SCIENTIA PLENA VOL. 20, NUM. 04 2024
www.scientiaplena.org.br doi: 10.14808/sci.plena.2024.040201

Assessment of graphene oxide as an attenuator of the toxic effect of aluminum on Persian clover seed germination

Avaliação do óxido de grafeno como atenuador do efeito tóxico do alumínio na germinação de sementes de trevo persa

R. Stefanello[1]*; W. J. S. Garcia[2]; T. R. Salles[3]; C. R. B. Rhoden[3]

[1]*Departamento de Biologia, Universidade Federal de Santa Maria, 97105-900, Santa Maria-RS, Brasil*
[2]*Departamento de Física, Universidade Federal de Santa Maria, 97105-900, Santa Maria-RS, Brasil*
[3]*Laboratório de Materiais Magnéticos Nanoestruturados, Universidade Franciscana, 97010-491, Santa Maria-RS, Brasil*

**raquelstefanello@yahoo.com.br*
(Recebido em 20 de março de 2023; aceito em 16 de abril de 2024)

The aim of this study was to analyze the effect of graphene oxide on the germination of Persian clover seeds and its possible attenuating effect on aluminum stress. The seeds were sown on substrate paper and tested at varying concentrations of graphene oxide, aluminum and/or graphene oxide + aluminum concomitantly. They were then stored in a germination chamber at a temperature of 20 °C with a photoperiod of 12 h. The nanomaterials were characterized by Raman spectroscopy, X-ray diffraction, Fourier transform spectroscopy, and scanning electron microscopic. Seed germination and growth parameters (length and dry mass) of seedlings were evaluated. The nanomaterial did not cause adverse effects on Persian clover seed germination. However, the addition of aluminum above 125 mg L^{-1} was found to be harmful resulting in a decrease in germination, total and root length, and seedling dry mass. Nevertheless, simultaneous exposure to graphene oxide/aluminum, did not affect the initial growth nor the germination parameters of the seeds.
Keywords: graphene, germination process, *Trifolium resupinatum* L.

O objetivo deste estudo foi analisar o efeito do óxido de grafeno na germinação de sementes de trevo Persa e seu possível efeito atenuante no estresse por alumínio. As sementes foram semeadas em papel substrato e testadas em concentrações variadas de óxido de grafeno, alumínio e/ou óxido de grafeno + alumínio concomitantemente. Em seguida, foram armazenados em câmara de germinação à temperatura de 20 °C e fotoperíodo de 12 h. Os nanomateriais foram caracterizados por espectroscopia Raman, difração de raios X, espectroscopia por transformada de Fourier e microscopia eletrônica de varredura. Foram avaliados a germinação das sementes e os parâmetros de crescimento das plântulas) (comprimento e massa seca). O nanomaterial não causou efeitos adversos na germinação de sementes de trevo Persa. Entretanto, a adição de alumínio acima de 125 mg L^{-1} foi considerada prejudicial, resultando em diminuição da germinação, do comprimento total e radicular e da massa seca das plântulas. No entanto, a exposição simultânea ao óxido de grafeno/alumínio não afetou o crescimento inicial nem os parâmetros de germinação das sementes.
Palavras-chave: grafeno, processo germinativo, *Trifolium resupinatum* L.

1. INTRODUCTION

The use of chemical compounds, mostly artificial, in crops, industries, and homes has led to an increase in the concentration of these substances in the environment, which can have harmful effects on plants and also on humans. The current trend in agriculture is to focus on more sustainable practices and fewer synthetic chemicals. It also highlights alternative strategies that can increase productivity and plant resistance to abiotic stresses while promoting environmental sustainability [1].

More recently, graphene-based nanomaterials and its derivatives, such as graphene oxide (GO), amino-Fe_3O_4-functionalized graphene oxide (GO-NH_2-Fe_3O_4), and reduced graphene oxide (GOr) have been utilized in various fields, such as medicine, physics, energy, agriculture, and environmental science, and have potential to reduce harmful substances in the environment [2-5]. In addition, it has a high potential to be used as a stress-relieving tool due to its excellent properties. These include water solubility, non-toxicity, low cost compared to existing materials, and production on a large scale from graphite [6].

However, its increased use in recent years has raised concerns about its potential biological and environmental risk [7], justifying a wide investigation regarding toxicity and safety assessments [8]. Studies on its positive or negative impacts on plants, as well as its possible role as an attenuator of stress caused by chemical elements are insufficient and sometimes controversial, for example, aluminum, which in excess, becomes toxic to plants and the surrounding environment.

Aluminum (Al) is present in acidic soils in the form of trivalent ions (Al^{3+}). At a pH below 5.0, Al dissociates into toxic trivalent forms, and its concentration in the soil is deleterious for Al-sensitive plants (between 2 and 5 ppm), while above 5 ppm it is toxic for aluminum-tolerant species [9]. Most crops are sensitive to the micromolar concentration of toxicity from microelements, such as Al, with the root tip being the main target [10]. In this context, Al causes toxicity and irreversible damage to plant growth, consequently affecting the quality and productivity of crops [11].

