# Cytotoxicity and Genotoxicity of Sulfur-Vegetable Oil Polymers and Urea-Loaded Composites Evaluated by the Allium Cepa Test

**Ana Sofía Farioli**
Universidad Nacional de Rio Cuarto Facultad de Ingenieria
**Maricruz Fernandez**
IITEMA: Instituto de Investigacion en Tecnologias Energeticas y Materiales Avanzados
**Pablo César Cavallo**
Universidad Nacional de Rio Cuarto Facultad de Ingenieria
**Diego Fernando Acevedo**
Universidad Nacional de Rio Cuarto Facultad de Ingenieria
**Edith Ines Yslas**
eyslas@exa.unrc.edu.ar

Universidad Nacional de Rio Cuarto Facultad de Ciencias Exactas Fisico Quimicas y Naturales
https://orcid.org/0000-0002-6371-8533

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# Abstract

This study evaluated the cytotoxicity and genotoxicity of a sulfur vegetable oil biopolymer (p-SF) and its urea-loaded biocomposites using the *Allium cepa* L. assay. Urea was incorporated at different loadings 79 mg and 92 mg per g of biopolymer (p-SF-79U, p-SF-92U) to obtain controlled-release biocomposites for potential agricultural applications. After 72 h exposure, root growth increased by up to 63% in p-SF-92U compared with – 2.6% in the negative control, without visible signs of citotoxicity. Mitotic index values remained comparable to the control (5.93%), ranging from 4.27% to 6.66%, while the positive control ($H_2O_2$) completely inhibited mitosis. Microscopic analyses aloe to determinate that chromosomal aberrations were consistently low (< 2.9%) in treated cells, even below the control (3.67%), confirming the absence of genotoxicity. Moreover, all mitotic phases in treated groups, and severe chromosomal damage induced by $H_2O_2$ were observed. These findings demonstrate that the evaluated biopolymers and biocomposites are non-cytotoxic and non-genotoxic to eukaryotic cells, while promoting root development. Overall, the *Allium cepa* assay proved to be a reliable, low-cost tool for preliminary toxicological assessment, and the results support the safe application of sulfur oil biocomposites as sustainable encapsulation matrices for urea, offering an eco-friendly strategy to improve fertilizer efficiency and minimize environmental risks.


# Introduction

The growing global population and the reduction of arable land have intensified the need for increased fertilizer use, particularly nitrogen (N), to meet the rising demand for food (Brown et al. 2009; Chien et al. 2009). Fertilizer is any material applied to soil that supplies one or more essential nutrients necessary for plant growth and development (Vanlauwe and Giller 2006). Among them, chemical fertilizers have been widely adopted due to their ability to rapidly release nutrients and promote efficient plant growth (Sneha 2018). However, the prolonged use has raised significant concerns regarding soil health, such as the degradation of soil quality and a gradual decline in soil fertility (Farnia and Hasanpoor 2015). Moreover, excessive fertilizer application contributes to environmental problems, including water pollution and biodiversity loss in soil ecosystems.

Nitrogen, one of the major essential nutrients, is vital for plant development and directly influences crop yield (Zayed et al. 2023). However, nitrogen fertilizers are subject to significant losses through leaching, denitrification, and erosion (Pal et al. 2020). Urea, the most commonly used nitrogen fertilizer worldwide, is especially prone to volatilization as ammonia ($NH_3$) following hydrolysis by urease enzymes (Carter et al. 2009). These inefficiencies highlight the urgent need for more sustainable fertilization strategies that preserve soil health and reduce environmental impacts.

One promising approach involves the encapsulation of nutrients in polymeric matrices that allow for controlled or slow release, enhancing nutrient uptake by plants while minimizing ecological harm (Tylkowski et al. 2020a; Vo et al. 2021; Das et al. 2023; Riswoko et al. 2024). Recent research in this area has led to the development of multifunctional, eco-friendly fertilizers systems designed to improve agricultural sustainability. These systems often employ biopolymeric carriers that control urea release through diffusion and gradual matrix degradation, thereby reducing leaching, improving nitrogen use efficiency (NUE), and decreasing application frequency (Ravindran et al. 2025). Despite these advances, a critical gap persists, the cytotoxicity and genotoxicity of the polymeric materials used remain largely unassessed. To ensure safe and sustainable implementation, it is necessary to evaluate the biological compatibility of these materials before widespread agricultural application.

An innovative alternative involves the use of sulfur-rich polymers synthesized via inverse vulcanization, a green chemistry technique where elemental sulfur, an abundant byproduct of the petrochemical industry, is chemically crosslinked with unsaturated triglycerides from renewable sources such as vegetable oils (Ghumman et al. 2023; Farioli et al. 2023). Unlike conventional vulcanization, where sulfur is used in minor proportions, inverse vulcanization uses sulfur as the primary component, forming durable polymeric networks through reactions with carbon–carbon double bonds in the oil structure (Chalker et al. 2019). This method enables the synthesis of cost-effective, functional materials aligned with circular economy principles. Consequently, biopolymer-sulfur-based systems have gained considerable attention in agro-biotechnology (Valle et al. 2019a).

Recent studies have demonstrated the potential of inverse vulcanization to produce sulfur-enriched copolymers for slow-release fertilizers that improve NUE, address sulfur deficiencies in soils, and reduce environmental burdens (Valle et al. 2019b; Zhao et al. 2020; Ghumman et al. 2021; Shaan Manzoor Ghumman et al. 2021; Nayeem et al. 2022). These materials were synthesized using different vegetable oils (e.g., soybean, jatropha, and rubber seed oil) and elemental sulfur ($S_8$), and characterized using advanced analytical techniques such as FTIR, TGA, DSC, and SEM to assess nitrogen release kinetics, biodegradability, and sulfur oxidation capacity.

