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ECOLOGICAL TECHNIQUES FOR STIMULATING PLANT GERMINATION AT SUBATMOSPHERIC PRESSURES AND CARBON NANOCLUSTERS

TEHNICI ECOLOGICE DE STIMULARE A GERMINAȚIEI PLANTELOR LA PRESIUNI SUBATMOSFERICE ȘI NANOCLUSTERI DE CARBON

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Abstract. *This study examined the impact of ecological stimulation on wheat and maize seeds under low-pressure conditions, using a composite material with C_{20}-C_{60} carbon nanoclusters. Seeds harvested in 2022 and maintained under standard conditions showed significant improvements compared to the control group when subjected to combined biostimulation at reduced pressure and immersion in a carbon nanocluster solution. Germination time was shortened, with germination capacity increasing by 35% for Zea mays L. and 15% for Triticum aestivum L. These findings suggest that nanotechnology could enhance seed performance, supporting sustainable agriculture and meeting the rising demand for healthy, sustainably produced food.*
Keywords: *biostimulation, carbon nanoclusters, germination, sustainable agriculture.*

Introduction

Sustainable agriculture presupposes a minimum use of agrochemicals that can ultimately protect the environment and preserve various species from extinction. The literature suggests that replacing chemicals to increase the productive potential of crops or as herbicides with physical seed pretreatment methods would help to minimize the anthropogenic impact on agrosystems and improve the quality of sowing material. Therefore, in recent decades, the search for biophysical techniques and technologies is aimed at achieving genetic potential, increasing nonspecific resistance to abiotic and biotic stress, strengthening the adaptive potential of plants to grow and stabilize the crop [1-5].

Environmental pollution is one of the major global challenges, as pollutants of a different nature contaminate urban and agricultural areas [6]. In order to improve pollutant remediation strategies for environmental sustainability, there is a need to in-crease the effectiveness of conventional methods or to introduce innovative approaches. In this context, nanotechnology and in particular carbon-based nanomaterials can contribute greatly as they possess enormous absorption potential due to their large surface area. Advances in nanotechnology have led to the development of new materials with various applications. For instance, carbon nanomaterials (CNMs) have been used in diverse fields, including agriculture. These CNMs are characterized by their ability to be easily absorbed by plant cells, positively impacting plant growth and development. They have been applied to remove pesticides from water, extract heavy metals from soil and water, and exhibit antifungal and antibacterial effects. In agriculture, CNMs have been employed to stimulate seed germination and act as growth regulators, also enhancing the antioxidant activity of plants [7-9]. For example, in the studies of Yadong Li et. al, 2020 [10], TR Shojaei et. al, 2019 [11] and Moşneaga A. et al, 2020 [12] Carbon-based nanomaterials played a role in root and stem elongation, bio-mass accumulation, and improved carbohydrate content and photosynthesis, in-creased germination and growth rate of some agricultural crops. Also in their work, Aacharya, R. et al. [13], demonstrate the utility of carbon nanofertilizers in improving seed germination,

seedling growth, shoot length, chlorophyll content, photosynthesis rate, and plant biomass increase in various cereals and horticultural crops. Additionally, the potential of carbon nanomaterials in agriculture is also discussed for the commercial production of nanocarbon as fertilizer.

Carbon-based nanomaterials have been utilized as efficient adsorbents to remove a wide range of contaminants for extended periods. Recently, increased attention has been given to nanoscale carbon-based adsorbents, which not only are capable of ad-sorbing pesticides or their degradation products but could also significantly contribute to enhanced pesticide degradation [14].

Thus, in the last decade, research into the application of carbon-based nano-materials in agriculture has been developed and deepened to monitor crop health, stimulate crop growth, enhance fertilizer efficiency, and manage diseases, pests, and environmental stress. Moreover, it has been found that carbon-based nanomaterials exhibit much lower toxicity to the environment and higher biocompatibility due to their non-toxic carbon backbone [15].

Research on seed processing at lower than atmospheric pressures has also shown a significant impact on germination. In a study conducted by NASA [16] it was found that subjecting seeds to frequent changes in atmospheric pressure can shorten germination time, improve the growth rate of young plants, and cause more massive and rapid root growth. One notable finding from the study is the observation of more massive and rapid root growth in plants subjected to pressure changes. Healthy root development is crucial for nutrient uptake and overall plant health, so this effect could significantly benefit plant growth and productivity. The effects of hypobaric (reduced atmospheric pressure) and hypoxia (reduced oxygen concentration) on seed germination have been studied in various plant species.