In recent years, there has been growing concern about growing plants in soils with high concentrations of aluminum. Among them are those of the *Trifolium* genus, which, not only fixes nitrogen, but also grows quickly, allowing for rapid soil coverage, significantly contributing to the invasive plant control. However, most species do not develop in poor, sandy, and acidic soils. Their development is aggravated by toxic levels of aluminum, manganese, and phosphorus deficiencies. Associated with this, previous studies have shown that GO has low ecotoxicity [5, 6, 8], raising the hypothesis that it may mitigate to the toxic effect of Al on seed germination. Considering that, so far, the studies on the phytotoxicity of isolated GO do not provide an understanding of the potential interactions of GO with other contaminants in the environment [12], the purpose of this study was to analyze the effect of GO on the germination of Persian clover seeds (*Trifolium resupinatum* L.) and its possible attenuating effect on Al stress.

2. MATERIAL AND METHODS

2.1 Plant material

The pelleted seeds of Persian clover (*Trifolium resupinatum* L.), cultivar Lightning, were purchased from a traditional seed trading company (PGG Wrightson Seeds Brasil).

2.2 GO (graphene oxide) synthesis

The GO synthesis followed the methodology of Salles et al. (2020) [13], and the final concentration resulted in approximately 1.5 g.

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 Graphene oxide in seed germination

Persian clover seeds were subjected to different concentrations of GO: 0 (control), 125, 125, 250, 500, and 1000 mg L^{-1}. In the control treatment (0), only distilled water was used.

2.4 Aluminum in seed germination

During germination and initial development, Persian clover seeds were exposed to 0 (control), 125, 250, 500, 1000, and 2000 mg L^{-1} of aluminum chloride ($Al_2Cl_3.2H_2O$).

2.5 Graphene oxide and aluminum stress

To analyze the potential of GO as a possible attenuator of the toxic effect of aluminum, concentrations of 0, 125, 250, 500, and 1000 mg L^{-1} of GO were joined and mixed with 125 mg L^{-1} of $Al_2Cl_3.2H_2O$ (the concentration considered toxic in the previous step considering the results obtained in the seedling length test).

2.6 Experiments with seed germination

In the three stages, the germination of Persian clover seeds were evaluated by subjecting them to graphene oxide and/or aluminum chloride, as previously mentioned. Four repetitions of 50 seeds were distributed on germitest paper and placed in transparent plastic boxes (gerbox). Then the boxes with the seeds were stored in a germination chamber (20 °C and photoperiod of 12 h). The count of normal seedlings was performed, according to Brasil (2009) [14], on the 7th day after sowing. Together with germination, the emerged seedlings were counted daily (until the 7th day), calculating the germination speed index (GSI) [15].

To determine the length (cm) and dry mass (mg) of the seedlings, the methodology described by Krzyzanowski et al. (2020) [15] was used. Four replications of 20 seeds were sown in two rows in the upper third of the germitest paper and maintained under the same condition as the germination test. On the 7th day after sowing, the lengths of 10 normal seedlings of each replication were measured. Following the procedure, ten normal seedlings were chosen from each replicate of the seedling length test to determine the seedling dry mass (mg). The seedlings were weighed on a precision balance (of 0.001 g) after drying the material in a forced ventilation oven at 60 ± 5 °C for 48 h.

2.7 Statistical analysis

The experimental design was completely randomized, with treatments consisting of different concentrations of graphene and/or aluminum oxide. The data was submitted to an analysis of variance using the F ($p \leq 0.05$) test and, when significant, a regression analysis was performed using the program Sisvar 5.6 software. The presentation of column graphs was chosen for better viewing of the results.

3. RESULTS AND DISCUSSION

3.1 Graphene oxide characterization

The GO characterization is shown in Figure 1. X-ray diffraction (XRD) (Figure 1a) of carbon nanomaterials showed complete oxidation of precursor material (graphite) for the absence of a peak around $2\theta \approx 26°$ and the appearance of an intense signal at $2\theta \approx 11.4°$ (001) which is characteristic of graphene oxide [16]. The functional groups of GO are shown in Figure 1b. According to the FTIR (Fourier-Transform Infrared Spectroscopy) a large signal at 3405 cm^{-1} related to the stretching vibration of OH groups [8]. The bands around 1734.02, 1650.71, 1342.65, and 1050 cm^{-1} correspond respectively to the C=O stretch vibration, C=C, C-OH, and C-O bonds [17].

The Raman Spectroscopy shows the three characteristic bands in the GO spectrum (Figure 1c). The D, G, and 2D bands are associated with the defects and disorder of graphite structure, sp^2 vibration by carbon atoms, and second order of the D band, respectively [18]. However, the ID/IG ratio of GO was 0.96, which reveals a high-quality carbon nanomaterial [16]. The morphological structure of GO is exhibited in Figure 1d. According to the SEM results, it is possible to observe a wrinkles sheet, corresponding to a nanomaterial with a few layers [19].