These innovations contribute to the global effort to develop eco-friendly fertilizers that support agricultural productivity while preserving environmental integrity. Reducing the dependance on synthetic agrochemicals, minimizing pollution, and optimizing resource efficiency are key goals in the pursuit of sustainable agriculture (Han et al. 2009; González et al. 2015; Tylkowski et al. 2020b). To fully realize the potential of these advanced materials it is necessary thorough toxicological evaluation to ensure their compatibility and safety for plant development (González et al. 2015; Maruyama et al. 2016) .

Therefore, cytotoxicity and genotoxicity testing are vital to evaluate whether these materials can induce cellular or genetic damage. Addressing these questions is essential for validating safety, closing knowledge gaps, and enabling the responsible use of innovative materials in agriculture.

The *Allium* test (AT) has a long-standing presence in the scientific literature for assessing the potential genotoxicity of various chemical, physical, and biological agents(Scherer et al. 2019; Mercado and Caleño 2020; Meetiyagoda et al. 2024).

The *Allium cepa* (AT) test has been widely used as a bioindicator of cytotoxicity and genotoxicity since Levan's pioneering study in 1938 (LEVAN 1938), later standardized by Fiskesjö (1985a). Its advantages include the presence of large chromosomes that are easily observed under light microscopy and its proven sensitivity for detecting chromosomal alterations, making it a reliable, low-cost tool for environmental and toxicological assessment (Khanna and Sharma 2013; Palsikowski et al. 2018; Çavuşoğlu and Çavuşoğlu 2024).

Also, the AT test is fast, easy and inexpensive method, proving valuable data for assessing genotoxicity in plants and other eukaryotes, including humans. Bosio *et at.*, shown that the results from *A. cepa* tests are comparable to those from animal cell lines when assessing the effects of ecotoxicological agents (Bosio and Laughinghouse IV 2012). Additionally, the test can be used to assess cytotoxicity by examining various macroscopic parameters, such as root length, changes in root shape, color, and texture, as well as the presence of broken root tips, tumors, and hooks (FISKESJÖ 1985b).

Despite the growing use of sulfur-based polymers in agriculture, their potential biological impact remains largely unknown. Therefore, assessing their cytotoxicity and genotoxicity is essential to ensure their safe implementation as controlled-release matrices for fertilizers. In this study, we applied the Allium cepa assay as a reliable, low-cost, and sensitive tool to detect chromosomal and mitotic alterations. Specifically, we evaluated a sulfur–vegetable oil biopolymer (p-SF) and its urea-loaded biocomposites (p-SF-79U and p-SF-92U) after 72 h of exposure, using distilled water as a negative control and $H_2O_2$ as a positive control. The findings provide critical evidence for validating these materials as safe and sustainable encapsulation matrices for urea, supporting their future use in agrochemical applications. This study addresses the unexplored biological impact of sulfur–based biopolymers by applying the Allium cepa assay as a sensitive tool for cytotoxicity and genotoxicity screening. The results validate their safe use as sustainable encapsulation matrices for urea, reinforcing their potential in future agrochemical applications.

## Materials and Method

# Materials:

Elemental sulfur (S) used as monomer was obtained from Aldrich (purity > 99%). Commercial sunflower oil Natura® (SF), from AGD (General Deheza, Cordoba, Argentina) was employed without further purifications. Urea (Aldrich > 99%) was used to produce biocomposites.

## Synthesis and Characterization

# Biopolymer synthesis (p-SF):

Biopolymers based on sunflower oil and elemental sulfur were synthesized through a reverse vulcanization process, adhering to the methodology described by Farioli *et al.* (Farioli et al. 2023) The resulting biopolymer, p-SF, was produced using a 50:50 mass ratio of SF to S.

# Synthesis and characterization Biocomposites: p-SF-Urea

The synthesis involved dispersing urea in sunflower oil (30 g), heating the mixture to 160°C, and then adding it to a reactor containing sulfur (30 g) at 160°C under stirring conditions (900 rpm). (Farioli et al. 2023) Biocomposites were synthesized by adapting the procedure used for p-SF preparation, incorporating two different amounts of urea into the biopolymer matrix: 28 g for the p-SF-79U formulation and 20 g for the p-SF-92U formulation.

# Fourier transforms infrared (FT-IR) spectroscopy

The biopolymers and biocomposites chemical structure was examined by Fourier transform infrared spectroscopy (FT-IR) in absorbance mode using a Bruker Tensor 27 spectrometer with a ZnSe single reflection attenuated total reflectance device. Duplicate spectra per sample were obtained with 25 scans per spectrum at a spectral resolution of 4 $cm^{-1}$. The wavenumber range employed was from 500 $cm^{-1}$ to 4000 $cm^{-1}$.

### Assay with Allium cepa

### Organism/Allium cepa test

The *Allium cepa* test was carried out to evaluate the toxic and genotoxic potential of the biopolymer p-SF and the biocomposites p-SF-79U and p-SF-92U (Fig. 1). Bulbs of *Allium cepa L.* (2n = 16) were selected for the assay, considering uniformity in size and similar morphological characteristics.

Fresh onion bulbs of uniform size were selected and the protective outer layers and old root tissue were removed, followed by washing each bulb before testing. The Allium cepa bulbs were then placed in beaker with distilled water for 48 hours until roots emerged, the roots at this time were 2.5–3.8 cm in length.

