CIÊNCIAeNATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, v. 46, e84266, 2024 • https://doi.org/10.5902/2179460X84266
Submissão: 28/06/2023 • Aprovação: 30/06/2023 • Publicação: 10/04/2024

Environment

Graphene oxide assessment on the germination of persian clover and buckwheat seeds

Avaliação do óxido de grafeno na germinação de sementes de trevo-persa e trigo mourisco

Raquel Stefanello[I], Wagner Jesus da Silva Garcia[I], Theodoro da Rosa Salles[II], Cristiano Rodrigo Bohn Rhoden[II]

[I] Universidade Federal de Santa Maria, Santa Maria, RS, Brazil
[II] Franciscan University, Santa Maria, RS, Brazil

ABSTRACT

The increase in the use of graphene oxide (GO) allows different studies in several fields, and raise concerns about its possible toxic effect on the environment, especially in the early growth of plants. Thus, this study aimed to evaluate the effect of GO on the germination of Persian clover and buckwheat seeds. The seeds were placed on germitest paper in different concentrations of graphene oxide (0, 125, 250, and 500 mg L^{-1}) and kept in a germination chamber at 20 °C (photoperiod of 12 hours). The evaluated parameters were seed germination and seedling growth (length and dry mass). Graphene oxide did not show toxic effects on seed germination and initial growth of both species up to 500 mg L^{-1}. In this context, understanding the role of graphene oxide in the germination process and the development of plants will be able to contribute positively to understanding its possible environmental impacts when used in large quantities in ecosystems.

Keywords: *Fagopyrum esculentum* moench; Graphene; Germination process; Nanomaterial; Toxicity; *Trifolium resupinatum* L

RESUMO

O aumento do uso do óxido de grafeno (GO), permite diversos estudos em diversas áreas, e levanta preocupações sobre seu possível efeito tóxico ao meio ambiente, principalmente no início do crescimento das plantas. Assim, este trabalho teve como objetivo avaliar o efeito do GO na germinação de sementes de trevo persa e trigo mourisco. As sementes foram colocadas sobre papel germitest em diferentes concentrações de óxido de grafeno (0, 125, 250 e 500 mg L^{-1}) e mantidas em câmara de germinação a 20 °C (fotoperíodo de 12 horas). Os parâmetros avaliados foram a germinação das sementes e o crescimento das plântulas (comprimento e massa seca). O óxido de grafeno não apresentou efeitos tóxicos na germinação de sementes e no crescimento inicial de ambas as espécies até 500 mg L^{-1}. Nesse contexto, compreender o papel do óxido de grafeno no processo de germinação e desenvolvimento das plantas poderá contribuir positivamente para o entendimento de seus possíveis impactos ambientais quando utilizado em grandes quantidades nos ecossistemas.

Palavras-chave: *Fagopyrum esculentum* moench; Grafeno; Processo germinativo; Nanomaterial; Toxicidade; *Trifolium resupinatum* L

1 INTRODUCTION

Graphene-based nanomaterials, such as graphene oxide (GO) and reduced GO (rGO), exhibit excellent physicochemical and structural properties, such as good water solubility, low toxicity, and a high specific surface area (Oliveira *et al.*, 2023). This class of nanomaterials is receiving great attention due to their applications in biomedicine, biotechnology, drug delivery, infection treatment, medical device coating, packaging, biosensors, energy storage, catalysis, and environmental remediation (Choudhary & Das, 2019; Priyadharshini *et al.*, 2022). In addition, they have been used in water remediation due to their high adsorption capacity for different pollutants, including heavy metals, pharmaceuticals, pesticides, oil spills, salts, chlorophenols, phenols, and microplastics (Ahamed & Loganathan, 2023; Nasiri, Ahmadzadeh, & Amiri, 2021; Nunes, Bruckmann, Salles, & Rhoden, 2023; Diraki, Mackey, McKay, & Abdala, 2019; Kaymak, Sevim, & Metin, 2022; Abu-Nada, Abdala, & McKay, 2021; Karamipour, Fathi, & Safari, 2021; Salles *et al.*, 2023; Sun, Wang, Zheng, Chen, & Li, 2021).