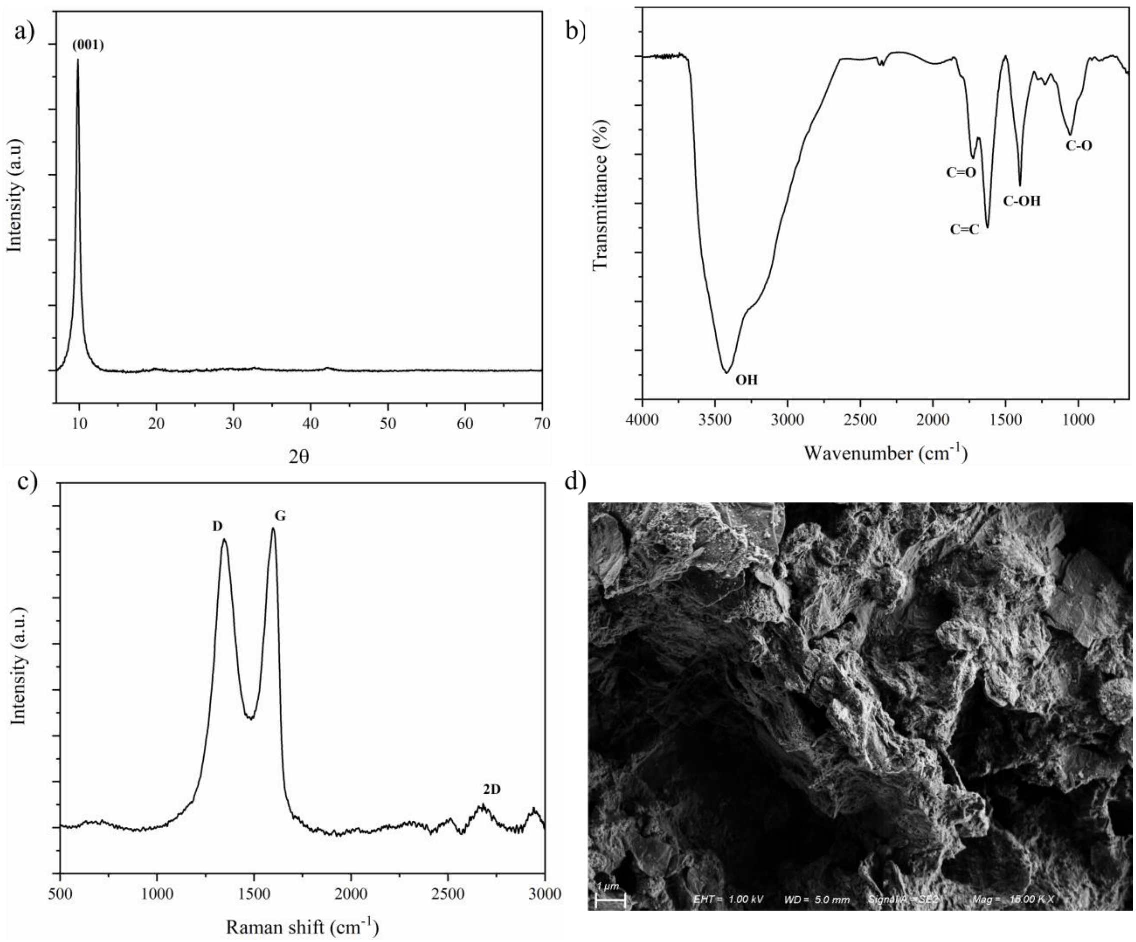

Figure 1. XRD (a), FTIR (b), Raman Spectroscopy (c) and SEM (d) of graphene oxide.

3.2 Graphene oxide in seed germination

Through data analysis, it is possible to observe that in the absence of GO (the control), the seeds presented an average of 97% of normal seedlings in the germination test (Figure 2a), with no significant decrease in the percentage of normal seedlings at the concentrations tested. Thus, it is possible to state that GO concentrations up to 1000 mg L^{-1} (the highest concentration used) did not have a toxic effect on the germination of Persian clover seeds. Furthermore, no significant difference was observed in germination speed and seedling dry mass (Figures 2b and 2d). On the other hand, seedling length showed a reduction only at the highest concentration used, with values ranging from 5.97 cm (control) to 4.21 cm (1000 mg L^{-1}) (Figure 2c).

Similar results to this study were observed by Park et al. (2020) [20], in which the seed germination of *Arabidopsis thaliana* (L.) Heynh. was not affected by the GO doses tested (100, 1000, and 10000 mg L^{-1}) and were similar to the results of Kazlauskas et al. (2023) [12] in *Lepidium sativum* L. (1-80 mg L^{-1}). According to Yang et al. (2022) [5], the action of GO on plant growth depends on the genotypic characteristics and the concentration used.