Research by Shuangsheng Guo et.al [17] investigated how these environmental factors influence the wheat germination process. The specific conclusions of the study would depend on factors such as the duration and intensity of hypobaric and hypoxic conditions, as well as the characteristics of the plant species studied. Photosynthetic rate, transpiration rate, biomass and wheat yield all increased, some nutrients also in-creased.

The experiment by Yongkang Tang et al demonstrated that lettuce (L. sativa) can be grown under long-term low pressure without adverse effects on seed germination, gas exchange and plant growth. Thus, under hypobaric conditions, the leaf area and root activity of lettuce plants were greater than those at ambient pressure, but the shoot/root ratio was lower [18].

The present study shows the results of biostimulation of seed germination of wheat and maize crops. Biostimulation was carried out in two steps: processing the seeds at lower pressure than the atmospheric one and immersing them in carbon nanocluster solution. Wheat and corn are not only among the important crops in the world, but also the primary candidate crop in CELSS (Closed Ecological Life Support System).

Material and method

Seed pretreatment at lower than atmospheric pressures

Preprocessing seeds under pressures lower than atmospheric pressure involves subjecting the seeds to environments where the pressure is reduced compared to the standard atmospheric pressure at sea level (101.3 kPa or 1 atm). The specificity of the treatment consists in the evacuation of air from the premises, where the seeds are placed, at a pressure of 10^{-3} Torri. The depression is maintained by a vacuum pump, and water vapor is periodically introduced into the premises. The duration of treatment is 1 h for each batch. The rationale behind preprocessing seeds at lower pressures is to potentially enhance their performance in terms of germination rate, seedling vigor, and overall crop yield. However, the specific effects of this preprocessing method can vary depending on factors such as the type of seeds, the duration and intensity of the pressure reduction, and environmental conditions during subsequent growth stages.

Preparation of composite material with carbon nanoclusters

To stimulate growth, materials of a mineral nature with the content of carbon clusters of the order of 3-5% were used. The primary substance, carbon clusters, were obtained by burning pure spectral graphite in helium atmosphere [19,20] The content of the clusters of different orders was determined by the gas pressure, the rate of combustion of the electrodes and the arc voltage.

The seeds of *Triticum aestivum L* and *Zea mays L* were used as model seeds, for reasons of growing conditions, to investigate the effect of individual and combined treatment on germination. The specifics of batch processing are given in Table 1.

Table 1. Experimental conditions used in the study

Sample series	carbon nanoclusters	pretreatment at less than atmospheric pressure
s_1	-	-
s_2	-	+
s_3	+	-
s_4	+	+

s_1 – control series of wheat and corn samples; s_2, s_3 and s_4 – wheat and corn seeds subjected to the treatment

Prior to commencing the research, a germination assessment was conducted to evaluate the seed quality. Four samples, each containing 25 seeds per crop species, were arranged in Petri dishes lined with filter paper. Approximately 10 ml of water was uniformly applied to each dish. Subsequently, the seeds were positioned in indirect natural light conditions at room temperature (20°C), and germination progress was monitored weekly over a span of four weeks. Adequate moisture levels were maintained by irrigating the samples as necessary. Radicle emergence served as the criterion for determining germination.

Seeds were deemed suitable for inclusion in the main study if they exhibited a minimum germination rate of 85%. After testing the quality of the seeds samples of 50 seeds were placed in Petri dishes containing a thin layer of gauze. The samples were watered with 10 mL of water every 24 hours and placed in a dark chamber at a temperature of (20 ± 2) °C and relative humidity of (75 ± 5) %.

The number of germinated seeds was observed and recorded every day. The rate of germination, defined as the number of germinated seeds divided by the total number of seeds, was traced up to 5 days.

Statistical processing of research results

Germination is a crucial step in establishing plants in agriculture and is fundamental for crop quality. Rapid seedling development ensures quick leaf expansion and root elongation, facilitating nutrient absorption, translocation through transpiration flow, and biomass production [21-23].

