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ARTICLE

Cytototoxic effects of paracetamol (acetaminophen) in *Allium cepa* L. meristematic cells and seed germination

Gisele ORTIZ-MOLINA[1], Jéssica GOUVÊA-BUCHWEITZ[1] & Rosiane Borba DE AGUIAR[2]

1 Licenciatura em Ciências Biológicas, Campus Pelotas - Visconde da Graça, Instituto Federal de Educação, Ciência e Tecnologia Sul-rio-grandense, Pelotas, Brazil.

2 Departamento de Educação Profissional e de Cursos Superiores de Graduação, Campus Pelotas - Visconde da Graça, Instituto Federal de Educação, Ciência e Tecnologia Sul-rio-grandense, Pelotas, Brazil.

Correspondence
RB de Aguiar
E-mail: rosianeaguiar@ifsul.edu.br
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Resumen

Efectos citotóxicos del paracetamol (acetaminofeno) en células meristemáticas y germinación de semillas de Allium cepa L.

El desecho de medicamentos en la basura doméstica puede ser muy peligrosa para el medio ambiente. El presente trabajo buscó evaluar parámetros de toxicidad del contaminante emergente paracetamol. En los ensayos se utilizaron tres dosis de paracetamol (0,4 mg L^{-1}, 0,5 mg L^{-1} y 0,6 mg L^{-1}). Los resultados mostraron que la exposición aguda al paracetamol induce varios daños clastogénicos y aneugénicos en el test de *A. cepa*. No se encontró ningún efecto en la germinación. Aunque el paracetamol es un fármaco importante, también puede representar un riesgo significativo para la producción agrícola y la preservación del medio ambiente. Los resultados del presente trabajo corroboran datos anteriores y refuerzan que la exposición a contaminantes farmacéuticos puede representar un problema significativo para la producción de alimentos, el equilibrio ambiental y la preservación de especies.

Palabras clave: Genotoxicidad; Germinación; Paracetamol; Medio ambiente.

Abstract

Disposing medicines in the common domestic waste can be very harmful to the environment. The present work aimed to evaluate toxicity parameters of exposure to micropollutant acetaminophen. Three paracetamol doses (0.4 mg L^{-1}, 0.5 mg L^{-1} and 0.6 mg L^{-1}) were used in the tests. Results showed that acute exposure to paracetamol causes several chromosomal aberrations of both clastogenic and aneugenic nature in the *A. cepa* test, which are indicative of paracetamol genotoxic action. No effect in germination was found. Although paracetamol is an important pharmaceutical that contributes to human wellbeing, it may also represent a significant risk to agricultural production and environmental preservation. The results in the present work corroborate data from previous publications and reinforce that exposure to pharmaceutical micropollutants may represent a significant problem to food production, environmental balance and species preservation.

Key words: Genotoxicity; Germination; Paracetamol; Environment.

Introduction

The voracious consumerism of modern society fuelled the exponential growth of industrial activity and, as a negative consequence of this event, we see whole ecosystems undergo intense physical and chemical modifications (Bridges & Oldeman 1999, Peters & Meybeck 2000, Sparemberguer & Da Silva 2005).

Among the various human activities with socioeconomic and environmental impact, the pharmaceutical industry stands out both positively and negatively. Pharmaceutical production is of great economic importance around the world. The supply chain, production, marketing, and private consumption of pharmaceuticals and cosmetics has a strong impact on the creation of contribution to the gross domestic product (GDP) and employment. Data from the Chamber for Drug Market Regulation in Brazil point out that in 2018, the pharmaceutical industry profit was over US$ 14 billion, making this industry an important economic pillar in developing countries.

More importantly, medicines and cosmetics are products that profoundly influence health, well-being, life expectancy, and many of the dynamics in our society. This industry has a considerable social impact by enabling disease treatment and increasing quality and life expectations. However, despite the benefits cited, the pharmaceutical sector is also considered a potential polluter. The development of new and more sensitive analytical techniques allowed the detection of thousands of pharmaceuticals in the aquatic environment, even in supposedly treated sewage and drinking water. The classical methodology for wastewater, sewage, and water treatment, which is required by legislation, is based on specific target compounds, but many pharmaceutical drugs exist in very low concentrations (Rogowska *et al.* 2020) and are not yet regulated by Brazilian current legislation. Thus, most wastewater, sewage, and drinking water are not investigated for the presence of these substances, much less treated adequately to remove these compounds (reviewed by Pinho *et al.* 2017, Rogowska *et al.* 2020).

Studies indicate that the huge consumption of pharmaceuticals by the Brazilian population, together with the inadequate sanitary structure in the country could explain the high concentrations of pharmaceuticals detected in Brazilian waters, especially in regions with high population density (reviewed by Lima *et al.* 2017). Moreover, the growing demand, associated with the habit of the population to dispose of these drugs irregularly in the common waste, adds up to the numerous problems of waste management of the country, contributing to the pollution of soil and water and further worsening the environmental impact derived from the pharmaceutical industry (Lessa & Cariello 2017).