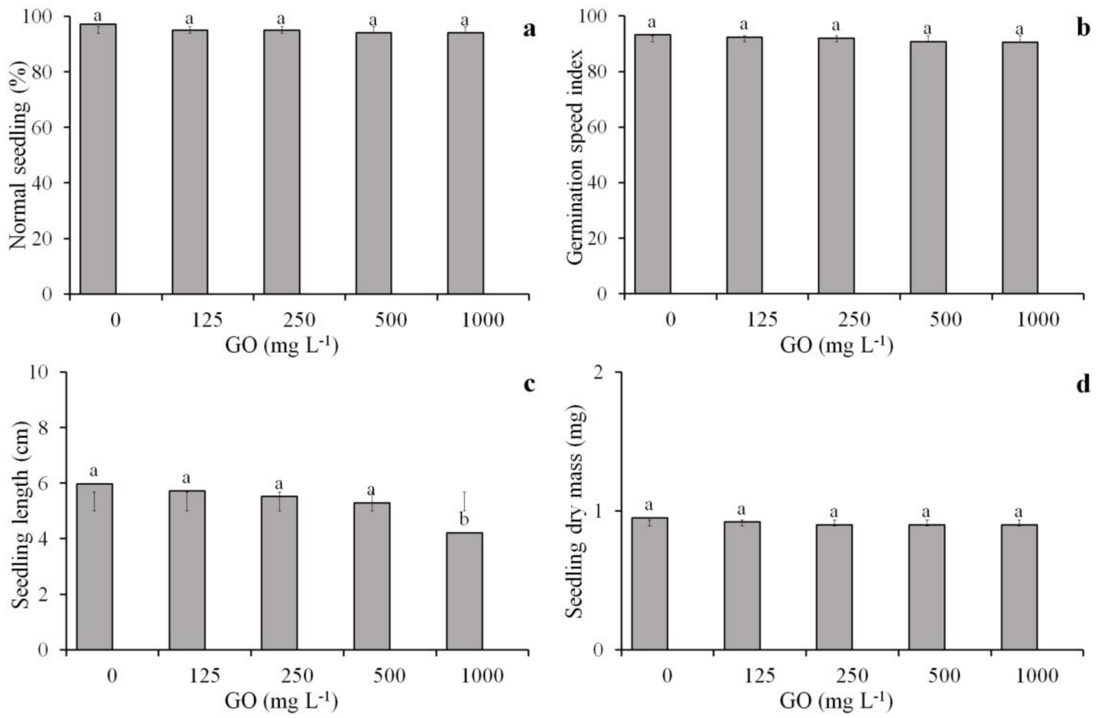

Figure 2. Germination (a) and seed germination speed (b), length (c), and dry mass (d) of Persian clover seedlings submitted to different concentrations of graphene oxide (GO).

The majority of studies show that the presence of GO can stimulate germination, which is the first stage of vegetable development, enabling the seeds to absorb water [21]. There are reports in the literature of the use of GO to promote seed germination in spinach [21]; the accumulation of root biomass and the morphological development of tomato seedlings [22]; the promotion of tap root length and lateral roots in *A. thaliana* [23], as well as positive effects on the germination of persian clover and buckwheat (*Fagopyrum esculentum* Moench) seeds [24], cotton - *Gossypium hirsutum* L. [22] and alfalfa - *Medicago sativa* L. [25]. The positive effect can be attributed to the fact that GO penetrates the seed coat, facilitating water absorption, resulting in stimulation of germination and root development [26]. In contrast, in other reports, GO inhibited seed germination in rice – *Oryza sativa* L. [27], had no effect on root growth in wheat – *Triticum aestivum* L. [28] and, in white clover (*Trifolium repens* L.), exposure to GO had negative effects on seedling growth, and nutrient uptake by the shoot system, with a more relevant effect observed with increasing concentration and exposure time [7].

3.3 Aluminum in seed germination

The percentage of seed germination and seedling growth of Persian clover decreased with increasing aluminum concentrations (Figure 3). In the absence of Al, the seeds showed a rate of average germination of 97% (Figure 3a), with a significant reduction in normal seedlings from 125 and 250 mg L^{-1} (90% each). The germination speed index decreased from 500 mg L^{-1} (Figure 3b). Root length, total length, and dry mass of seedlings decreased from 125 mg L^{-1} (Figures 3c and 3d). Furthermore, the roots presented a brown color and a fragile structure.