Slow germination can expose young seedlings, one of the most vulnerable stages in the plant life cycle, to various environmental stresses or pathogens, leading to decreased vigor and crop productivity, ultimately resulting in economic losses. Thus, the precise quantification of germination, achieved through the meticulous analysis of data collected during the germination process, is of interest.

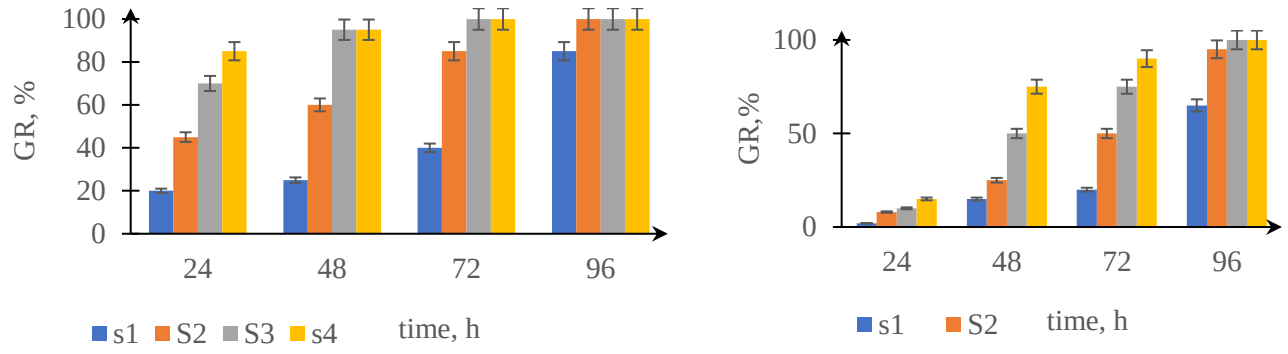
These indicators allow a rigorous evaluation of the ability of a plant organism to express its protective potential in the first stages of development.

Results and discussions

The performance assessment of a seed lot can be achieved through precise quantification of cumulative germination data. The germination process involves seed imbibition with liquid, activation

process, and intra-seed growth culminating in embryonic emergence. Germination parameters analyzed include germination rate (GR), coefficient of variation of germination time (CVt), and mean germination time (MGT). Seeds are considered to be germinated when the emergence of radiculae was about 2 mm. Figure 1 shows the general germination results of the seeds of *Triticum aestivum L* and *Zea mays L* during the first 5 days after treatment.

As depicted in Figure 1, employing a combined treatment involving carbon nanoclusters and reduced pressure conditions than the atmospheric pressure has led to an augmentation in both the germination rate and efficiency. Notably, for both analyzed crops, the highest germination rate of 100% was attained by the batches treated with the combined method, denoted as s_4. A comparative analysis against the control batches clearly demonstrates the superior performance of the combined treatment across both species. This enhanced performance can be attributed to the optimization of physiological processes, consequently resulting in a shortened germination period and a notable increase in production potential, by 35% for *Triticum aestivum L*. and 15% for *Zea mays L*.



(a) **(b)**

Figure 1. Evolution of the seed germination rate (GR) of: (a)- *Triticum aestivum* L. and (b)- *Zea mays* L.

As observed, there is a difference in the response of the seeds of the two re-searched crops to stimuli. Thus, Triticum aestivum L. exhibited a higher early vitality compared to Zea mays L, even within the first 24 hours. Seed germination provides a suitable basis for plant growth, development, and yield. Mean germination time (MGT) is a measure of germination speed and temporal spread of germination, considering viable seeds. Figure 2 presents the results of mean germination time for seeds of *Triticum aestivum L*. and *Zea mays L*.

This metric provides valuable insight in to the overall germination process and can be used to compare the germination performance of different treatments or seed varieties. A lower mean germination time indicates faster and more uniform germination across the sample. The MGT recorded for each treatment showed lower values, compared to the control group, for both crops following the same trend.