Several reports employing GO showed significant improvement in plant growth, aroused favorable interest in its possible applications in agriculture, positively influencing developmental stages such as seed germination, plant physiological and biochemical responses and root and shoot growth (Zhang, Gao, Chen, & Li, 2015; González-García *et al*., 2019; Park, Choi, Kim, Gwon, & Kim, 2020; Samadi, Lajayer, Moghiseh, & Rodríguez-Couto, 2021; Guo *et al.*, 2021; Yang *et al.*, 2022; Zhao *et al.*, 2023; Kazlauskas *et al.*, 2023). Furthermore, GO it can act as a water carrier to promote seed germination due to its hydrophilic nature (He *et* *al.*, 2018). Some studies even suggest that GO can be used as a fertilizer carrier to reduce the release rate, resulting in greater nutrient efficiency and, therefore, reducing the application of agrochemicals (Bhattacharya, Cahill, Yang, & Kochar, 2022).

On the other hand, previous research indicates that physicochemical properties, exposure time, size, number of layers, mode of application, and genotype play an important role in determining GO toxicity's fate in plants (Fadeel *et al.*, 2018; Yang *et al.*, 2022). However, the most important factor from a toxicological point of view is the dose/concentration of GO applied (Chen, Mu, & Tian, 2019). In some species, GO in high concentrations can have a negative impact on shoot length, root development, fresh root biomass, and shoot dry weight, inducing oxidative stress, decreasing nutritional level, and compromising structural development (Song, Cao, Duan, Luo, & Cui, 2020; Zhang, Gao, Chen, & Li, 2020; Zhu, Weng, Zhang, Liu, & Du, 2022; Zhao *et al.*, 2023).

In recent years, the release of graphene-based nanomaterials into the environment has raised concerns about their potential biological and environmental risks (Zhao, Zhu, Mou, Wang, & Duo, 2022; Yang *et al.*, 2022). With the growing application of GO in biomedicine and industry, the possible implications for animals, microorganisms, and human health have been frequently studied; however, the effects of the nanomaterial on plants, especially in the early stages of development, are rarely considered, justifying the need for prior studies before its use in the agricultural environment (Yin, Wang, Wang, Xu, & Bao, 2018). This study aimed to evaluate the effect of nanomaterials such as graphene oxide on seed germination of forage species of relevant economic importance, such as Persian clover (*Trifolium resupinatum*) and buckwheat (*Fagopyrum esculentum*).

2 MATERIAL AND METHODS

2.1 Plant material and synthesis of graphene oxide (GO)

To conduct the experiment, seeds of Persian clover (*Trifolium resupinatum*), cultivar Lightning, and buckwheat (*Fagopyrum esculentum*), cultivar ALTAR 92, acquired from traditional companies selling seeds, were applied.

2.2 Synthesis and characterization of graphene oxide (GO)

The GO synthesis followed the methodology of Salles *et al.* (2020): 1 g of flake graphite (Sigma-Aldrich®) was added to a 60 mL of 98% H_2SO_4 (Synth®) under magnetic stirring for 10 min at room temperature. Successively, 6 g of $KMnO_4$ (Synth®) were added (during 20 min). The resulting solution was heated to 40 °C and kept under stirring for 5 h. Sequentially, at room temperature, distilled water (180 mL) was added slowly and under stirring for 12 h. Next, the reaction was heated at 40 °C for 2 h, and 300 mL of distilled water and 10 mL of hydrogen peroxide (Synth®) were added. The yellow solution was washed to pH 7.0 and dried in an oven (DeLeo) at 50 ºC for 7 days. Finally, 1.5 g of final product was dispersed in 1000 mL of distilled water, resulting in a concentration of 1.5 g L^{-1}.

GO was characterized by different techniques. Crystallinity was determined by Raman spectroscopy (Renishaw inVia spectrometer system) and X-ray diffraction (Bruker diffractometer, model D2 Phaser). Functional groups were established using Fourier Transform Spectroscopy (Perkin-Elmer, Spectro One model), and the morphology was analyzed in scanning electron microscopy (Zeiss Sigma 300 VP).

2.3 Experiments for seed germination

To evaluate the effect of nanomaterials (GO) on germination, concentrations of 0 (only distilled water), 125, 250, and 500 mg L^{-1} were used. In the first stage, pelleted Persian clover seeds were sown on germitest paper only moistened with different concentrations of GO, following a methodology adapted from Gao, Zhang e Liu (2019). In the second stage, the buckwheat seeds were soaked for 24 hours in the dark at room temperature in different concentrations of GO, according to the methodology adapted from Kaymak *et al.* (2022). After conditioning with GO, the seeds were dried at room temperature in the laboratory (24 hours). This experiment aimed to evaluate the effect of GO, not the methodologies.