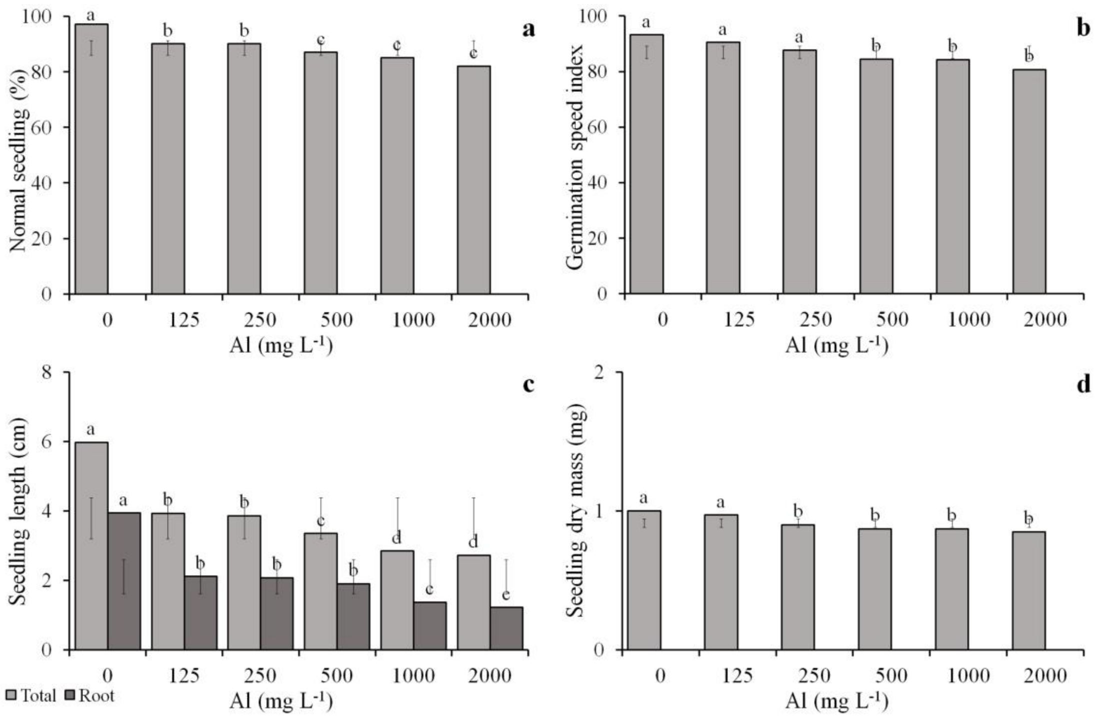

Figure 3. Germination (a) and germination speed (b) of seeds, total seedling and root lengths (c), and dry mass (d) of Persian clover seedlings submitted to different aluminum concentrations ($Al_2Cl_3.2H_2O$).

Aluminum toxicity in acidic soils is regarded as one of the main restrictions on seed germination and plant development [29]. As the first interaction zone of germinated seeds and metals is the root [30], these organs are the first to find and respond to soil pollutants, and their anatomical structure may reflect their adaptation strategy to the external environment [31]. When plants are exposed to Al stress, the inhibition of root elongation is the most prominent symptom, consequently, these changes in root structure will directly affect the absorption of water and nutrients [32]. Al toxicity disrupts the root system by inhibiting cell elongation and cell division, disorganizing various metabolic processes, and promoting cell death, leading to a decrease in growth, a loss of yield, or total crop damage [33].

3.4 Graphene oxide and aluminum stress

The analysis of variance indicated that there was no significant influence of the simultaneous application of graphene oxide and aluminum (125 mg L^{-1}) on the germination, germination speed, length, and dry mass of Persian clover seedlings (Figure 4).

The absorption and accumulation of metals in plant tissues depend on several factors, such as the stability of the metal in the biotic and abiotic environment, its bioavailability in water, and the transfer rate through the plant membrane [34]. The exposition of GO combined with cadmium (Cd^{2+}) on rice seed germination were investigated by Yin et al. (2018) [35] in which they concluded that graphene oxide can adsorb cadmium in solution and, increased concentrations of GO, proportionally lower the residual concentration of the metal.

Additionally, Hu et al. (2018) [36] evaluated the influence of GO (1 and 5 mg L^{-1}) on copper (Cu) stress in *Lemna minor* L. They found that GO with concentrations under 5 mg L^{-1}, did not have adverse effects on the species. The authors noted that graphene oxide's unique properties and wide application cause it to interact with other toxic elements, subsequently changing its behavior and toxicity.