Following this, the Allium cepa assay was initiated (t = 0 h). The onion bulbs were positioned atop each beaker with their roots oriented downward, allowing direct contact with the treatment solutions for a period of 72 hours. Treatment 1 was carried out using 0.301 g of p-SF, while Treatments 2 and 3 employed 0.316 g of p-SF-79U and 0.289 g of p-SF-92U, respectively. Considering the composition of the biopolymers and the mass employed, the treatment (p-SF-79U) contributed with 0.079 g of urea, while p-SF-92U contributed with 0.092 g of urea. Also, a negative control (NC) was performed with distillated water and a positive control (PC) using a solution 7% w/v of hydrogen peroxide ($H_2O_2$) (Cabuga Jr et al. 2017). The bioassay was performed using two independent exposures in two different batches, each with three replicates per treatment.

# Evaluation of Macroscopic parameters

The macroscopic parameter assessed in this study was the root length. Each individual root from every bulb was measured and recorded; also, statistical analysis was employed to calculate the mode of the root's length in each experiment. Additionally, the total number of roots was counted to provide a comprehensive assessment of the toxic impact on plant growth. These measurements offered valuable insights into the extent of toxicity affecting root development and overall plant health. Another parameter considered was the turgescence, which refers to the firmness of the root tips and indicates toxicity levels. The color change was also monitored throughout the experiment, as both the root tips and the entire plant may exhibit discoloration due to toxic effects. For instance, the browning of root tips indicates cell death caused by exposure to toxic substances.

## Microscopic Observation

For the microscopic assay, the roots were thoroughly washed with distilled water and then fixed in a solution of ethanol-acetic acid (3:1 volumetric ratio) for 6 hours. Following fixation, the roots were rinsed again with distilled water and treated with 0.1 M HCl for 15 minutes at room temperature. Following this step, 3 to 4 roots were carefully selected and trimmed to isolate the root apex. The apical sections were then placed on a microscope slide and stained with a 2% orcein-acetic acid solution for 5 minutes to facilitate microscopic analysis. The stain excess was carefully removed, and the roots were covered with a coverslip, which was gently pressed using the thumb to flatten the sample (Wandscheer et al. 2017). Each prepared slide was then examined under an optical microscope at a magnification of 40X. Micrographs were taken for subsequent analysis of the determination of the mitotic index and chromosomal aberrations.

The mitotic index (%MI) was calculated as the ratio between the number of cells undergoing mitosis divided and the total number of cells (Eq. 1). On the other hand, the percentage of chromosomal aberration (% CA) was calculated as the ratio between the number of aberrant cells and the total number of cells in each sample (Eq. 2). The number of cells analyzed from 5 separate slides and calculated as mitotic cells per 1000 cells examined per each bulb (Leme and Marin-Morales 2009; Ikechukwu et al. 2024).

$$\%MI = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cells} * 100\ \ (Equation\ 1)$$

$$\%\ CA = \frac{Number\ of\ Aberrant\ cells}{Total\ number\ of\ \ cells} * 100\ \ (Equation\ 2)$$

# Statistical analysis

The data obtained from the *Allium cepa* assay were statistically analyzed using one-way analysis ANOVA, followed by Tukey multiple comparison test to assess significant differences between treatment groups. Statistical analysis was performed using Origin® software. The experimental design included two independent exposure trials, each conducted in two separate batches, with three replicates per treatment to ensure reproducibility and statistical robustness. The results are reported as mean ± standard deviation (SD). A p-value of $< 0.05$ was considered statistically significant.

## Results and discussion

# Biopolymer and biocomposites Synthesis and Characterization

During the synthesis, the yellow sulfur powder initially melted at 115°C, transitioning into a liquid state. As the temperature increased to approximately 160°C, the molten sulfur turned into an orange liquid. At this stage, preheated unsaturated vegetable oil (heated to 160°C) was added to the sulfur reaching 180°C in 5 minutes, and the two phases combine leading a homogeneous red phase. After 20 minutes, a significant increase in viscosity was observed, indicating the completion of the polymerization reaction. This process yielded a flexible, brown rubber-like material. These observations are consistent with previous findings reported by Farioli et al. and Chalker et al., further supporting the reproducibility and reliability of the synthesis process (Chalker et al. 2021; Farioli et al. 2023).

The biocomposite was synthesized following the same procedure employed for the biopolymer synthesis. All the observations noted during the synthesis of the biopolymer were replicated, and the resulting material displayed visual characteristics consistent with those of the biopolymer. This indicates a successful integration of the components and uniformity in the synthesis process.

In Fig. 2, the FTIR spectrum of p-SF exhibits characteristic bands typical of this class of materials, aligning with previously reported spectroscopic fingerprints of typical triglycerides. In the region of 3000 to 1100 $cm^{-1}$ (Johnston and Rogers 2017); bands at 2921 $cm^{-1}$ and 2852 $cm^{-1}$ correspond to the antisymmetric and symmetric stretching of C-H from methylene groups; at 1746 $cm^{-1}$ it is possible to observe the C = O stretching of the ester. Also, is possible to detect bands at 960 $cm^{-1}$ and 725 $cm^{-1}$, that are assigned to the S-S stretching and C-S stretching (Luo et al. 2020). Also, Fig. 2, shows the FTIR of the urea and p-SF-92U. The FTIR spectrum of urea displays characteristic absorption bands at 1670 $cm^{-1}$, 1590 $cm^{-1}$, 1463 $cm^{-1}$, and 1159 $cm^{-1}$, which are attributed to $NH_2$ bending vibrations, C = O stretching, antisymmetric C–N stretching, and $NH_2$ deformation, respectively (Grdadolnik et al. 2002)

The biocomposite polymer presents all the band present in p-SF, additionally is possible to observe an intense and sharp peak at 1590 $cm^{-1}$ corresponding to the carbonyl group of the urea, likewise the $NH_2$ bending vibration (1670 $cm^{-1}$ and 1159 $cm^{-}$1) deformation, also the C-N stretching at 1463 $cm^{-1}$. Based on this result, it is possible to conclude that the incorporation of urea in the synthesis by inverse vulcanization does not alter the chemical structure of the biopolymer and confirm the presence of urea encapsulated in the matrix of the biopolymer.