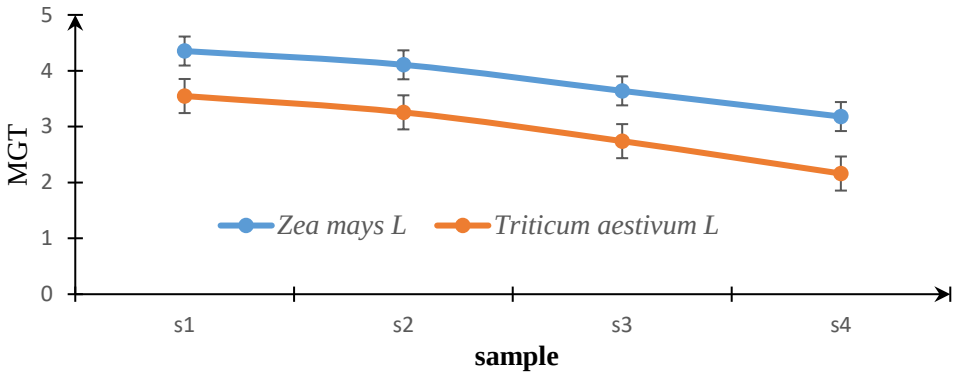


Figure 2. The mean germination time for seeds of *Triticum aestivum L.* and *Zea mays L.* depending on the pretreatment

The combined treatment led to higher results, so the s4 series showed the earliest development in both species. As expected, not all species respond similarly to stimuli. However, in terms of germination time, the trend was the same for both species.

Figure 3 shows the variation of the germination rate coefficient CVt depending on the applied treatment. CVt gives us an indication of the rapidity of seed germination.

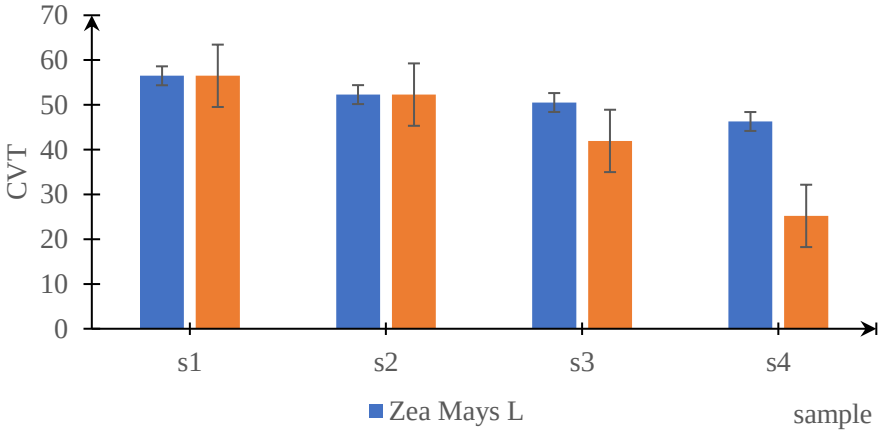


Figure 3. The coefficient of variation of the germination time of *Triticum aestivum L.* and *Zea mays L.* seeds

The CVt index increases as the number of germinated seeds increases and the time required for germination decreases. A higher CVt indicates greater variability in the germination times among the seeds in the sample, while a lower CVt suggests more uniformity in the germination process. Theoretically, the highest possible CVt is 100%, which occurs if all tested seeds in a treatment germinate on the same day. The seeds of all studied species do not have a CVt value higher than 56.48% for any of the tested treatments. The lowest CVt value, 25.19%, was obtained for *Triticum aestivum L.* seeds from lot 4. Additionally, lots s2 and s3, compared to the control lot, recorded lower CVt values. This may indicate a greater stimulation of germination and a shorter germination time for the studied seeds.

The picture of seeds germinated at different periods is shown in Figure 4. The obtained results suggest that the treatments administered to the investigated seeds, employing the specified methods, outperform the values observed in the control group samples. Consequently, these treatments have spurred a swifter development of the coleoptile compared to the control group, while concurrently fostering a more robust embryonic root system in the batches treated with the composite material.