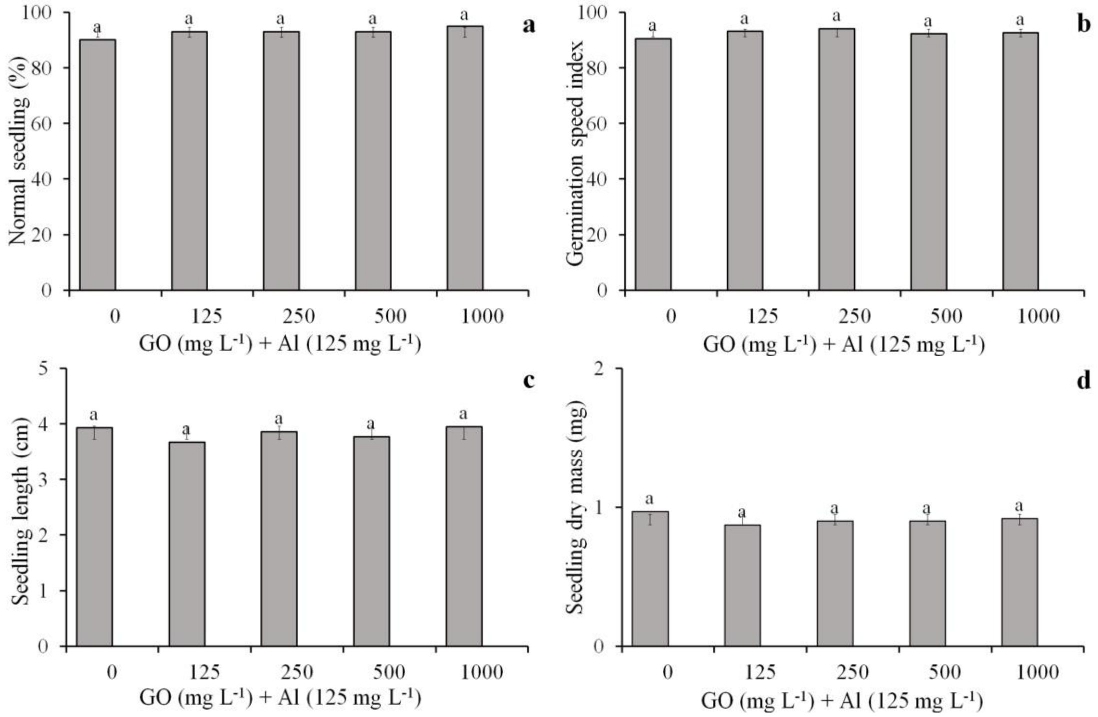

Figure 4. Germination (a) and germination speed (b) of seeds, length (c) and dry mass (d) of Persian clover seedlings submitted to different concentrations of graphene oxide (GO) and aluminum (Al - 125 mg L^{-1}).

Complementarily, GO affected the accumulation of tested metals (GO + Ni, Zn, Cr, and Cu) in the root and shoot systems of *L. sativum* due to its ability to adsorb metals from the growth medium [12]. In another report, Kaymak et al. (2022) [6], analyzed different doses of GO (0, 0.25, 0.50, 0.75, and 1.0 mg mL^{-1}) under saline stress (0, 5, 10, and 15 g L^{-1}) verified that the effect of GO impacted on melon seed germination (*Cucumis melo* L.) based to the concentrations. Furthermore, the simultaneous application of GO and sodium chloride favored the germination of Persian clover seeds [8].

Graphene oxide has the potential to stimulate seed germination under stressful conditions due to its capability to dissolve in water, versatility and high ionic charge property that eliminates the antagonistic effects of alkaline ions by removing them from the seed during the germination process [6]. The mechanism behind this event is not clear, however, GO may play the role of a protective shield by interfering with the hydrophilic groups of carbohydrates and proteins in the seed coat, thus attenuating the inhibition of seed germination induced by exposure to the metal [12].

In this study, the addition of aluminum above 125 mg L^{-1} represented a stress condition, which was evidenced by the decrease in germination percentage, length, and dry mass of Persian clover seedlings. When seeds were simultaneously exposed to GO and Al, no reduction in germination and initial growth parameters was observed. Although GO did not have a mitigating effect on Al stress, these study provide an understanding of the role of graphene oxide in Persian clover seed germination, indicating nontoxic effect.

Future studies including different methodologies and analyses, as well as longer exposure times to the nanomaterial, are necessary and may help to understand the role of graphene oxide in other stages of the development of species of the genus *Trifolium* and possible interactions with other pollutants.

4. CONCLUSIONS

The graphene oxide did not cause adverse effects on Persian clover seed germination. It was observed that the addition of aluminum above 125 mg L^{-1} resulted in harmfully stress condition, leading to a decrease in germination, total and root length, and seedling dry mass. Nevertheless, when exposed to graphene oxide/aluminum simultaneously, there were no changes observed in the initial growth or germination parameters of the seeds.

5. ACKNOWLEDGMENTS

The authors would like to thank the Federal University of Santa Maria and Franciscan University for financial support.