# Analysis of Macroscopic parameters

The macroscopic analysis of the *Allium* test was performed descriptively, focusing on the number, length and appearance of the roots (Fig. 3). The parameters were measured at the start of the experiment (t = 0 h) and after 72 hours.

The root number between 0 and 72 hours is show in the Fig. 3A. The change in number of roots, obtained from Fig. 3A, is as follows: for PC, an increase of + 8.75%; for NC, a decrease of -2.6%; and for treatments 1, 2, and 3, increases of + 4%, + 19%, and + 63%, respectively. These results indicate that all treatments, except NC, led to an increase in root number. In the analysis of root length, the mean root length was calculated for each treatment (Fig. 3B). For PC, a decrease in the mean root length was observed. However, this analysis must be interpreted in the context of the number of the roots. Based on this, it can be concluded that while the mean root length decreases over time, this reduction in the average is due to the presence of new roots (+ 8.75%), which lowers the overall mean. For NC and p-SF-79U, the mean root length did not show significant changes after 72 hours. In NC, there was a 2.6% decrease in number of roots, indicating that the roots present at 72 hours were the original ones from t = 0 and had not increased in length. In contrast, p-SF-79Ushowed a 19% increase in number of roots while maintaining the mean root length, suggesting that the new roots had reached a significant size. The analysis of p-SF-79U and and p-SF-92U revealed that both treatments resulted in a higher number of roots (4% and 63%, respectively). However, the mean root length for both treatments decreased. For p-SF (biopolymer alone), the decrease in mean root length and the limited growth of new roots suggest that the treatment did not significantly influence these parameters. In contrast, for p-SF-79U, despite the decrease in mean root length, the 63% increase in number of roots indicates that the treatment strongly promotes root growth.

The visual analysis of the roots, the positive control presents a weaker and thinner appearance compared to those in the NC group. Moreover, the roots of the bulbs treated with the biopolymer p-SF and the biocomposites p-SF-79U showed no signs of root length inhibition. On the contrary, for p-SF-79U a clear increase in both roots number and length was observed. Moreover, any color change was observed and the roots displayed, healthy, hardened, and turgid appearance. These findings are promising, as the macroscopic analysis suggests that treatments with p-SF, biocomposites p-SF-79U and p-SF-92U do not induce general cellular toxicity and promote more and healthy roots.

However, only a macroscopic analysis is insufficient to confirm the safety and suitability of these materials for agricultural use. Therefore, a complementary microscopic analysis is required to assess the potential genotoxic effects of the materials.

# Genotoxic analysis

The genotoxic effect of p-SF, p-SF-92 and p-SF-79 on eukaryotic cells of onions was analyzed by calculating the % MI and % CA whose values are shown in Table 1. The term genotoxic refers to agents that induce DNA damage. Chromosomal aberrations that can be classified as genotoxic include C-metaphases, polyploid metaphases, metaphases with adhesions, metaphases exhibiting chromosomal loss, as well as anaphases and telophases characterized by losses, delays, and chromosomal bridges. In addition to chromosomal aberrations, nuclear abnormalities such as lobulated nuclei, nuclei with buds, and multinucleated cells can also be considered endpoints of genotoxicity (Souza et al. 2013).

Table 1
Mitotic index (% MI) and chromosomal aberrations (% CA) in *Allium cepa* meristematic cells after 72 h exposure to distilled water (negative control, NC), $H_2O_2$ (positive control, PC), biopolymer (p-SFand its urea biocomposites (p-SF-79U; p-SF-92U). Values expressed as presented as mean ± SD (n = 6 bulbs per treatment; 1500 cells per bulb).

| Treatment | Nº total cells | Nº dividing cells | Nº Aberrantes cells | %MI (Mean ± SD) | %CA (Mean ± SD) |
|---|---|---|---|---|---|
| NC | 1500 | 89 | 55 | 5.93 ± 0.04[a] | 3.67 ± 0.02[a] |
| PC | 1500 | 0 | 177 | 0 ± 0.0 [b] | 11.80 ± 0.05[b] |
| p-SF | 1500 | 97 | 44 | 6.47 ± 0.05[a] | 2.93 ± 0.03[a] |
| p-SF-79U | 1500 | 64 | 32 | 4.27 ± 0.05 [c] | 2.13 ± 0.04 [a] |
| p-SF-92U | 1500 | 100 | 36 | 6.66 ± 0.03 [a] | 2.40 ± 0.05 [a] |

Different letters in the same column indicate statistically significant differences between treatments ($p < 0.05$; ANOVA followed by Tukey multiple comparison test).

The results of mitotic index (%IM) values for each treatment are presented in Table 1. Notably, the %IM value for the positive control (PC) is zero, while the other treatments display non-zero values. This behavior indicates that $H_2O_2$ administration inhibits root growth, manifesting as a disruption in cell division. Evidently, the %IM for T3 and p-SF are nearly similar to that of NC, while the %MI value for p-SF-79U is lower than that of NC. The %MI is an indicator of cell proliferation whose decrease or increase is good toxicity biomarkers. In contrast, the %CA value for PC is 11.80, significantly higher than those observed in the other treatments. Additionally, the %CA value for NC exceeds those recorded for p-SF, p-SF-79U, and p-SF-92U. This result suggests that p-SF, p-SF-92U, and p-SF-79U promote cellular development, reduce the cellular deformations and aberrations.