Many substances are included in the category called emergent micropollutants. These persistent, organic, and damaging chemical compounds are imperceptible to the naked eye and difficult to detect in the environment due to their low concentrations. The fact that they are persistent is related to their molecular structure that provides greater stability, hindering their degradation and removal from the environment (Lessa & Cariello 2017). Some of these drugs cause significant environmental contamination and can be found frequently in industrial and domestic effluents.

Paracetamol (acetaminophen) is among the various potential micropollutants of pharmaceutical origin and has an analgesic and antipyretic action. This chemical belongs to the group of "non-selective oxygenase cycle inhibitors", and is very popular among the Brazilian population (Garrido 2012, Remião 2020). The metabolic processing of paracetamol in the human body is performed in the liver, resulting in the production of N-acetyl-p-benzoquinone imine (NAPQI). This compound has the ability to bind to different types of molecules, such as DNA, proteins, and unsaturated lipids, inactivating them and, if a decrease in glutathione levels in the body is established, the effect of intoxication is more severe (B. Pereira 2018). Although pharmaceutical waste pollution is a current and worrying reality, little has been done in Brazil to address this issue.

Several studies reported paracetamol toxicity, but most of them evaluated the cytotoxic effects of paracetamol are in animal cells or models (B. Pereira 2018, Chrois *et al.* 2020) mainly because this drug is widely used by the human population and there are a significant number of incidents due to excessive intake of this compound (Fisher & Curry 2019). Studies with crustaceans *Daphnia magna* Straus, 1820 and *Daphnia longispina* (O.F.Müller, 1776) have shown the activity of this compound on catalase and GSTs (glutationa-S-transferase) activities, with significant consequences on the prevention of oxidative damage

(Sousa & Nunes 2021). In addition, research with cell biomarkers in liver tissue has shown that acetaminophen, in acute concentrations, may result in glutathione reduction, hepatocyte lesions (Da Silva Junior *et al.* 2019), or even apoptosis (M. Pereira 2018).

Surprisingly, there are not many studies investigating the toxicity of acetaminophen in plant cells as a parameter to access the environmental and socioeconomic impact of this micropollutant. Micronucleus and chromosomal changes have already been reported (Sturbelle *et al.* 2010), but very few studies evaluated the effects of this drug on economically and ecologically important parameters such as seed germination. Plant models are widely used as bioindicator models. These organisms allow scientists to conduct several types of toxicity tests, which are the most common researches (eg. chromosome aberration trials and cytogenetic tests). However, they also allow the evaluation of many chemical substances on important physiological processes such as those that have significant influence over reproduction, development, and survival (eg. germination and growth analysis) (Cuchiara *et al.* 2012).

The *Allium cepa* system is used worldwide as a model for cytotoxicity assessment (Bagatini 2007, Lessa *et al.* 2017). Onion is one of the main products of Brazilian family horticulture, promoting sustenance and permanence of families in the rural area since small farmers are responsible for more than half of Brazilian production (Monteavaro 2014). The seeds are coated with a thick layer, are very sensitive, and can easily deteriorate when in contact with moisture or inadequate substrate (Rossetti *et al.* 2019).

The *A. cepa* test is considered an effective methodology for detecting important cytological changes such as DNA damage to eukaryotes, mutagenic potential of a compound, etc. (Fiskesjö 1958, Bagatini *et al.* 2007). The mitotic index, the chromosomal change index, and the nuclear abnormalities index are used as parameters for the evaluation of toxicity caused by cell disrupters as well as mutagenic agents (Bagatini *et al.* 2007).

Adult plants of *A. cepa* are widely used in toxicological studies. The OECD guideline (OECD 2006) lists *A. cepa* seeds as a monocotyledonae model for the testing of chemicals, but a quick research in the Science Direct database shows that, in the last 10 years, there were not many studies that include the germination of onion seeds as a study parameter.

A group of scientists found that *A. cepa* seeds germination is significantly affected by fungicides difenoconazole and tebuconazole (Bernardes *et al.* 2015), but only a few studies focus on this parameter when studying micropollutants. Seed germination depends on various physiological events influenced by internal and external factors. At the beginning of germination, several processes occur that may be affected by the presence of pollutants, resulting in changes in the normal development of the plant, and endangering its survival. One of the critical factors for seed germination, development, and survival is water availability. Coincidentally, water is one of the main natural elements affected by irregular waste disposal and accumulation of micropollutants.

Brazilian population presents a massive and indiscriminate use of pharmaceuticals. In addition, there is a significant fragility of the legislation regarding the regulation and supervision of these substances disposal. Considering all the above, there are direct and indirect risks for Brazilian biodiversity and the balance of native ecosystems. Thus, the purpose of this study was to evaluate cytotoxicity parameters of exposure to micropollutant paracetamol, through both the classic *A. cepa* test and the germination of its seeds. Comprehending the toxic effects of micropollutants on plant models may help the identification of environmental risks, especially on the native flora that is the basis of any ecosystem.

Materials and methods

Most protocols used in the present work were established in previous work, and very few adaptations were necessary.