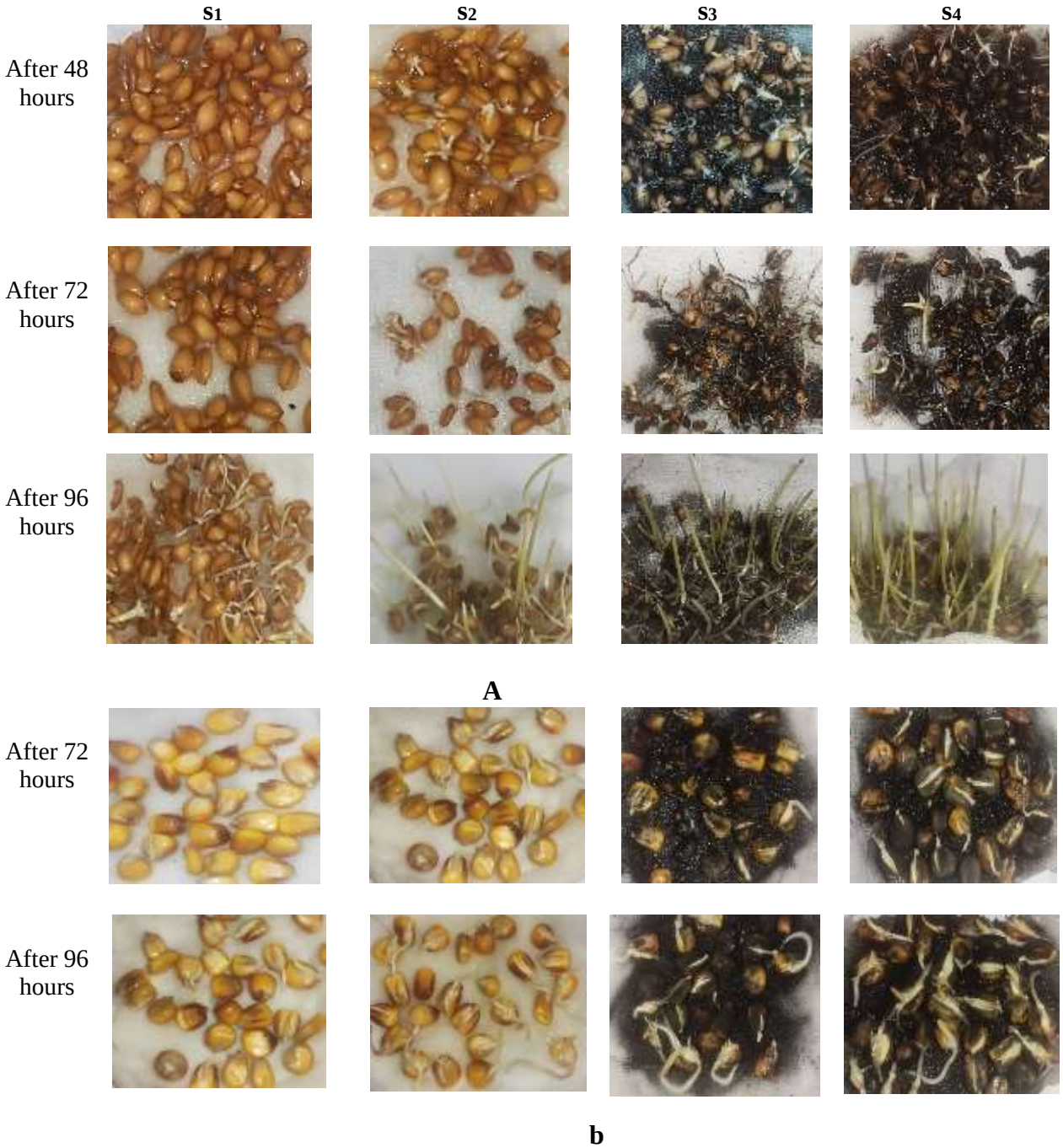


Figure 4. Image of seeds of (a)- *Triticum aestivum* L and (b)- *Zea mays* L at different germination periods

The experimental findings reveal that seedlings arising from treated seeds demonstrate superior performance when compared to their untreated counterparts across a spectrum of parameters. These encompass not only germination rates and seedling stature but also root architecture, water absorption kinetics, respiratory activity, and photosynthetic efficiency. The onset of primary root emergence served as a crucial milestone in the germination process. Noteworthy is the observation that both the application of carbon nanoclusters and seed preconditioning under sub-atmospheric pressure proved to be effective stimulants for the germination of both plant species.