In both experiments, four repetitions of 50 seeds were distributed on germitest paper, in gerbox boxes (Persian clover) or rolls (buckwheat) that were stored in a germination chamber (BOD - Biochemical Oxygen Demand) at a temperature of 20 °C and 12-hour photoperiod. The count of normal seedlings was performed according to Ministério da Agricultura, Pecuária e Abastecimento (2009) on the 7th day after sowing. To evaluate the length and dry mass of the seedlings, the Krzyzanowski, França-Neto, Gomes-Junior e Nakagawa (2020) methodology was followed.

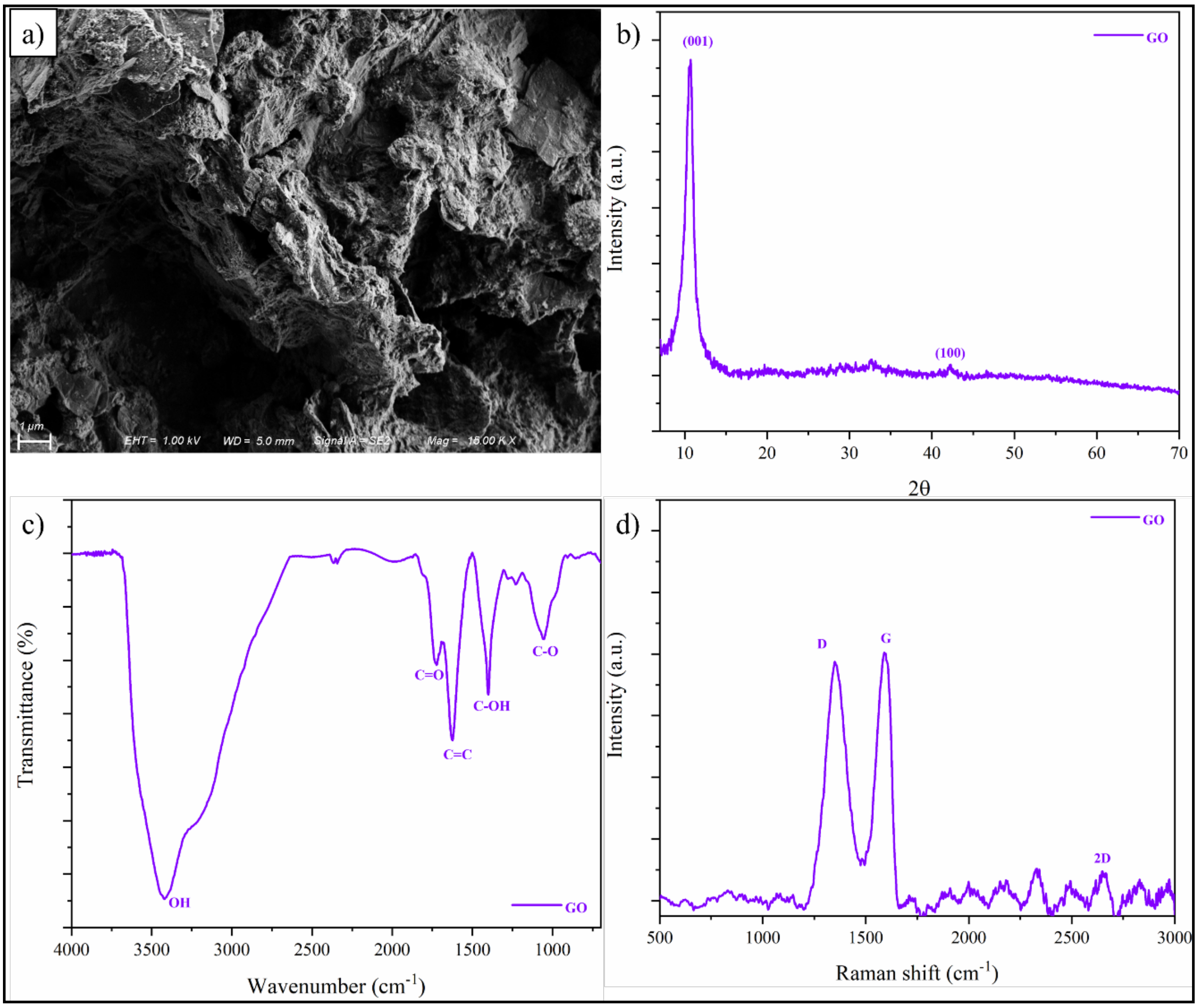
The test was in a completely randomized design, with treatments consisting of different concentrations of graphene oxide and data analyzed using the Sisvar software (version 5.6).

