, and 96 h, seeds of A. cepa were randomly placed in each Petri dish (0.05 M). A SolvC condition was also performed. Thirty seeds of A. cepa were randomly placed in each Petri dish (90 mm × 1.5 mm), and then, the dishes were arranged in a BOD incubator at 22 ± 2 °C for 96 h (Palmieri et al. 2014). After periods of 24, 32, 40, 48, 56, 64, 72, 80, 88, and 96 h, the number of germinated seeds was assessed.

To calculate the germination speed index (GSI), the following formula was used:

\[ \text{GSI} = \frac{(N_1 \times 1) + (N_y - N_1) \times \frac{1}{2} + (N_3 - N_1) \times \frac{1}{3} + (N_4 - (N_3 - N_1)) \times \frac{1}{y}}{N_y} \times 100 \]

where Ny is the number of seeds germinated in a given period (Palmieri et al. 2014). After 96 h, root lengths were measured to determine initial development.

The percentage of germinated seeds was also calculated:

\[ \% = \frac{\text{number of germinated seeds}}{\text{total seeds distributed}} \times 100 \]

**Statistical analyses**

Statistical analyses were performed using the IBM SPSS Statistics 22 program. For each analysis, normality and homogeneity tests were performed using the Shapiro–Wilk test and Levene test, respectively. When the data showed a normal and homogeneous distribution, parametric tests (t-tests) were performed at a probability level of 5% (p < 0.05); data with nonnormal and nonhomogeneous distributions were assessed using a nonparametric test (Kruskal–Wallis/Dunn test) at a probability level of 5%.

**Results**

**Phytotoxicity**

According to a statistical assessment of the data, all phytotoxic parameters were homogeneously and normally distributed, and none differed significantly in the NC and SolvC conditions.

The germination rate remained constant throughout all treatment groups, differing significantly only in regard to the PC (p = 0.000) (Table 3). Initial root development was affected at most concentrations of eprinomectin when compared with NC. Only treatments C₃ Phyto, C₆ Phyto, and C₇ Phyto did not show significant reductions in relation to the NC. Seeds of the PC did not reach a sufficient length to be measured (Table 3).

| Treatments | Germinated seeds(%) | GSI       | Root growth(cm) |
|------------|---------------------|-----------|-----------------|
| NC         | 94.0 ± 0.4          | 5.5 ± 0.36| 1.66 ± 0.51     |
| SolvC      | 93.0 ± 0.0          | 5.2 ± 0.39| 1.57 ± 0.43     |
| PC         | 40.0 ± 3.9*         | 1.3 ± 0.46| 0.00            |
| C₁ Phyto   | 92.5 ± 2.5          | 5.2 ± 0.84| 1.47 ± 0.40*    |
| C₂ Phyto   | 91.0 ± 1.1          | 5.0 ± 0.22| 1.51 ± 0.47*    |
| C₃ Phyto   | 96.5 ± 1.2          | 5.3 ± 0.16| 1.55 ± 0.49     |
| C₄ Phyto   | 96.0 ± 0.8          | 4.6 ± 0.25| 1.41 ± 0.38*    |
| C₅ Phyto   | 92.5 ± 1.3          | 4.9 ± 0.50| 1.49 ± 0.44*    |
| C₆ Phyto   | 89.0 ± 2.4          | 4.9 ± 0.28| 1.47 ± 0.55     |
| C₇ Phyto   | 89.0 ± 2.3          | 4.7 ± 0.35| 1.58 ± 0.45     |
| C₈ Phyto   | 89.0 ± 1.6          | 4.7 ± 0.35| 1.48 ± 0.39*    |
| C₉ Phyto   | 94.0 ± 0.8          | 5.1 ± 0.24| 1.39 ± 0.35*    |

GSI, germination speed index; NC, negative control; SolvC, solvent control; PC, positive control; C₁, 0.5 μg/g; C₂, 1.0 μg/g; C₃, 2.0 μg/g; C₄, 4.0 μg/g; C₅, 8.0 μg/g; C₆, 16.0 μg/g; C₇, 32 μg/g; C₈, 64 μg/g; C₉, 128 μg/g. *p < 0.05
The majority of the treatments caused delayed germination of seeds when compared to the NC. Significant differences in the speed of germination relative to the NC were observed for the PC (\( p = 0.000 \)), C2\textsubscript{Phyto} (\( p = 0.027 \)), C4\textsubscript{Phyto} (\( p = 0.003 \)), C6\textsubscript{Phyto} (\( p = 0.027 \)), C7\textsubscript{Phyto} (\( p = 0.012 \)), and C8\textsubscript{Phyto} (\( p = 0.010 \)) treatment groups (Table 3).