Figures 4 and 5 illustrate the phases of mitosis: prophase, metaphase, anaphase, and telophase across each treatment. In Fig. 4, which corresponds to the PC microscopy, the eukaryotic cells of *Allium cepa* exhibit significant deformities and aberrations, such as nuclear disintegration, micronucleus, and nuclear budding additionally, also, none of the cells are observed in the reproductive phase (mitosis). Pandir *et al.* (Pandır et al. 2018) established that $H_2O_2$ induced toxicity results in cell death and chromosomal aberrations in the meristematic root cells of *Allium* cepa. The inhibition of the number of root growth was found to be dependent on decrease of % mitotic index.

In contrast, the microscopy images of the eukaryotic cells from the negative control, as well as treatments 1, 2, and 3 (Figs. 5A) to D)), display predominantly normal morphology. In these cases, all phases of mitosis are present, indicating normal cell division.

The increasing demand for sustainable and environmentally safe materials in agriculture has driven the development of novel encapsulation matrices derived from renewable resources. In this context, the present study contributes to the field of environmental sciences by evaluating the cytotoxic and genotoxic potential of biopolymers and biocomposites synthesized via inverse vulcanization, using elemental sulfur and sunflower oil, both abundant, low-cost, and eco-friendly raw materials.

The results provide strong evidence of the biocompatibility and non-toxic nature of the evaluated materials, supporting their safe application as encapsulation matrices for agronomic applications. Their use holds significant potential not only to enhance soil fertility and crop productivity but also to reduce the environmental contamination typically associated with conventional agricultural inputs. Furthermore, this work demonstrates the importance of reliable toxicity screening in the development of new biomaterials, by integrating materials science, plant biology, and environmental toxicology, the study presents a robust model for advancing sustainable agricultural practices and promoting the responsible use of bio-based technologies. The biocomposites examined here offer an eco-friendly alternative that can help reduce soil and water pollution, improve soil health, and contribute to the long-term sustainability of ecosystems.

In summary, this research validates the safety and environmental compatibility of sulfur-based biocomposites and underscores the critical importance of thorough toxicological evaluations assessments supporting their practical application. These findings support the adoption of innovative, renewable, and safe materials in agriculture, contributing to the broader goal of ecosystem protection and sustainable development. Moreover, the results demonstrate that the *Allium cepa assay* is an effective, low-cost, and straightforward method for assessing both the cytotoxicity and genotoxicity of such materials, making it a valuable tool for preliminary environmental risk assessment in agricultural applications.

Taken together, these findings provide preliminary nonetheless valuable evidence that sulfur–vegetable oil biopolymers and their urea composites may represent safe and efficient carriers for controlled nitrogen release in sustainable agriculture. The absence of detectable cytogenotoxic effects in *A. cepa* highlights their promise as environmentally compatible materials. Nevertheless, the present study was limited to a single bioindicator species, a short-term (72 h) exposure, and discrete treatment concentrations, which restricts the extrapolation of results to complex field conditions. To rigorously establish their environmental safety and long-term agronomic viability, future work will prioritize extended exposure assays under realistic soil conditions, together with evaluations in maize and other relevant crops, thereby addressing both ecological risks and agronomic performance within the framework of sustainable nutrient management.

# Conclusion

The growing demand for environmentally safe and efficient encapsulation matrices in agriculture highlights the importance of evaluating their potential cytotoxic and genotoxic effects to ensure their safe application. In this study, biopolymers and biocomposites synthesized via inverse vulcanization were assessed using the *Allium* cepa assay to evaluate DNA damage, chromosomal aberrations, and disruptions to the mitotic cycle.

The materials were successfully synthesized, and their chemical structure, including effective urea incorporation, was confirmed by Fourier-transform infrared spectroscopy (FTIR). The *Allium* cepa assay revealed that, in contrast to the positive control ($H_2O_2$), which induced significant chromosomal abnormalities, the biopolymeric matrix (p-SF) and urea-containing biocomposites (p-SF-79U and p-SF-92U) did not produce cytotoxic or genotoxic effects. All mitotic phases observed in the negative control were also present in the treated samples, indicating normal cell division. Additionally, the percentage of chromosomal aberrations (%CA) was low in treated groups, suggesting that these materials may even promote normal cellular development by reducing the occurrence of abnormal or deformed cells.

These findings confirm the biocompatibility of p-SF and its urea based derivatives, providing a strong foundation for their safe use in agricultural applications. Furthermore, the results demonstrate the effectiveness of the *Allium* cepa assay as a low-cost, sensitive, and reliable method for preliminary screening of the toxicological properties of polymer based materials proposed for their real-world agricultural implementation. In conclusion, this study supports the potential of this kind of biocomposites as viable and eco-friendly alternatives for soil fertilization, contributing to the advancement of sustainable agriculture. The use of the *Allium* cepa test not only confirms the non-toxic nature of these materials but also offers a practical approach to assessing the safety of novel biopolymers synthesized through inverse vulcanization. Also, this approach provides valuable insights into the safety and efficacy of these materials for agricultural use and underscores the crucial role of toxicological assessments in developing renewable, sustainable technologies that enhance productivity while minimizing environmental impact.

# Declarations

### Ethical approval

This is not applicable.

### Consent to participate

This is not applicable.

### Consent for publication

This is not applicable.

#### Competing interests

The author declares no competing interests.