3 RESULTS AND DISCUSSION

The characterization of graphene oxide by different techniques (SEM, XRD, FTIR, and Raman) are presented in Figure 1. According to the SEM results (Figure 1a), the morphological of GO demonstrated a wrinkled sheet, suggesting a material with few layers (Hatel, Majdoub, Bakour, Khenfouch, & Baitoul, 2018). The XRD data (Figure 1b) reveals a signal at $2\theta \approx 10.50^o$ (001), characteristic of GO (Oliveira *et al.*, 2023). The absence of peak around $2\theta \approx 26.0^o$ correspond to the complete oxidation of precursor material (Vargas *et al.*, 2023). The signal at $2\theta \approx 43^o$ interfere that the nanomaterial presents a small distance between layers (Salles *et al.*, 2020).

Also, FTIR results can be seen in Figure 1c. The intense band around 3400 cm^{-1} corresponds to the stretching vibration of OH groups (Bruckmann *et al.*, 2022). Other signals are attributed to the C=O stretch vibrations (1734.02 cm^{-1}), C=C bonds (1650.72 cm^{-1}), C-OH (1342.22 cm^{-1}), and C-O (1050 cm^{-1}), characteristic of graphene oxide (Oliveira *et al*., 2023). Raman spectroscopy partner (Figure 1d) reveals three peaks: D, G, and 2D, corresponding respectively to defects and disorder of graphite structure, sp^2 vibration by carbon atoms, and second order of D band (Kellici *et al.*, 2014). In addition, the I_D/I_G reveals a value of 0.97, which suggest a GO with a high-quality structure (Rhoden, Bruckmann, Salles, Kaufmann, & Mortari, 2021).

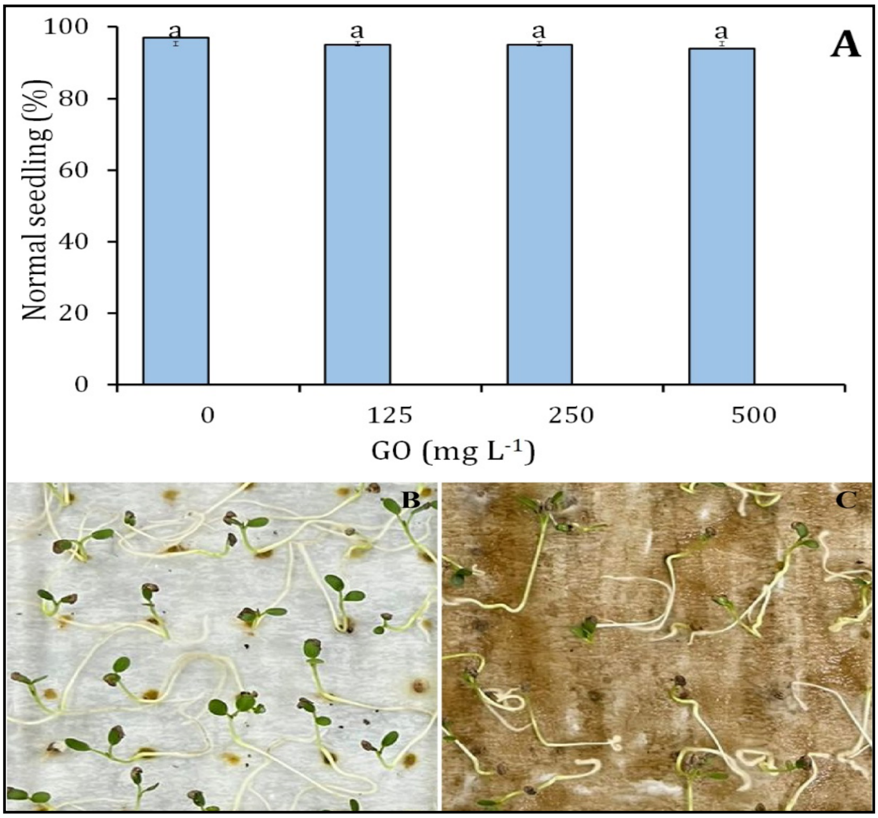
Figure 1 – SEM (a), XRD (b), FTIR (c) and Raman spectroscopy (d) of graphene oxide



Source: Authors (2023)

Through data analysis, it is possible to observe that in the absence of GO (control), Persian clover seeds presented, on average, 97% of normal seedlings in the germination test (Figure 2A), with no significant reduction in the percentage of normal seedlings at the highest concentration used - 500 mg L^{-1} – (Figures 2B and 2C).