Paracetamol

Paracetamol (N-(4-hydroxyphenyl) ethanamide), also known as acetaminophen, was purchased locally in the most common consumable form: 750 mg tablets. A main solution of 750 mg L^{-1} was prepared and serial dilutions were performed until the testing concentrations of 0.60 mg L^{-1} (*P0.6*), 0.50 mg L^{-1} (*P0.5*). and 0.40 mg L^{-1} (*P0.4*) were achieved. The control group (CTR) was exposed to distilled water. Testing concentrations were based on maximum paracetamol dosage (533.64 μg L^{-1}) found in Brazilian surface waters in a post-Covid-19 study (Nascimento *et al.* 2023).

Experimental Models

For toxicity tests, bulbs of *A. cepa* (Crioula variety produced in the South of Brazil) were used. The bulbs with similar size and shape were acquired from a local supplier. For the germination tests, 2,600 *A. cepa* seeds (Crioula variety, ISLA®, lot.128770-001-52, Dec/21) acquired from local specialized suppliers were used.

Root Growth

Healthy medium-sized onion bulbs were sanitized and all the dry scales and dry roots of the bulbs were removed carefully with a sharp knife without destroying the roots primordial. After, onions were placed in distilled water to evaluate root growth. The roots that emerged in water were measured (pre-treatment growth – Pr-TG) and removed. Bulbs with no root growth were discarded and the remaining bulbs (N = 80) were divided into treatments (CTR, *P0.6*, *P0.5*, *P0.4*) and exposed for 96h. Root growth was evaluated once again.

For exposure to water (pre-treatment and CTR) and paracetamol, bulbs were placed in beckers containing their respective treatments so that only the roots area was in contact with the solutions. All specimens were kept in an incubator with controlled temperature (25 ± 2 ºC) and 12:12 (Light:Darkness) photoperiod. The roots were protected from direct light. Four replicate per treatment and five repetitions per replicate and were used (eg. CTR – Bulb 1A, 1B, 1C, 1D and 1E; Bulb 2A, 2B, 2C, 2D and 2E, etc.) to a total of 20 bulbs per treatment. After 96h, the new roots were measured with the help of a pachymeter and root growth was registered.

Allium cepa test

The biological material from the root growth post-treatment exposure were used in this test. The methodology was based on published articles (Ancia & Romão 2016, Machado 2013, Rodrigues *et al.* 2016 & Sturbelle *et al.* 2010), with modification. Briefly, after exposure, the terminal root meristems were removed and washed with distilled water. Then the fixation, hydrolysis and staining procedures began, and between all steps, the roots were washed with distilled water. Fixation was performed with Carnoy's solution for 5 min. Then the roots were exposed to HCL 1N for hydrolysis. Finally, the root tips were treated with 2% acetic orcein solution under heating to perform the staining. The roots were then placed in slides and the crushing technique was performed. Slides were made for each bulb and in each slide, 500 cells were analyzed under the microscope (DI-136T Digilab ® LED) with a 40x objective. Non-dividing cells and cells in mitosis were counted.

Mitotic index and abnormalities index

For both calculations, the total number of cells analyzed was n=500 cells. Mitotic index (MI) was established according to Machado (2013). Briefly, the mitotic index was calculated as the ratio between the number of dividing cells and the total number of cells analysed. To determine the MI, the following equation was used:

$$IM = NCM/NTC \times 100$$

NCM: number of cells in mitosis

NTC: total number of cells analysed.

The abnormality index (AI) was calculated:

$$AI = NCA/NTC \times 100$$

NCA: number of cell abnormalities

NTC: total number of cells analysed.

Considered aberrations included: anaphase bridges, chromosomal delays, chromosomal breaks, micronucleus, isolated chromosomes, fuse changes, and others found in cells division or interphase.

Germination Test

Previously disinfected *A. cepa* seeds (Pinheiro *et al.* 2014) were used for the germination test. The seeds were distributed in germination boxes, in double-layered germination paper (Germitest®) moist with 10 mL of paracetamol solution or distilled water. The boxes were kept in an incubator for 12d at 23±2 ºC and 12/12h photoperiod. The count of germinated seeds was held on the 12th day, according to the Rules for Seed Analysis (Brasil, 2009). A number of 13 repeats, of 50 seeds each, were made for treatments and control groups. Every 3 days, boxes were checked for humidity levels and, if necessary, 2 mL of solution were added.

Statistical analysis

Statistical analysis, including analysis of variance (ANOVA) with the appropriate post hoc test

(Tukey HSD) were carried out with BioEstat 5.3 (Ayres *et al.*, 2007). The level of statistical significance was $p \leq 0.05$ in all cases and each data point represents Mean ± SEM.

Results

Exposure to acute doses of paracetamol resulted in darkening of the root tissue in both seed and bulbs roots as shown in the figure 1.

In addition, it was possible to notice a change in the root texture, which became more rigid in paracetamol-treated groups. In addition, paracetamol significantly reduces root growth, as shown by the results presented in the table 1.