Genotoxicity

According to the Levene and Shapiro–Wilk tests, the assessment of GenI and FMN (both meristematic and F\textsubscript{1} regions) produced data that demonstrated nonnormal and nonhomogeneous distributions. Assessment of MI produced data that were characterized as having a normal and homogeneous distribution. There was no statistically significant difference between NC and SolvC for any of the genotoxicity parameters analyzed.

Regarding meristematic cells, a significant decrease in MI was observed only for treatment C2\textsubscript{Gen} (\( p = 0.012 \)) (Table 4). Analyses of chromosomal and nuclear aberrations (GenI) revealed that the C3\textsubscript{Gen} (\( p = 0.042 \)) group was the only one which showed statistically significant damage (Table 4), such as chromosomal loss (Fig. 1A), chromosomal breakage (Fig. 1B), and nuclear bud (Fig. 1C). Induction of MN (FMN) (Fig. 1D) was significant regarding treatments C2\textsubscript{Gen} (\( p = 0.012 \)), C3\textsubscript{Gen} (\( p = 0.037 \)), and C4\textsubscript{Gen} (\( p = 0.035 \)) (Table 4).

An assessment of the FMN of the cells of the F\textsubscript{1} region revealed no significant increase in MN formation (Table 4).

Discussion

The use of A. cepa as a bioindicator organism allows for the analysis of several parameters to determine whether different substances released into the environment produce toxic effects. The tests allow researchers to determine whether a substance is toxic through the assessment of seed germination and root growth (Palmieri et al. 2014) and, at the cellular level, allow for the evaluation of the ability of toxic substances to interact and damage DNA (Leme and Marin-Morales, 2009).

Parameters such as seed germination and root growth, used to determine phytotoxicity, have been gaining popularity in recent years (Ruttkay-Nedecky et al., 2017; Pinho et al. 2017; Delerue et al. 2019; Garcia et al. 2019). The inhibition of seed germination is considered a lethal effect if it is a result of embryo death (Sobrero and Ronco 2004), while root length analysis is considered a sensitive indicator for plant development (Ratsch and Johndro 1986). A number of studies have been carried out using these parameters for the evaluation of several potentially toxic substances, such as agro-industrial waste (Luo et al. 2018), treated water (Priac et al., 2017), and metals (Gharebaghi et al. 2017); however, data of this nature are limited or nonexistent regarding the use of avermectins.

Previous reports have shown that avermectins do not have a negative effect on plants (Dybas 1989; Bloom and Matheson, 1993; Halley et al. 1993), but detailed parameters used to reach such a conclusion were not provided by these studies. Moreover, previous reports have also shown that the penetration of these substances into plant bodies, either directly through the leaf surface or through absorption from contaminated soils, was insignificant because it was believed that avermectins were rapidly photodegraded (Halley et al. 1993; McKellar 1997). However, a recent study concluded that plants grown close to feces contaminated with ivermectin had a high internal concentration of the drug, and, therefore, the capacity of plants to absorb significant quantities of these drugs was demonstrated (Iglesias et al. 2018).

According to our data, eprinomectin is not able to make the embryo nonviable, since all concentrations of the eprinomectin treatment germinated at levels similar to the NC. However, the eprinomectin treatment did alter the initial development and root growth of the species since a reduction in the lengths of roots of treated plants was observable even at low concentrations of the drug. The results herein regarding the phytotoxic effects of

### Table 4

| Treatments | MI | GenI | MNF | Meristematic cells | F\textsubscript{1} cells |
|------------|----|------|-----|-------------------|------------------------|
| NC         | 7.71 ± 3.27 | 0.09 ± 0.23 | 0.22 ± 0.35 | 0.42 ± 0.46 |
| SolvC      | 6.40 ± 3.20 | 0.07 ± 0.25 | 0.18 ± 0.48 | 0.54 ± 0.67 |
| CP         | 7.54 ± 3.98 | 2.25 ± 2.12* | 2.65 ± 2.28* | 1.41 ± 1.25 |
| C1\textsubscript{Gen} | 6.89 ± 3.12 | 0.16 ± 0.28 | 0.31 ± 0.70 | 0.43 ± 0.45 |
| C2\textsubscript{Gen} | 5.91 ± 3.34* | 0.09 ± 0.18 | 0.45 ± 0.47* | 0.66 ± 0.87 |
| C3\textsubscript{Gen} | 6.59 ± 3.66 | 0.16 ± 0.21* | 0.46 ± 0.60* | 0.58 ± 0.93 |
| C4\textsubscript{Gen} | 7.43 ± 3.36 | 0.08 ± 0.17 | 0.45 ± 0.54* | 0.62 ± 0.68 |