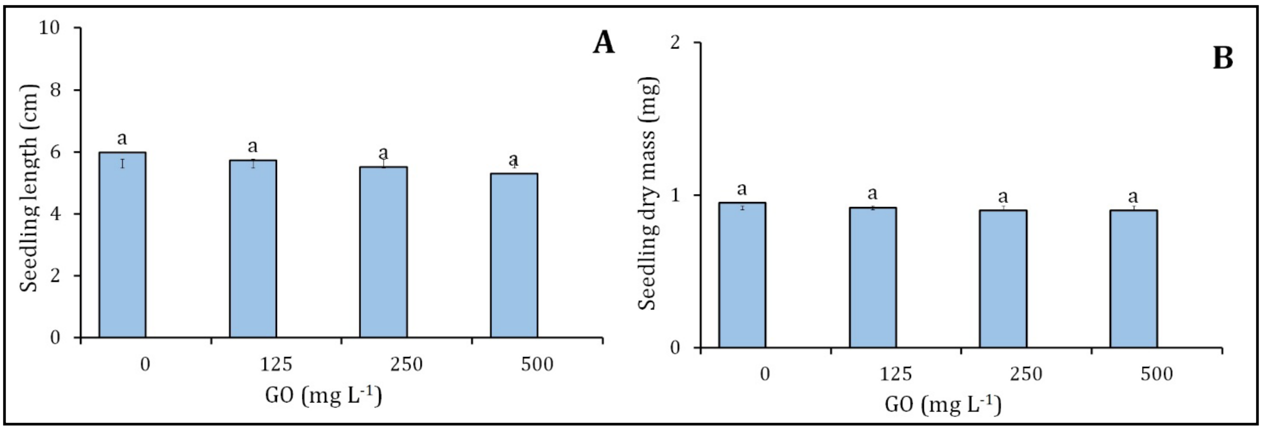
Figure 2 – Germination of Persian clover seeds (A) submitted to different concentrations of graphene oxide (GO). Absence of GO (B) and 500 mg L^{-1} (C)



Source: Authors (2023)

Furthermore, the total length and dry mass of Persian clover seedlings did not differ significantly, with values ranging from 5.97 cm to 5.29 cm (Figure 3A) and 0.95 mg to 0.90 mg (Figure 3B), respectively.

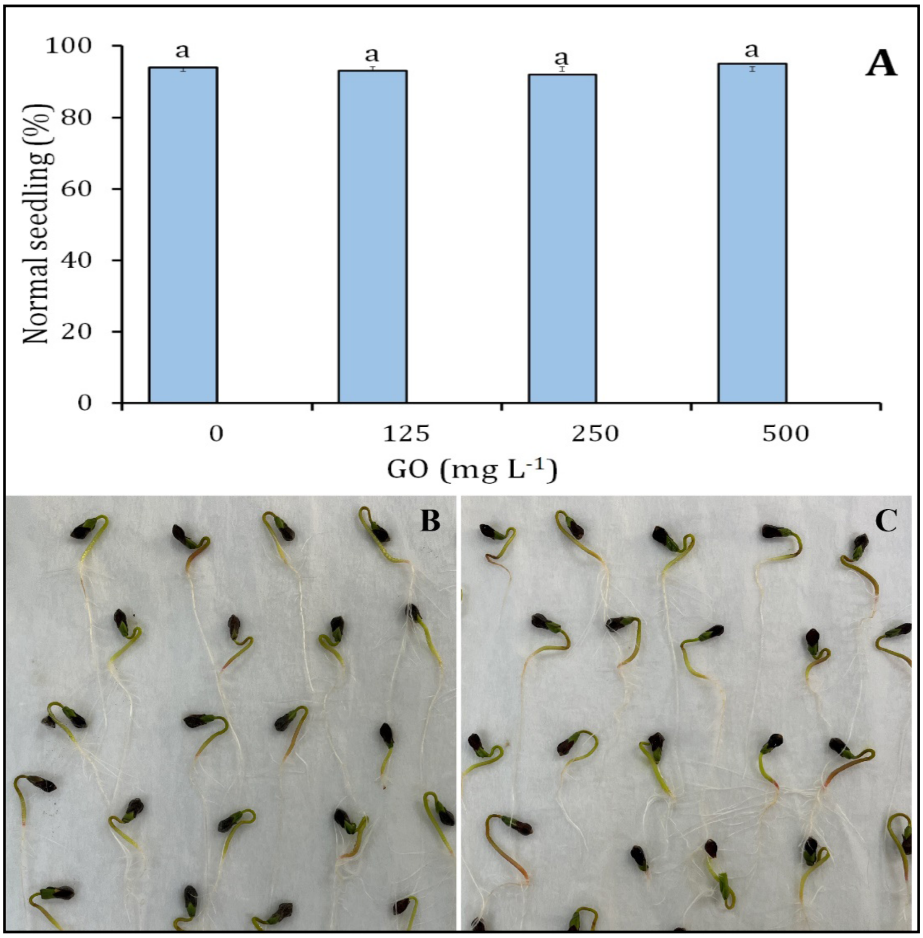
Figure 3 – Total length (A) and dry mass (B) of Persian clover seedlings submitted to different concentrations of graphene oxide (GO)