Additionally, seeds originating from the treated groups exhibited pronounced shoot elongation and increased root biomass, highlighting the multifaceted advantages conferred by the treatment methodologies. Both the root system and shoot system remained healthy, and no negative effects of carbon nanoclusters or low-pressure treatment on seedling growth were observed. The average length of the root system and shoot system is greater in the treated plants compared to the control batches, as depicted in Figure 4.

Conclusions

The combined effects of seed processing with carbon nanoclusters at lower pressure than atmospheric pressure have been investigated. The combined treatment of immersion biostimulation in a solution with carbon clusters and the exposure of the seeds to pressures of 10^{-3} Torr with of the seeds of the species *Triticum aestivum L.*, and *Zea mays L.* contributed to the reduction of the germination time, also increasing the germination rate by 35% for wheat and 15% for maize. So, composite materials with carbon nanoclusters cause changes in plant metabolism, such as the intensification or reduction of physiological processes with consequences on reducing the period of shaving and increasing productive potential. Following the analyses of the results and the conclusions presented in this paper, it may be recommended to use composite materials with carbon nanoclusters, in order to improve the percentage of germination indicators of the or seed.

The studies and results obtained demonstrate that carbon-based nanomaterials have potential applications in nanoactivated agriculture. However, the physiological and molecular mechanisms underlying plant growth mediated by carbon clusters remain unclear, requiring further investigation.

References:

1. ALADJADJIYAN, A. Physical factors for plant growth stimulation improve food quality. *Food Production - Approaches, Challenges and Tasks*, *Publisher InTech* 2012, pp.145-168 doi.org/10.5772/32039.
2. ARAÚJO, S. DE S., PAPARELLA, S., DONDI, D., BENTIVOGLIO, A., CARBONERA, D., BALESTRAZZI, A. Physical methods for seed invigoration: advantages and challenges in seed technology. *Front Plant Sci*. 2016; *7(646)*, pp.1-12 ,doi.org/10.3389/fpls.2016.00646.
3. REINA, F. G., PASCUAL, L. A., FUNDORA, I. A. Influence of a stationary magnetic field on water relations in lettuce seeds. Part II: experimental results. *Bioelectromagnetics* 2001*, 22(8),* pp. 596-602, doi.org/10.1002/bem.89.
4. HERNÁNDEZ, A.C., DOMINGUEZ, P.A., CRUZ, O.A., IVANOV, R., CARBALLO, C.A., ZEPEDA, B.R. Laser in agriculture. *Int. Agrophys,* 2010, *24*, pp. 407- 422.
5. VASILEVSKI, G. Perspectives of the application of biophysical methods in sustainable agriculture. *Bulgarian Journal of Plant Physiology* 2003, *29(3)*, pp.179-186.
6. GONZÁLEZ-GARCÍA, Y., LÓPEZ-VARGAS, E.R., CADENAS-PLIEGO, G., BENAVIDES-MENDOZA, A., GONZÁLEZ-MORALES, S., ROBLEDO-OLIVO, A., ALPUCHE-SOLÍS, Á.G., JUÁREZ-MALDONADO, A. Impact of carbon nanomaterials on the antioxidant system of tomato seedlings. *Int. J. Mol. Sci.* 2019, *20*, 5858. doi.org/10.3390/ijms20235858.
7. VERMA, S.K., DAS, A.K., GANTAIT, S., KUMAR, V., GUREL, E. Applications of carbon nanomaterials in the plant system: A perspective view on the pros and cons. *Sci. Total Environ.* 2019, 667, pp. 485-499. doi.org/10.1016/j.scitotenv.2019.02.409.
8. GHORBANPOUR, M., HADIAN, J. Multi-walled carbon nanotubes stimulate callus induction, secondary metabolites biosynthesis and antioxidant capacity in medicinal plant Satureja khuzestanica grown in vitro. *Carbon N. Y.* 2015, *94*, pp.749-759. doi.org/10.1016/j.carbon.2015.07.056.
9. PATEL, A., TIWARI, S., PARIHAR, P., SINGH, R., PRASAD, S.M. Carbon nanotubes as plant growth regulators: impacts on growth, reproductive system, and soil microbial community. In