6. REFERENCES

1. Pareek A, Dhankher OP, Foyer CH. Mitigating the impact of climate change on plant productivity and ecosystem sustainability. J Exp Bot. 2020;71(2):451-6. doi: 10.1093/jxb/erz518
2. Guo R, Jiao TF, Li RF, Chen Y, Guo WC, Zhang L, et al. Sandwiched Fe_3O_4/carboxylate graphene oxide nanostructures constructed by layer-by-layer assembly for highly efficient and magnetically recyclable dye removal. ACS Sustain. Chem Eng. 2018;6(1):1279-88. doi: 10.1021/acssuschemeng.7b03635
3. Xie LL, Chen F, Zou XL, Shen SS, Wang XG, Yao GX, et al. Graphene oxide and ABA cotreatment regulates root growth of *Brassica napus* L. by regulating IAA/ABA. J Plant Physiol. 2019;240:153007. doi: 10.1016/j.jplph.2019.153007
4. Altan O, Metin Ö. Boosting formic acid dehydrogenation via the design of a Z-scheme heterojunction photocatalyst: The case of graphitic carbon nitride/Ag/Ag_3PO_4-AgPd quaternary nanocomposites. Appl Surf Sci. 2021;535:147740. doi: 10.1016/j.apsusc.2020.147740
5. Yang Y, Zhang R, Zhang X, Chen Z, Wang H, Li PCH. Effects of graphene oxide on plant growth: A Review. Plants. 2022;11:2826. doi: 10.3390/plants11212826
6. Kaymak HÇ, Sevim M, Metin Ö. Graphene oxide: A Promising material for the germination of melon seeds under salinity stress. Turk J Agric For. 2022;46(6):863-74. doi: 10.55730/1300-011X.3048
7. Zhao S, Zhu X, Mou M, Wang Z, Duo L. Assessment of graphene oxide toxicity on the growth and nutrient levels of white clover (*Trifolium repens* L.). Ecotoxicol Environ Saf. 2022;234:113399. doi: 10.1016/j.ecoenv.2022.113399
8. Stefanello R, Garcia WJS, da Rosa Salles T, Rhoden CRB. Graphene oxide decreases the effects of salt stress on Persian clover seed germination. J Toxicol Environ Health Sci. 2024;85(2):47-56. doi: 10.1080/15287394.2023.2274338
9. Gazey C. Effects of soil acidity [Internet]; Australia: Departament of Primary Industries and regional Development; 2018 Sep 17 [cited 2023 Mar 10]. Available from: https://www.agric.wa.gov.au/soil-acidity/effects-soil-acidity
10. Yamamoto Y. Aluminum toxicity in plant cells: mechanisms of cell death and inhibition of cell elongation. Soil Sci Plant Nutr. 2019;65(1):41-55. doi: 10.1080/00380768.2018.1553484
11. Chauhan DK, Yadav V, Vaculík M, Gassmann W, Pike S, Arif N, et al. Aluminum toxicity and aluminum stress-induced physiological tolerance responses in higher plants. Crit Rev Biotechnol. 2021;41(5):715-30. doi: 10.1080/07388551.2021.1874282
12. Kazlauskas M, Jurgelėnė Ž, Šemčuk S, Jokšas K, Kazlauskienė N, Montvydienė D. Effect of graphene oxide on the uptake, translocation and toxicity of metal mixture to *Lepidium sativum* L. plants: Mitigation of metal phytotoxicity due to nanosorption. Chemosphere. 2023;312:137221. doi: 10.1016/j.chemosphere.2022.137221
13. Salles TR, Rodrigues HB, Bruckmann FS, Alves LCS, Mortari SR, Rhoden CRB. Graphene oxide optimization synthesis for application on laboratory of Universidade Franciscana. Discip Sci, Ser Cienc Nat Tecnol. 2020;21:15-26. doi: 10.37779/nt.v21i3.3632
14. Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Brasília (DF): MAPA; 2009. Available from: https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise__sementes.pdf
15. Krzyzanowski FC, França-Neto JB, Gomes-Junior FG, Nakagawa J. Testes de vigor baseados em desempenho de plântulas. In: Vigor de sementes: Conceitos e testes. Londrina (PR): Abrates; 2020. p. 601.