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# Author contribution

A.S. Farioli: Visualization, Methodology, Data curation, Investigation and Formal analysis. M. Fernandez: Visualization, Formal analysis and Writing – review & editing. P.C. Cavallo: Writing – review & editing, Data curation and Formal analysis. D.F. Acevedo: Writing – review & editing, Funding acquisition, Project administration, Formal analysis. E. I Yslas: Conceptualization, Supervision, Resources, Investigation, Funding acquisition, Project administration, Formal analysis, Writing – original draft and Writing – review and editing.


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# Data availability

The datasets used and analyzed during the current study will be available from the corresponding author on reasonable request.

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# Figures

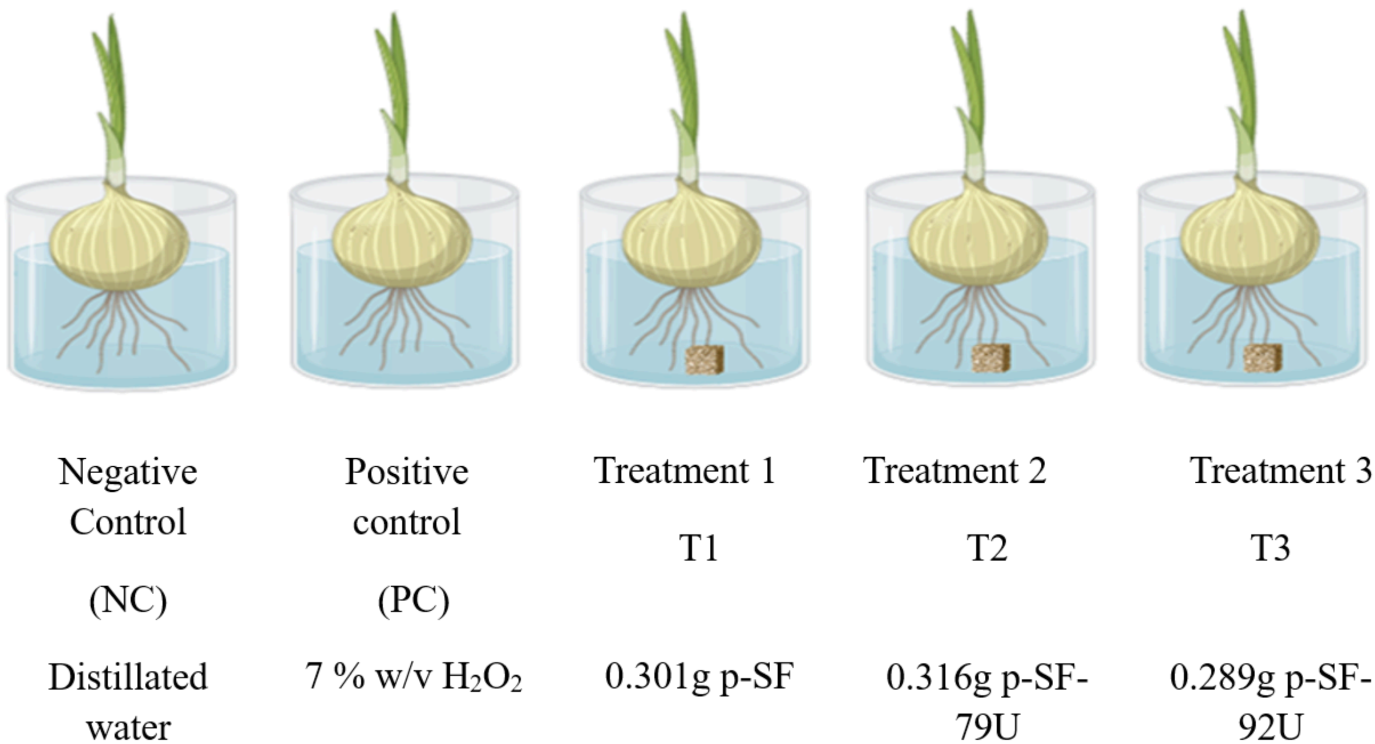



**Figure 1**

*Allium cepa* bioassay scheme.