nanomaterials in plants, algae and microorganisms; *Elsevier: London, UK*, 2017, pp. 23-42. doi.org/10.1016/B978-0-12-811488-9.00002-0.
10. LI, Y., XU, X., WU, Y., ZHUANG, J., ZHANG, X., ZHANG, H., LIU, Y. A review on the effects of carbon dots in plant systems. *Materials Chemistry Frontiers*2020, *4(2),* pp. 437-448. doi.org/10.1021/acsomega.7b00657.
11. SHOJAEI, T.R., SALLEH, M.A.M., TABATABAEI, M., MOBLI, H., AGHBASHLO, M., RASHID, SA., TAN, T. Synthesis, Technology and applications of carbon nanomaterials, SA Rashid, RNI Raja Othman and MZ Hussein, Elsevier, 2019, pp. 247-277. doi.org/10.1016/B978-0-12-815757-2.00011-5.
12. MOSNEAGA, A., NEDEFF, V., SANDU, I., LOZOVANU, P., MOSNEGUTU, E., LEHADUS, M.P., SANDU, I.G. Utilization of ozone and composite materials in the seed treatment to stimulate the germination and growth of agricultural crops*, Rev. Chim*. 2020, *71(2*), pp. 365-370. doi.org/10.37358/RC.20.2.7938.
13. RUCHI, A., HEMRAJ, C. Nanocarbon fertilizers: Implications of carbon nanomaterials in sustainable agriculture production, In *Micro and Nano Technologies*, Carbon Nanomaterials for Agri-Food and Environmental Applications, *Elsevier*, 2020, pp. 297-321. doi.org/10.1016/B978-0-12-819786-8.00015-3.
14. JAMPÍLEK, J., KRÁĽOVÁ, K. Potential of nanoscale carbon-based materials for remediation of pesticide-contaminated environment, In *Micro and Nano Technologies*,Carbon Nanomaterials for Agri-Food and Environmental Applications, Elsevier, 2020, pp. 359-399. doi.org/10.1016/B978-0-12-819786-8.00017-7.
15. MUHAMMAD, A., TAHIR, F., JASON, C., WHITE, Y. H., ZIFU, H., YUKUI, R. Carbon-based nanomaterials suppress tobacco mosaic virus (TMV) infection and induce resistance in Nicotiana benthamiana, *In Journal of Hazardous Materials,* 2021, *404, Part A*, 124167. doi.org/10.1016/j.jhazmat.2020.124167.
16. https://sbir.nasa.gov/SBIR/abstracts/87/sbir/phase1/SBIR-87-1-12.06-3053.html.
17. SHUANGSHENG, G., YONGKANG, T., FENG ,G., WEIDANG, A., LIFENG, Q. Effects of low pressure and hypoxia on growth and development of wheat, *Acta Astronautica*, 63, *Issues 7–10,* 2008, pp. 1081-1085. doi.org/10.1016/j.actaastro.2008.02.006.
18. YONGKANG, T., FENG, G., SHUANGSHENG, G., FANG, L. Effects of hypobaria and hypoxia on seed germination of six plant species. In: *Life Sciences in Space Research*, 2014, *V. 3,* pp. 24-31. doi.org/10.1016/j.lssr.2014.08.001.
19. LOZOVANU, P. Technology of preparation of C60 fullerenes. *Physics and Modern Technologies* 2005, 9*(1-2),* pp. 25-34.
20. LOZOVANU, P., MOŞNEAGA, A., ZICHIL, V. Soot processing technology containing carbon clusters of a lower order than fullerenes in voltaic arc. In: *Integration through research and innovation*. 2013, pp.118-119.
21. ESPIRITO SANTO PEREIRA, A., CAIXETA OLIVEIRA, H., FERNANDES FRACETO, L., SANTAELLA, C. Nanotechnology potential in seed priming for sustainable agriculture. *Nanomaterials* 2021, *11*, 267. doi.org/10.3390/nano11020267.
22. NONOGAKI, H. Seed dormancy and germination—emerging mechanisms and new hypotheses. *Front. Plant Sci*. 2014, 5. doi.org/10.3389/fpls.2014.00233.
23. NONOGAKI, H., BASSEL, G.W., BEWLEY, J.D. Germination—Still a Mystery. *Plant Sci.* 2010, *179*, pp. 574-581. doi.org/10.1016/j.plantsci.2010.02.010.