MI, mitotic index; GenI, genotoxicity index; MNF, micronuclei frequency; NC, negative control; SolvC, solvent control; PC, positive control; C1, 0.5 μg/g; C2, 2.5 μg/g; C3, 12.5 μg/g; C4, 62.5 μg/g. *\( p < 0.05 \)
eprinomectin corroborate the results determined by Vokřál et al. (2019) and Ahmed (2014) for other avermectins. No effect on seed germination was observed in Sinapis alba after exposure to ivermectin (Vokřál et al., 2019) or in A. cepa after exposure to a mixture of abamectin and emamectin benzoate. Root growth, however, was reduced even at low concentrations in S. alba and in concentrations ranging from 0.6 to 1.0 mL/L in A. cepa.

Although eprinomectin did not completely inhibit seed germination, the germination process was affected by delayed root development, as demonstrated by the reduction in germination speed. Unlike other xenobiotics, there is no information in the scientific literature concerning the action of avermectins in the physiology of plants. For example, it has previously been established that heavy metals can alter the distribution of auxin, a phytohormone responsible for several aspects of plant growth and development (Bücker-Neto et al. 2017). But, for avermectins, further studies must be performed to clarify their mechanisms of action on plant cells. Regarding the phytotoxicity of eprinomectin, lethal effects were not observed, but the drug affected root development and the speed of germination, which demonstrated its significant potential to harm the species.

The decreased root growth and delayed germination speed caused by eprinomectin may be due to its potential mito-depressive effects, as demonstrated by assessment of the MI. In our experiments, MI was reduced after treatment with all concentrations of eprinomectin, and the reduction was statistically significant in C2Gen. This parameter is used as an indicator of cytotoxicity and can be determined by the increase or decrease in the number of cells in the division process. Thus, a reduction in MI relative to the control indicates that differences are derived from the chemical effect of the substance, which results in the inhibition of the growth and development of the organism (Leme and Marin-Morales...
2009). Mito-depressive effects have already been reported regarding the reduced elongation of A. cepa roots exposed to the herbicide imazethapyr (Liman et al. 2015), metals (Gupta et al. 2018), and mining waste (Andrade-Vieira et al., 2017).

Along with MI analysis, the A. cepa test allows for the evaluation of other cellular parameters, such as the formation of chromosomal and nuclear aberrations in meristematic cells and MN induction, both in meristematic and F1 cells (Leme and Marin-Morales 2009). In meristematic cells, chromosomal aberrations demonstrate the genotoxic effects of substances, whereas MN induction is an indicator of chromosomal instability (Souza et al. 2017).

A significant increase in the number of aberrations was observed posttreatment with C3Gen within the meristematic cells. The principal effects included the formation of chromosome losses (Fig. 1A), chromosomal breakage (Fig. 1B), and nuclear buds (Fig. 1C). According to Fernandes et al. (2007), the presence of nuclear buds occurs as a result of the elimination of excessive genetic material derived from the polyploidization process. Chromosome losses indicate that eprinomectin may also act on the mitotic spindle of the cell. During cell division, an interruption or malformation of the mitotic spindle may produce inappropriate chromosome segregation when daughter cells are formed (Andrade-Vieira et al. 2012; Freitas et al. 2016), resulting in chromosome loss and other abnormalities, such as lagging chromosomes, chromosome stickiness, and disturbed anaphases (Tkalec et al. 2009).

The formation of MN, which can occur spontaneously or due to exposure to contaminants, is strongly related to the occurrence of lost and broken chromosomes (Heddle et al. 1983; Ma and Xu 1986). Since our data demonstrated both of these types of chromosomal aberrations, the induction of MN was expected, and it was observed significantly within the meristematic cells for treatments C2Gen, C3Gen, and C4Gen (Fig. 1D).

However, in cells from the F1 region, the presence of MN was not significantly different. The analysis of the cells of this region is important because it allows for the evaluation of whether damage present in cells of the meristematic region was repaired and therefore not transferred to daughter cells. According to Ma et al. (1995), after mitotic division within the meristematic region, DNA damage from the region could be visualized as MN in the F1 region. As there was no significant MN increase in this region, these results suggest that the cell repair system was able to neutralize the damage caused by eprinomectin. To confirm this evidence, recovery tests should be conducted in future studies.