Root meristematic cells analysis showed that mitotic index (MI) of treated tissue was significantly different from control in all doses studied (Fig. 2A). It was possible to see an MI elevation tendency in the *P0.5* group however, no statistical difference was found (*P0.4*=5.35±1.51; *P0.5*= 6.79±1.5; *P0.6*=3.13±1.51). The abnormality index (AI) found in the CTR group was significantly lower, as shown in the figure 2B, compared to treated groups. Results possibly indicate a dose-related tendency of increase in AI, but no statistical difference among treatments was found. Data showed that all doses of paracetamol in the present work reduced the number of meristematic cells in division, significantly reducing MI when compared to control. A significant increase in the number of abnormalities in the root meristematic cells could be identified in groups exposed to the test drug

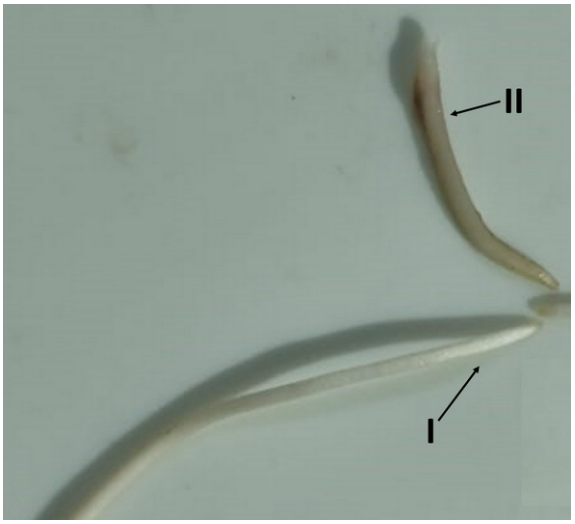

Figura 1. Color de la raíz de *Allium cepa*. Grupo control (CTR): con color claro típico (I); Oscurecimiento: Puntas de las raíces tratadas con un color marrón claro (II) en toda su longitud.

Figure 1. *Allium cepa* root color. Control group (CTR): with typical clear color (I); Darkening: treated root tips displaying a light brown color (II) along the entire length.

Treatment	Pr-TG (cm)	TG (cm)
CTR	2.5 ± 0.3	1.7 ± 0.15
P0.4	4.3 ± 0.5*	0.5 ± 0.12**
P0.5	5.1 ± 0.3*	0.5 ± 0.06**
P0.6	3.7 ± 0.3*	0.4 ± 0.05**

Tabla 1. Valores de crecimiento radicular antes y después de 96h de exposición al tratamiento. Pr-TG: crecimiento durante el pretratamiento. TG: crecimiento durante el tratamiento. *P0.4*, *P0.5*, *P0.6*: grupos tratados con Paracetamol 0,4, 0,5 y 0,6 mg L^{-1}. *Valor significativamente mayor que CTR. ** Valor significativamente más bajo que CTR.

Table 1. Values of root growth before and after 96h exposure to treatment. *P0.4*, *P0.5, P0.6*: groups treated with Paracetamol 0.4, 0.5, 0.6 mg L^{-1}. *Value significantly higher than CTR. ** Value significantly lower than the CTR.

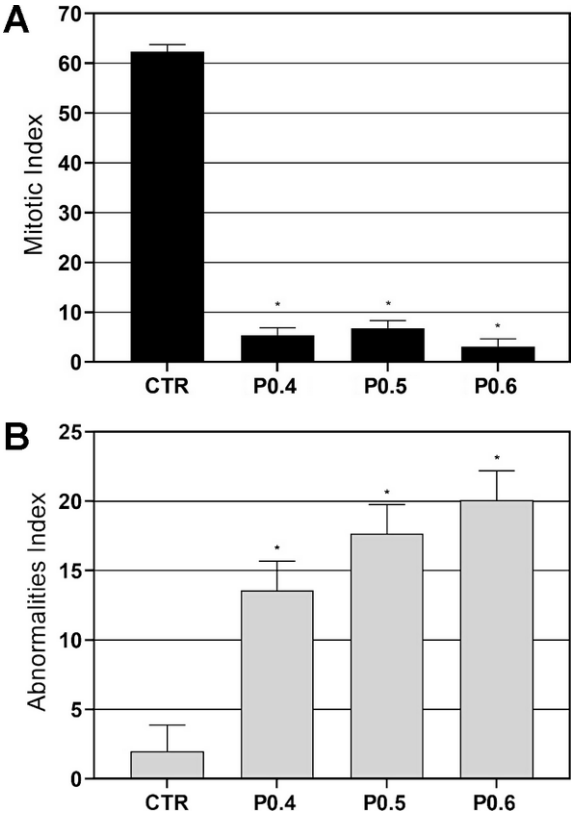

Figura 2. Índice mitótico e índice de anomalías en la mitosis de células meristemáticas de la raíz de *A. cepa*. Datos como media ± SEM. **A:** Índice mitótico de los grupos tratados y el control. **B:** Índice de anormalidad de los grupos tratados y el control. CTR: Grupo control. *P0.4*, *P0.5*, *P0.6*: grupos tratados con Paracetamol 0,4, 0,5 y 0,6 mg L^{-1}. *Diferencias significativas con CTR.