Source: Authors (2023)

Similarly, buckwheat seeds soaked in GO for 24 hours showed no difference in germination percentage at the tested concentrations (Figures 4A-C).

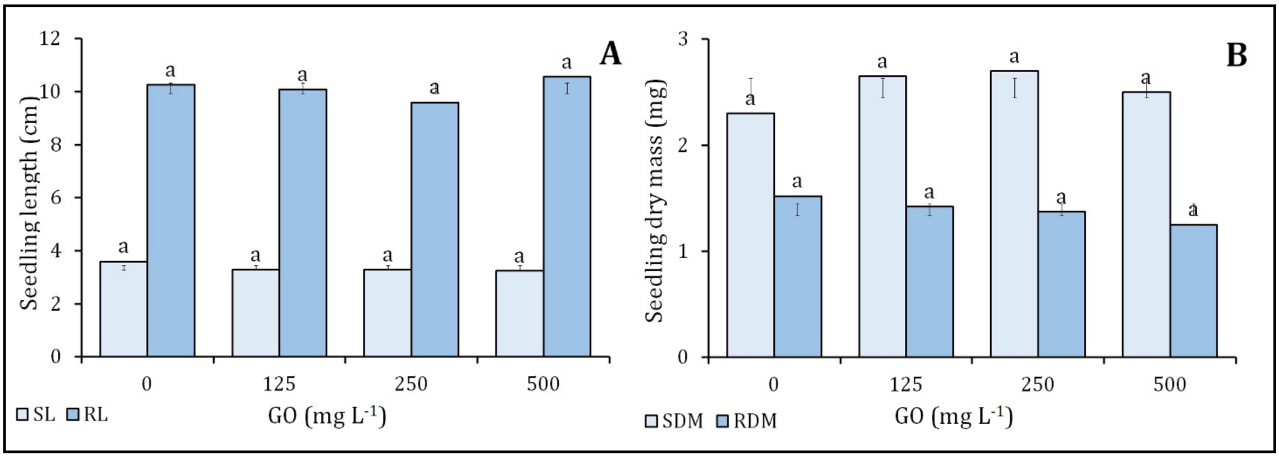
Figure 4 – Germination of buckwheat seeds (A) submitted to different concentrations of graphene oxide (GO). Absence of GO (B) and 500 mg L^{-1} (C)



Source: Authors (2023).

Likewise, the initial growth (shoot and root) and dry mass (shoot and root) of buckwheat seedlings were not influenced by different concentrations of graphene oxide (Figure 5A and B).

Figure 5 – Shoot length (SL) and root length (RL) (A) and shoot dry mass (SDM), and root dry mass (RDM) (B) of buckwheat seedlings submitted to different concentrations of graphene oxide (GO)



Source: Authors (2023)

During the germination process, graphene can penetrate the seed coat, causing its rupture, facilitating the absorption of water, thus resulting in the stimulation of germination and root elongation (Zhang *et al.*, 2015; Samadi *et al.*, 2021). In addition, GO, when present, penetrates the seed coat, retaining more water than the seeds of the treatment control (Zhang *et al.*, 2015).