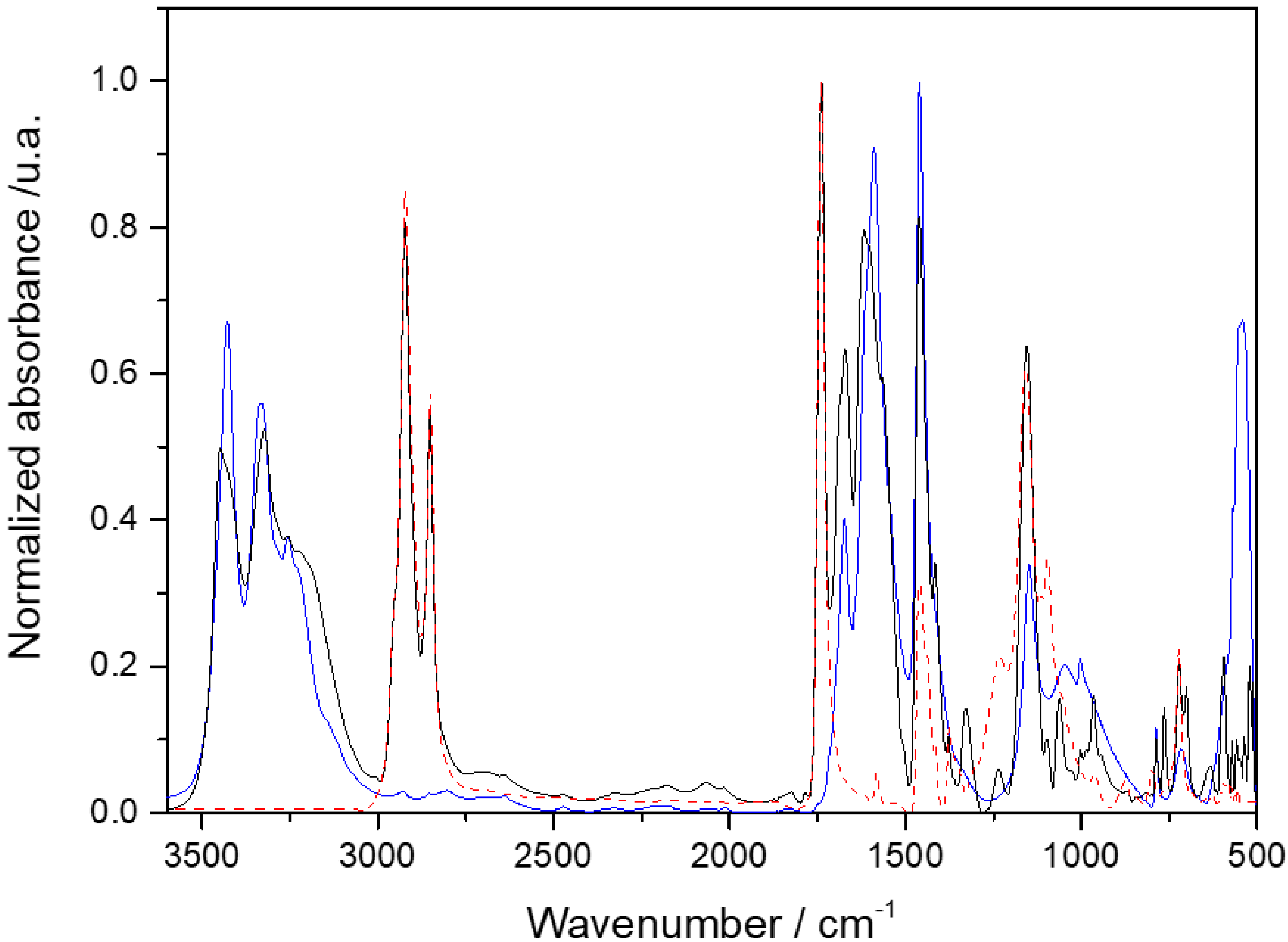
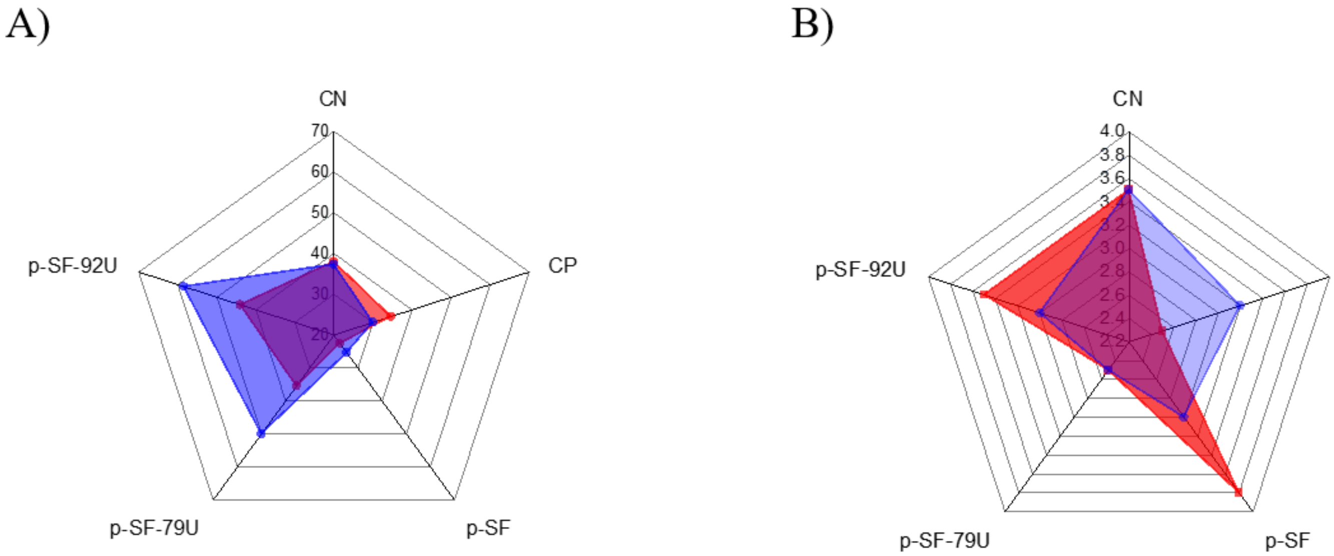