Figure 2. Mitotic index and abnormalities index in *A. cepa* root meristematic cell mitosis. Data as mean ± SEM. **A:** Mitotic index for treated and control groups. **B:** Abnormality index for treated and control groups. CTR: Control group. *P0.4*, *P0.5, P0.6*: groups treated with Paracetamol 0.4, 0.5, 0.6 mg L^{-1}. *Significant difference with CTR.

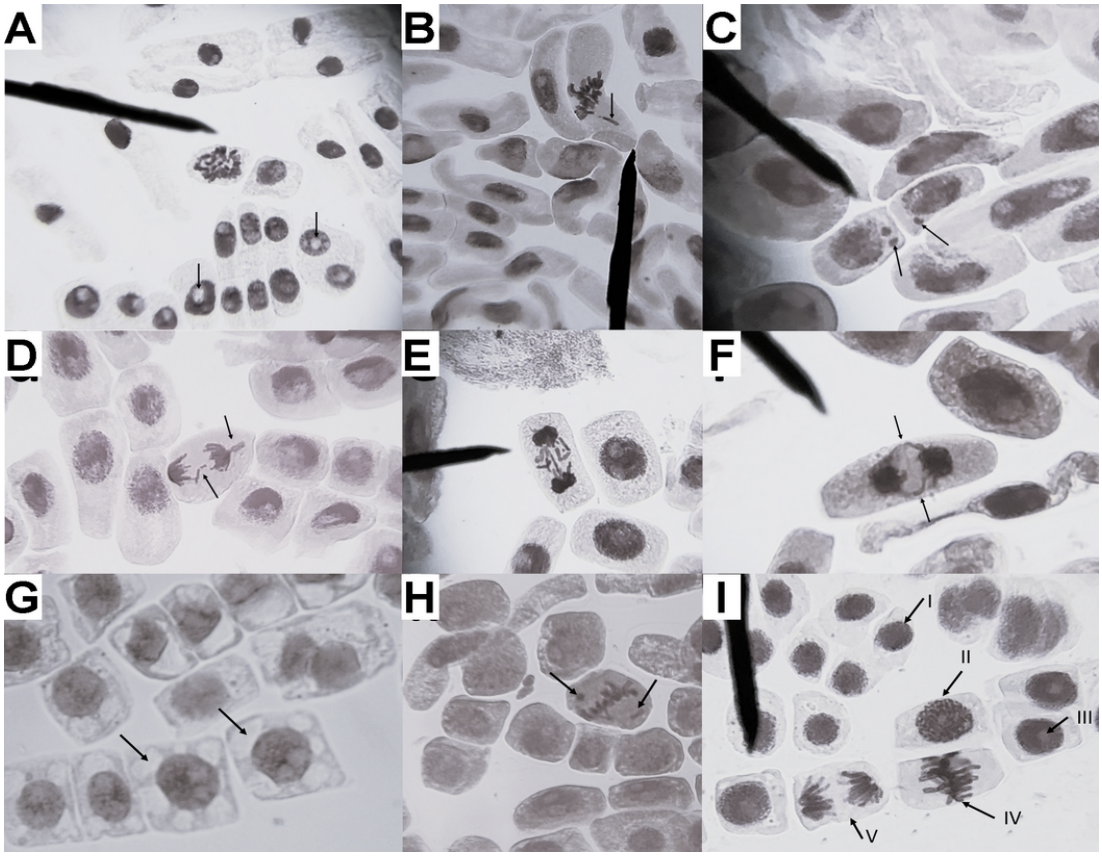

Figura 3. Alteraciones cromosómicas más frecuentes en células de raíces meristemáticas de *A. cepa* tratadas con paracetamol; **A:** Lesiones nucleares (flechas) y alteración del huso en anafase; **B:** Anafase diagonal temprana con rotura y cromosoma errante; **C:** Micronúcleo; **D:** Anafase con rotura cromosómica; **E:** Telofase con puente cromosómico y cromosomas errantes; **F:** Telofase con puentes cromosómicos dobles; **G:** Célula necrótica, **H:** Micronúcleo y cromosoma perdido; **I:** Algunas etapas celulares normales: Interfase (I), profase (II), célula con nucléolo (III), metafase (IV) y anafase (V).

Figure 3. Most frequent chromosomal alterations in *A. cepa* meristematic root cells treated with paracetamol. **A:** C-mitosis and nuclear lesions (arrows) and spindle disturbance in anaphase; **B:** Early diagonal anaphase with breakage and vagrant chromosome; **C:** Micronucleus; **D:** Anaphase with chromosomal breakage; **E:** Telophase with chromosome bridge and vagrant chromosomes; **F:** Telophase with double chromosome bridges; **G:** Necrotic cell; **H:** Micronucleus and lost chromosome; **I;** Few normal cellular stages: Interphase (I), prophase (II), cell with nucleolus (III), methaphase (IV) and anaphase (V).