The positive effects of graphene oxide (100, 1000, and 10000 mg L^{-1}) on plant growth were observed by Park *et al.* (2020) in watermelon and *Arabidopsis thaliana* L., where GO favored root length, number of leaves, and flower buds, and can therefore, be used as a regulatory tool to promote the growth of these plants. Likewise, González-García *et al.* (2019) reported a positive effect of graphene on optimizing the absorption of water and nutrients; activating the synthesis of abscisic and indole acetic acids; promoting the elongation of the cell wall, and the expression of genes responsible for cell division. In addition, the increasing in the enzyme activity and the accumulation of proteins, consequently, relaying in greater growth.

Similar results were observed by Kazlauskas *et al.* (2023), where the germination of *Lepidium sativum* L. seeds was not affected by the GO doses tested

(1-80 mg L^{-1}). Guo *et al.* (2021), also reported a study on cotton (*Gossypium hirsutum* L.) germination, in addition to positive effects verified on alfalfa (*Medicago sativa* L.) (Zhao *et al.*, 2023).

In contrast, high concentrations of GO (>100 mg L^{-1}) decreased the length, volume, and dry mass of the root of seedlings of *Larix olgensi* A. Henry (Song *et al.*, 2020) and, inhibiting seed germination in wheat (1000-2000 μg mL^{-1}) (Vochita *et al.*, 2019). Additionally, the exposure of white clover (*Trifolium repens* L.) to GO showed harmful effects on seedling growth and nutrient absorption by shoots, with a more significant effect being verified with increasing exposure time and concentration (Zhao *et al.*, 2022). Thus, for some species, high concentrations of GO can cause toxic effects, such as the destruction of the root cell structure, the inhibition of cell division, and the prevention of root growth (Chen, Yang, Li, & Ding, 2018).

With the widespread of GO applications, it is inevitably, in some way, the nature discard, intentionally or not, with adverse effects on ecosystems and human health (Goodwin *et al.*, 2018). Therefore, for agricultural research, it is essential to clarify the potential toxicity of GO and the related response of plants before applying large amounts of graphene oxide in production fields. Considering the results of this study on germination and initial growth of Persian clover and buckwheat, it is possible to affirm that concentrations of GO up to 500 mg L^{-1} did not have a toxic effect on seed germination, corroborating that its positive or negative effect depends on the concentration and species studied (Yang *et al.*, 2022). Understanding the role of graphene oxide in the germination process and plant development may contribute to understanding its possible environmental impacts in ecosystems when used in large quantities.

4 CONCLUSIONS

This work reported that graphene oxide did not show toxic effects on seed germination and initial seedling growth of Persian clover and buckwheat up to 500 mg

L^{-1}. Understanding the role of graphene oxide in the germination process and plant development may contribute to comprehending its possible environmental impacts in ecosystems and stimulating further studies using large quantities of GO increasing production with higher efficiency and environmental responsibility.

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Authorship contributions

1 – Raquel Stefanello

Biologist, PhD in Agronomy

https://orcid.org/0000-0003-3079-2099 • raquelstefanello@yahoo.com.br

Contribution: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization ,Writing – original draft, Writing – review & editing

2 – Wagner Jesus da Silva Garcia

Physicist, PhD in Physics

https://orcid.org/0000-0003-4782-2741 • wagnerjgarcia@hotmail.com

Contribution: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing

3 – Theodoro da Rosa Salles

Graduating in Chemical Engineering.

https://orcid.org/0000-0003-2157-2289 • theodoro.rsalles@gmail.com

Contribution: Methodology, Visualization, Writing – original draft, Writing – review & editing

4 – Cristiano Rodrigo Bohn Rhoden

Master’s degree in Chemistry and PhD in Nanosciences

https://orcid.org/0000-0001-6171-4224 • cristianorbr@ufn.edu.br

Contribution: Conceptualization, Visualization, Writing – original draft, Writing – review & editing

How to quote this article

Stefanello, R.; Garcia, W. J. da S.; Salles, T. da R.; Rhoden, C. R. B.. Graphene oxide assessment on the germination of persian clover and buckwheat seeds. Ciência e Natura, Santa Maria, v. 46, 2024. DOI: https://doi.org/10.5902/2179460x84266.