The mechanism of action of avermectin in plant cells remains unknown, but our results indicate that eprinomectin may interact with DNA, causing nuclear abnormalities and chromosomal breaks. Additionally, eprinomectin may act on cellular components required for cell division, which could cause chromosomal losses. Based on these results, eprinomectin acts as a clastogenic and aneugenic compound. The clastogenic effects are related to chromosomal breaks, and the aneugenic mode of action occurs as a consequence of failures in chromosomal attachment to the mitotic spindle (Leme and Marin-Morales 2009), herein observed as the loss of chromosomes.

Clastogenic alterations can be explained by the increased level of intracellular reactive oxygen species (ROS) generated by avermectins (Li et al. 2013; Zhang et al. 2017). When produced in abundance, ROS can lead to oxidative stress, which results in DNA damage (Schins and Knaapen 2007). According to Yi et al. (2007), ROS can also damage purine and pyrimidine bases and the deoxyribose unit in DNA, generating chromosomal breaks.

Regarding aneugenic action, there is some evidence that suggests that ivermectin can disrupt spindle formation in invertebrate organisms. In Caenorhabditis elegans, ivermectin can alter the expression of dyneins and kinesins, two proteins that play important roles in mitosis and meiosis, since they are associated with meiotic and mitotic spindle poles (Ballesteros et al. 2016); in Onchocerca volvulus and Haemonchus contortus, ivermectin changes the frequency of β-tubulin alleles (Eng et al. 2006), a structural protein of microtubules (Downing and Nogales 1998). Additionally, for H. contortus, ivermectin can bind to and alter the tubulin polymerization equilibrium, which can lead to mitotic arrest, as demonstrated by Ashraf et al. (2015). The aneugenic action of eprinomectin can also explain the reduction in MI, since mito-depressive effects may be related to the inhibition of microtubule formation or arresting of the 24-h cycle of A. cepa at the G1 and G2 phases, impaired nucleoprotein synthesis, and reduced levels of ATP that provide energy for spindle elongation, microtubule dynamics, and chromosomal movement (Türkoğlu 2012).

Studies testing the effects of avermectins on cells of vertebrate and invertebrate organisms have already shown that these substances may interact with DNA, corroborating our results. In these studies, low concentrations of avermectins were also responsible for damaging genetic material, as observed by Shen et al. (2011) and Al-Šarar et al. (2015), in silkworm hemocytes and in cell culture (CHOk1), respectively. In bovine peripheral lymphocytes, Anchordoquy et al. (2019) observed an increased presence of MN and level of nuclear buds after treatment with 20 ng/mL, 40 ng/mL, and 60 ng/mL doramectin. The production of effects at low concentrations of these compounds is expected since veterinary drugs are designed to produce biological effects at low doses (Arnold et al. 2013). It is important to note that these studies were not performed in the soil and, therefore, were not able to evaluate the effects of the combination of soil and avermectins.

The physical characteristics of soil, such as pH, carbon content, and grain size, may interfere with the bioavailability of xenobiotics and alter the toxicity of the substances (Moreira et al. 2019). Additionally, interpretation
of the results obtained with plant bioassays in contaminated soils is complex as a result of the variety of factors that can influence the results (Delerue et al. 2019). According to the physicochemical analysis (Table 1), the presence of contaminants in soil, such as metals, was below the maximum allowed by Brazilian legislation, suggesting that the observed results were caused exclusively by eprinomectin. Information regarding the role of pH on the sorption/desorption of eprinomectin in soil is absent in the scientific literature, but it is known that eprinomectin will be adsorbed to the soil material at neutral pH; the degree of this adsorption will be determined by the physicochemical properties of the soil (Vássilis et al. 2016). Granulometry analysis (Table 2) showed that the soil used in this study presented low amounts of clay minerals and organic matter, characteristics that could contribute to a higher level of desorption, indicating toxic risk to the environment (Vássilis et al. 2016), as observed for A. cepa.

The results presented here suggest that eprinomectin has phytotoxic and genotoxic effects on A. cepa. The phytotoxicity was indicated by observed delays in the initial development of the plants and reduced germination speed. Genotoxicity was indicated by observed delays in the initial development of chromosomal and nuclear aberrations and MN. It is important to note that these effects were observed even at low concentrations, in accordance with recent studies assessing drugs belonging to the same family. Since limited information regarding the effects of avermectins, mainly eprinomectin, on plants is available, it is necessary to perform further investigations, such as recovery tests, to better understand the mechanisms of action responsible for producing the effects observed here. Generally, the release of eprinomectin into the environment should be minimized.

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Author contribution RBS designed, carried out, and analyzed the experiments with support from CPS. The manuscript was written by RBS with input from all authors. JRG supervised the project. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable

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Conflict of interest The authors declare no competing interests.

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