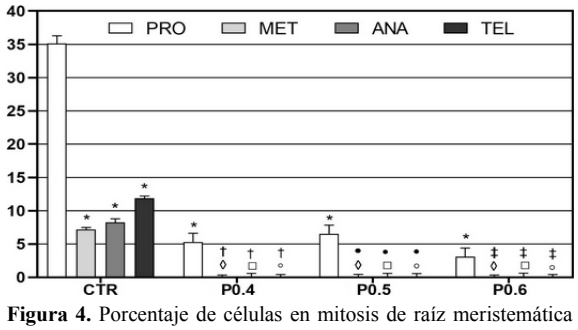

Figura 4. Porcentaje de células en mitosis de raíz meristemática de *A. cepa*. Solo se incluyen células sin anomalías. PRO: profase, MET: metafase, ANA: anafase, TEL: telofase. CTR: Grupo control. *P0.4*, *P0.5*, *P0.6*: grupos tratados con Paracetamol 0,4, 0,5 y 0,6 mg L^{-1}. Diferencias significativas: *CTR-PRO, † *P0.4*-PRO, • *P0.5*-PRO, ‡ P-0.60-PRO, ◊ CTR-MET, ▫ CTR-ANA.◦ CTR-TEL.

Figure 4. Percentage cells in mitosis phases in meristematic root of *A. cepa*. Only cells without abnormalities are included. PRO: prophase, MET: metaphase, ANA: anaphase, TEL: telophase, CTR: Control group. *P0.4*, *P0.5, P0.6*: groups treated with Paracetamol 0.4, 0.5, 0.6 mg L^{-1}. Significant difference: *CTR-PRO, † *P-0.4*-PRO, • *P0.5*-PRO. ‡ *P0.6*-PRO, ◊ CTR-MET, ▫ CTR-ANA, ◦ CTR-TEL.

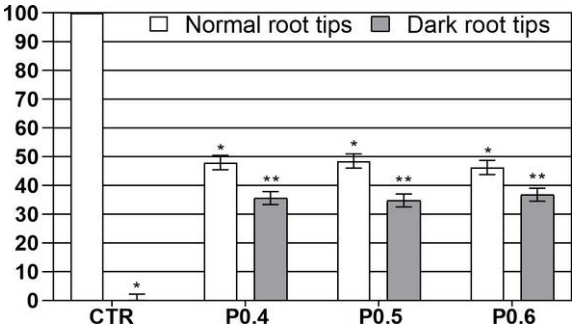

Figura 5. Puntas de raíces de *A. cepa* de semillas germinadas. Porcentaje de células oscurecidas por el tratamiento de paracetamol. CTR: Grupo control, sin oscurecimiento. *P0.4*, *P0.5*, *P0.6*: grupos tratados con Paracetamol 0,4, 0,5 y 0,6 mg L^{-1}. *Diferencia significativa en comparación con CTR. **Diferencia significativa en comparación con CTR y en comparación con el número de puntas de raíces normales tratadas.

Figure 5. *A. cepa* root tips from germinated seeds. Percentage of cells with darkening caused by paracetamol. CTR: Control group, without darkening. *P0.4*, *P0.5, P0.6*: groups treated with Paracetamol 0.4, 0.5, 0.6 mg L^{-1}. *Significant difference compared to CTR. **Significant difference compared to CTR and compared to treated normal root tip number.

The present study identified several types of alterations in *A. cepa* root meristematic cells exposed to the micropollutant paracetamol and the most frequent ones are highlighted in the figure 3A-H.

Paracetamol caused a significant reduction in the occurrence of mitosis in *A. cepa* root. In addition, a significant reduction in the occurrence of metaphase, anaphase, and telophase was registered in paracetamol groups (Fig. 4). Prophase was predomination in all groups, but significantly lower in treated roots.

Treatment with paracetamol did not affect *A. cepa* seed germination compared to control group. Nevertheless, it was possible to notice that treatment with paracetamol resulted in a significant amount of dark root tips (Fig. 5), compared to control which has not presented signs of abnormality.

Discussion

Emergent micropollutants can cause significant environmental contamination. The effects on native species could result in irreparable damage to ecological balance. The hazard effects of these substances may also affect the most sensitive area of the world's economy, which is food production. Plants are the base of our nutrition whether consumed directly or used to feed the animals we use as protein sources.

Even though the risks are very important, stopping the production and consumption of substances that originate micropollutants is not a possibility. Pharmaceutical drugs have increased our longevity and our quality of life throughout the last centuries and our society needs to act in the sense of improving both industry and domestic residue management.

In the scientific world, the *Allium cepa* system is widely used as a model for cytotoxicity assessment (Bagatini *et al.* 2007, Lessa *et al.* 2017). Adult plants of *A. cepa* are used in a variety of toxicological studies, providing reliable data on important parameters such as DNA damage and mutagenic potential of compounds (Bagatini *et al.* 2007).

Nevertheless, onions are also one of the most produced and consumed vegetables worldwide. Onions (paired with garlic, potato, tomato, and watermelon) accounted for over 60% of global vegetable production in 2013-14 (Camargo & Camargo 2017). This means that this species has a significant impact on both nutrition and the economy worldwide, and substances that can disrupt vegetable life cycle have to be carefully managed.