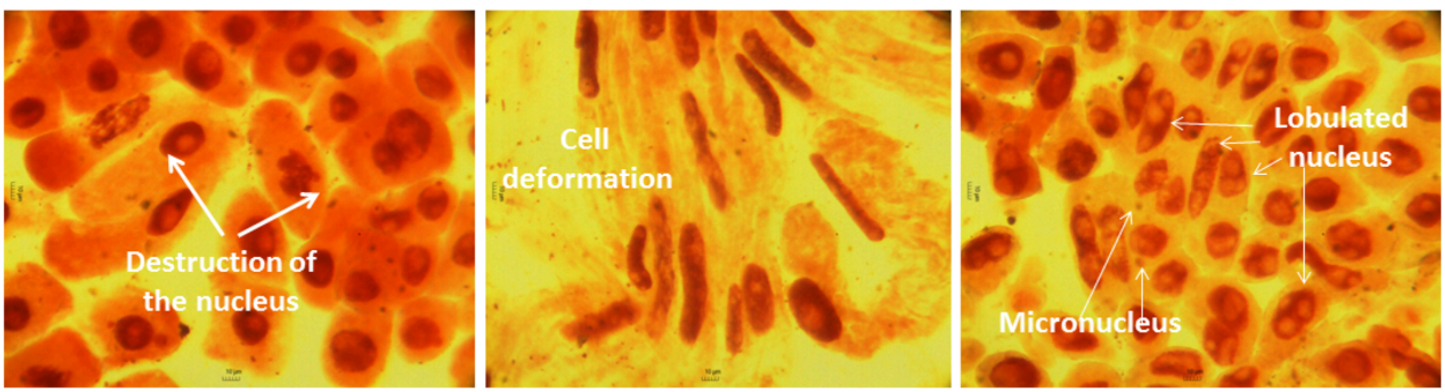

**Figure 2**

FTIR spectra of biopolymer p-SF (red-dot), urea (blue) and its urea-loaded composites p-SF-92U (black).



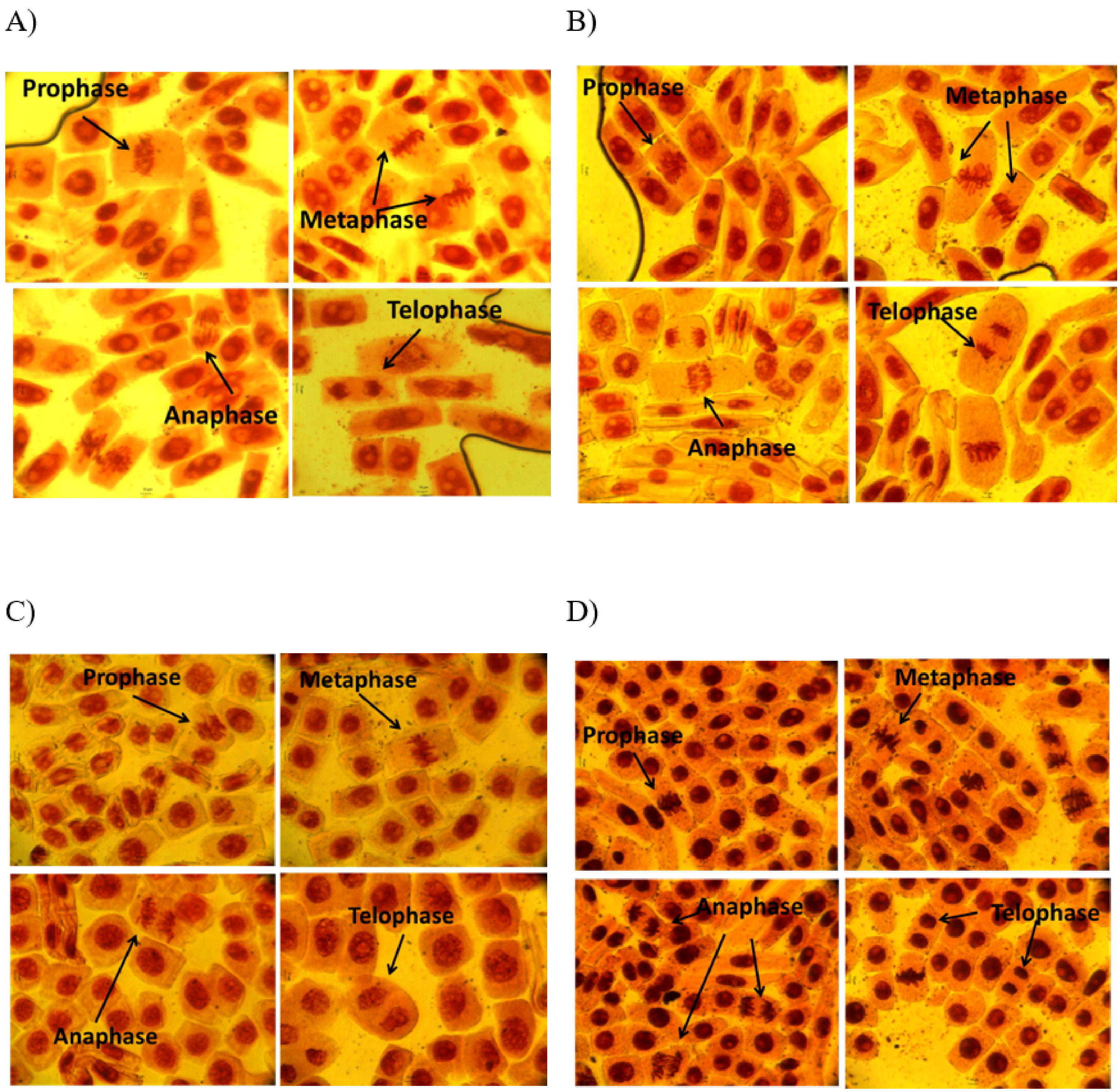

## Figure 3

Effect of treatments, p-SF, p-SF-79U, p-SF-92U, CP and CN on root growth of *Allium cepa* at time 0 hours (red) and at time 72 h (blue) **A)** Root number, **B)** Root length in centimeters.




## Figure 4

Microscopy images of eukaryotic cells of *Allium cepa* from positive control.




## Figure 5

Microscopy images of eukaryotic cells of *Allium cepa* from treatment: A) NC, B) p-SF, C) p-SF-79U and, D) p-SF-92U.