16. Rhoden CRB, Bruckmann FS, Salles TR, Kaufmann Junior CG, Mortari SR. Study from the influence of magnetite onto removal of hydrochlorothiazide from aqueous solutions applying magnetic graphene oxide. J Water Process Eng. 2021;43:102262-75. doi: 10.1016/j.jwpe.2021.102262
17. Ossonon BD, Bélanger D. Synthesis and characterization of sulfophenyl-functionalized reduced graphene oxide sheets. RSC Adv. 2017;7:27224-34. doi: 10.1039/c6ra28311j
18. Kellici S, Acord J, Ball J, Reehal HS, Morgan D, Saha B. A single rapid route for the synthesis of reduced graphene oxide with antibacterial activities. RSC Adv. 2014;4:14858. doi: 10.1039/c3ra47573e
19. Hatel R, Majdoub SE, Bakour A, Khenfouch M, Baitoul M. Graphene oxide/Fe_3O_4 nanorods composite: Structural and Raman investigation. J Phys Conf Ser. 2018;1081:012006. doi: 10.1088/1742-6596/1081/1/012006
20. Park S, Choi KS, Kim S, Gwon Y, Kim J. Graphene oxide-assisted promotion of plant growth and stability. Nanomaterials. 2020;10(4):758. doi: 10.3390/ nano10040758
21. He Y, Hu R, Zhong Y, Zhao X, Chen Q, Zhu H. Graphene oxide as a water transporter promoting germination of plants in soil. Nano Res. 2018;11(4):1928-37. doi: 10.1007/s12274-017-1810-1
22. Guo X, Zhao J, Wang R, Zhang H, Xing B, Naeem M, et al. Effects of graphene oxide on tomato growth in different stages. Plant Physiol Biochem. 2021;162:447-55. doi: 10.1016/j.plaphy.2021.03.013
23. Gao C, Xiao CJ, Lu S, Wang SR, Yuan HH, Cao YY. Promoting effect of graphene oxide on the root growth of *Arabidopsis thaliana*. Biotechnol Bull. 2022;38(6):120-8. doi: 10.13560/j.cnki.biotech.bull.1985.2021-1188
24. Stefanello R, Garcia WJS, Salles TR, Rhoden CRB. Graphene oxide assessment on the germination of Persian clover and buckwheat seeds. Ci Nat. 2024;46:e84266. doi: 10.5902/2179460x84266
25. Zhao S, Wang W, Chen X, Gao Y, Wu X, Ding M, et al. Graphene oxide affected root growth, anatomy, and nutrient uptake in alfalfa. Ecotoxicol Environ Saf. 2023;15:250:114483. doi: 10.1016/j.ecoenv.2022.114483
26. Samadi S, Lajayer BA, Moghiseh E, Rodríguez-Couto S. Effect of carbon nanomaterials on cell toxicity, biomass production, nutritional and active compound accumulation in plants. Environ Technol Inno. 2021;21:101323. doi: 10.1016/j.eti.2020.101323
27. Chen J, Mu Q, Tian X. Phytotoxicity of graphene oxide on rice plants is concentration-dependent. Mater Express. 2019;9:635-40. doi: 10.1166/mex.2019.1538
28. Zhu Y, Weng Y, Zhang S, Liu L, Du S. The nitrate uptake and growth of wheat were more inhibited under single-layer graphene oxide stress compared to multi-layer graphene oxide. Ecotoxicol Environ Saf. 2022;247:114229. doi: 10.1016/j.ecoenv.2022.114229
29. Ofoe R, Lokanadha R, Gunupuru LR, Wang-Pruski G, Fofana B, Thomas RH, et al. Seed priming with pyroligneous acid mitigates aluminum stress, and promotes tomato seed germination and seedling growth Plant Stress. 2022;4:100083. doi: 10.1016/j.stress.2022.100083
30. Yang Y, Zhang L, Huang X, Zhou Y, Quan Q, Li Y, Zhu X. Response of photosynthesis to different concentrations of heavy metals in *Davidia involucrata*. PLoS One. 2020;15(3):e0228563. doi: 10.1371/journal.pone.0228563
31. da Cunha Cruz Y, Scarpa ALM, Pereira MP, de Castro EM, Pereira FJ. Root anatomy and nutrient uptake of the cattail *Typha domingensis* Pers. (Typhaceae) grown under drought condition. Rhizosphere. 2020;16:100253. doi: 10.1016/j.rhisph.2020.100253
32. Lima JV, Tinôco RS, Olivares FL, Chia GS, de Melo Júnior JAG, da Silva GB. Rhizobacteria modify root architecture and improve nutrient uptake in oil palm seedlings despite reduced fertilizer. Rhizosphere. 2021;19:100420. doi: 10.1016/j.rhisph.2021.100420
33. Bojórquez-Quintal E, Escalante-Magaña C, Echevarría-Machado I, Martínez-Estévez M. Aluminum, a friend or foe of higher plants in acid soils. Front Plant Sci. 2017;8:1767. doi: 10.3389/fpls.2017.01767
34. Usman K, Al-Ghouti MA, Abu-Dieyeh MH. The assessment of cadmium, chromium, copper, and nickel tolerance and bioaccumulation by shrub plant *Tetraena qataranse*. Sci Rep. 2019;9:5658. doi: 10.1038/s41598-019-42029-9
35. Yin L, Wang Z, Wang S, Xu W, Bao H. Effects of graphene oxide and/or Cd^{2+} on seed germination, seedling growth, and uptake to Cd^{2+} in solution culture. Water Air Soil Pollut. 2018;229:151. doi: 10.1007/s11270-018-3809-y
36. Hu C, Liu L, Li X, Xu Y, Ge Z, Zhao Y. Effect of graphene oxide on copper stress in *Lemna minor* L.: evaluating growth, biochemical responses, and nutrient uptake. J Hazard Mater. 2018;5:341:168-76. doi: 10.1016/j.jhazmat.2017.07.061