Results in the present work show that the micropollutant paracetamol is significantly toxic for both seed and adult *A. cepa*. The mitotic index reduction found in the adult meristematic root tips is strong and reliable proof that paracetamol is cytotoxic to onions roots. The chemical action in cell cycle affected root growth and impaired bulb development. Studies have shown the pro-oxidative nature of paracetamol (Nunes 2020). Through oxidative stress, paracetamol may induce changes in the intracellular Ca^{2+} homeostasis, DNA fragmentation and, consequently mitosis impairments (Bergman *et al.* 1996). Early exposure to such micropollutants could result in delay or even a full interruption of plant development, causing significant economic losses.

Results how both cytoplasmic and nuclear alterations in cells treated with paracetamol. The nucleus is vital for many cellular functions and alterations in this structure might affect the physiology and the survival of the eukaryotic cell. Interphase aberrations are suggestive of toxic effect of a substance and may be attributed to the presence of a significantly toxic substance (Sutan *et al.* 2014). Programmed cell death, or apoptosis, is a complex process involving a sequence of biochemical reactions that result in cell modifications and, eventually, death. Among the modifications that are indicative of an apoptotic event are cytoplasmic vacuolation and disintegration, ghost cell, nuclear vacuolation, and nuclear fragmentation (Prajitha & Thoppil 2016).

Nuclear vacuolization was registered in plants exposed to known toxic substances such as heavy metals (Sabeen *et al.* 2020). The presence of nuclear lesions offers cytological evidence for the inhibitory action of chemicals on DNA biosynthesis, and disruptions at the beginning of cell division could result in complete inhibition of the cell mitosis. Another alteration found in plant cells exposed to toxic substances is related to nucleoli. A recent study found that exposure to some pollutants increased nucleoli in *A. cepa* while reduced its area and showed that both nucleoli number and area are effective parameters to determine cytogenotoxicity (Lima *et al.* 2019). In the present work, we found nuclear alterations in treated cells

that could be an increase of nucleoli or even the beginning of vacuolization. Even though no specific cytological study was conducted to differentiate this nuclear alterations, they were only present in treated cells, which strongly indicates they were an effect related to paracetamol exposure.

Cytoplasmic vacuolation is another type of cellular lesion commonly reported in cells treated with toxic substances. This kind of lesions have been reported in *A. cepa* meristematic root cells after both heavy metal (Liu & Kottke 2003) and organophosphate insecticides (Cortés-Eslava *et al.* 2018) exposure and it is considered indicative of imminent cell death (Bianchi *et al.* 2011). The lesions seen in treated cells the present work, are a strong indicative that the paracetamol dosages found in Brazilian surface waters after the Covid19 pandemic may be a serious environmental problem that should be addressed very carefully.

The significant reduction in root growth verified in the present work can be related to the significant cytotoxic effect from paracetamol treatment, which caused an important disturbance in both nuclear and cytoplasm structure and function. Previous work found that *A. cepa* root cells are very sensitive to pollutants (Ancia & Romão 2016, Rosculete *et al.* 2019) and therefore provide a reliable method for cytotoxic and genotoxic evaluations.

The present work found several chromosomal aberrations of both clastogenic and aneugenic nature, which are indicative of paracetamol genotoxic effect in plant cells. Aneugenic chromosomal aberrations induced by toxic compounds may result from dysfunction of nuclear spindle (Sutan *et al.* 2014). Results found after paracetamol exposure show spindle disturbances during anaphase and telophase, resulting in vagrant chromosomes that would originate cells with altered chromosome numbers.

Chromosome stickiness, which may result from improper condensation and entanglement of chromatin fibers (Rosculete *et al.* 2019) was also found. Stickiness may originate from alterations in chromosome contraction or condensation, or DNA polarization changes, and reflects toxic effects, which may lead to cellular death (Sabeen *et al.* 2020). Stickiness can originate chromosomal bridges, cause serious alterations in the nucleus, loss of genetic material, and disruptions in cell division.

Another common aberration induced by paracetamol exposure in the present work was micronucleus. Micronuclei were detected in *A. cepa* after exposure to common pollutants such as wastewaters, sewage, and organophosphate insecticide (Grover *et al.* 1999, Anacleto *et al.* 2017 & Cortés-Eslava *et al.* 2018). Micronucleus may originate from a lagging or a fragment of a broken chromosome and serve as a source of genome instability (Rosculete *et al.* 2019, Krupina *et al.* 2021). This type of aberration is a clear manifestation of genotoxicity and may suggest a spindle inhibitory or a clastogenic action of a particular substance. In plant cell division, specific processes of extensive and continuous reorganization of microtubule arrays occur and mitotic spindles emerge as structures primarily reliant on microtubules, which are responsible for the separation of chromosomes during mitosis (Liu & Lee 2022). Interaction of some chemicals with the spindle apparatus may lead to the origination of chromosome aberrations. Alkaloids, for example have been reported to alter mitosis by binding to tubulin and preventing spindle formation during cell division (Fatemeh & Khosro 2012) so that the presence of alkaloid at an appreciable concentration in a plant extract may be responsible for its cytotoxicity. Even though paracetamol is not an alkaloid, it provoked a similar effect as these substances on darkening *A. cepa* roots (Fatemeh & Khosro 2012) which could mean that, at least at some level, paracetamol presents a similar mechanism of toxicity in roots meristematic cells.

It has also been established that reactive oxygen species (ROS) are implicated in the regulation of cell cycle development, organization of microtubule and other cell structural dynamics (Livanos *et al.* 2012). Since oxidative stress plays is significant in a large number of biological processing and cell signaling pathways, significant changes in this process usually result in cell disturbance, which can be interpreted as a sign of toxic effects. Paracetamol has been shown to induce ROS generation and could lead to significant alterations in antioxidant status in in vitro models, resulting in an oxidant state that could be related to toxicities induced by this micropollutant (Wang *et al.* 2017). Among the effects, protein peroxidation was one of the remarkable oxidative stress indices reported

to be induced by paracetamol and that could pose a significant menace to the protein-based spindle apparatus in plant cells (Wang *et al.* 2017).

Even though all the above disruptions occurred in cells exposed to paracetamol in the present work, chromosomal breakages were the most frequent. This aberration is also seen in cells exposed to effluents containing metals such as Pb, Ni, and Cu (Bianchi *et al.* 2011) and in organophosphorus insecticides exposure (Cortés-Eslava *et al.* 2018).

The presence of vagrant chromosomes during cell division may be the result of failure in the spindle mechanism or could be due to the interference in the DNA synthesis, a process that may be affected by paracetamol through a specific inhibition of ribonucleotide reductase in a concentration-dependent manner (Hongslo *et al.* 1990). The paracetamol metabolite (NAPQI) also binds to most macromolecules inactivating them, which could cause severe intoxication (B. Pereira 2018).

Genotoxic agents interact with DNA producing modifications that may result in altered chromosomes. Outcomes may include increasing cell apoptosis, which leads to development impairments, lower viability, or even increased mortality index. For commercial species, this damage can provoke productivity decrease, which leads to significant socioeconomic impact. However, for the environment, this represents potential damage to fauna and flora biodiversity.

Chemical stress may induces perturbations in several cellular functions, initiating signalling cascades that orchestrate the modulation of gene expression and other intracellular activities (Zhu 2016). Even though seed germination analysis did not reveal significant differences between treated and control groups, the darkening of root tips in paracetamol-treated groups is a possible indicative of alterations. The alteration in color of plant root after exposure to paracetamol has been identified as a toxic effect in other species (Mercado & Galvis 2023). Authors showed that pharmaceutical micropollutant diclofenac also does not affect seed germination percentual (Sousa *et al.* 2020). Nevertheless, this might have more to do with dosage utilized and time of exposure than with a possible safety of these substances. Recent work found that germination decreases as the concentration of diclofenac increases (Priyan *et al.* 2021). In addition, it was reported that, even though both diclofenac and paracetamol may not affect seed germination and primary root length, older plants exhibited a decrease in biomass production and leaf area.

Recent work showed that the antiproliferative effect of paracetamol in onion roots does not decrease by solution filtration with activated carbon filters (Lessa & Cariello 2017). Drinking water treatments use chlorination and activated carbon, but those conventional treatments are unable to completely remove pharmaceutical micropollutants from water (Couto *et al.* 2019). Other technologies, such as biodegradation, solar photolysis, and sedimentation, were tested for the removal of paracetamol from water with low efficiency, a phenomenon attributed to the unique characteristics of the molecule (Erba *et al.* 2012, Pacheco-Álvarez *et al.* 2022). Paracetamol is continuously introduced into the aquatic environment as a parent compound, metabolites or conjugate of both by pharmaceutical industrial effluents and human use. Ozonation processes have emerged as a promising alternative to significantly remove paracetamol from both wastewaters and drinking waters however, strategies for dealing with the by-products of paracetamol destruction are still the subject of speculation by researchers (Peralta-Hernández & Brillas 2023).

Acetaminophen has been detected in groundwater, in drinking waters and in surface waters around the world (Wadhah Hassan *et al.* 2017) and the inefficiency of regular treatment combined with the lack of specific legislation and parameters for this group of substances aggravates the environmental and economic hazard that this class of substances represents in the world.

The outcomes of studies conducted on different biological models unequivocally reveal that paracetamol possesses the capacity to disrupt the oxidative balance within eukaryotic cells. This interference manifests in the modification of cell division processes, ultimately leading to cytotoxic and genotoxic effects. While the majority of investigations into the mechanisms of paracetamol toxicity are conducted using animal models, it is essential to acknowledge that the chemical can induce adverse effects in both animals and plants. Nevertheless, the detailed pathways of its specific actions in plant cell remain to be established.

Although paracetamol is an important analgesic and antipyretic drug that contributes to human wellbeing, it may also represent a significant

risk to agricultural production and environmental preservation. Present data corroborate previous studies, showing clear evidence that pharmaceutical products may cause significant damage to cell DNA, disrupting cellular division, reducing root growth, and, possibly, plant development. These effects may represent a significant problem to food production, environmental balance and species preservation.

Emergent micropollutants represent a significant health, socioeconomic and environmental problem. The cytotoxic and genotoxic effects of these substances on several biological models reinforce the urgency of their inclusion in the regular chemical screening in effluent and water treatment plants in order to minimize the introduction of critical pollutants into the aquatic environment.

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