

EXTRACTS DERIVED FROM SPIRULINA CULTIVATED ON MEDIA WITH Cu AND CuO NANOPARTICLES AS ACTIVE AGENTS FOR TRITICALE SEED PRIMING

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Summary. This study investigates the effects of seed priming in triticale using extracts from *Arthrospira platensis* cultivated with copper nanoparticles (CuNP) and copper oxide nanoparticles (CuONP). Results demonstrate significant increases in chlorophyll, carotenoid, and phenolic content, alongside enhanced antioxidant activity in the leaves. Notably, the treatment with nanoparticle-enriched extracts reduced malondialdehyde (MDA) levels, indicating a protective effect against oxidative stress. The findings suggest that spirulina-based extracts, even with the addition of nanoparticles, can enhance crop health, offering a sustainable approach to agriculture.

Keywords: priming, triticale, *Arthrospira platensis* (spirulina) extract, CuNP, CuONP, pigments, malondialdehyde.

Introduction

Microalgae and cyanobacteria are seen as the future of agricultural production due to their high growth rates, efficiency in solar energy conversion, and ability to recover nutrients from wastewater [13]. They produce biomolecules such as phytohormones, amino acids, and vitamins that enhance plant growth and productivity [19]. As biofertilizers and soil conditioners, microalgae improve soil fertility and transform agriculture into a more sustainable practice by reducing the need for chemical fertilizers and increasing plant tolerance to abiotic stresses [11, 16]. These characteristics make microalgae a promising and innovative resource for sustainable agricultural production [2].

Microalgae also serve as biostimulants, enhancing plant resistance to stresses like drought, salinity, and temperature extremes through antioxidant defense and adaptation mechanisms [4]. Their natural antioxidants protect plants from oxidative stress, promoting healthier growth [9]. Using microalgae extracts for seed priming improves germination rates and growth [21]. For instance, *Chlorella vulgaris* and *Scenedesmus quadricauda* extracts boost germination and plant vigor [17], while *Spirulina platensis* extracts enhance root development and vegetative mass [5]. Priming with *Dunaliella salina* extracts can improve salt stress response and germination rates [4].

Nanoparticle priming is an emerging technique using nanoparticles to enhance seed germination and plant growth by stimulating physiological and biochemical processes [3]. Nanoparticles provide essential microelements, improving early growth and resistance to abiotic stresses like drought and salinity [18]. Common nanoparticles include silver, silicon, and zinc oxide, each affecting plants based on their chemical and physical properties [7, 8]. Further research is needed to understand the long-term impacts of nanoparticles on the environment and human health [7, 12, 14].

This study explores an alternative seed priming method for triticale using products derived from *Arthrospira platensis* grown on media supplemented with copper and copper oxide nanoparticles, avoiding direct seed contact with nanoparticles.

Materials and methods

The *Arthrospira platensis* CNMN-CB-02 (spirulina) strain, stored in the National Collection of Non-Pathogenic Microorganisms at the Institute of Microbiology and Biotechnology, Technical University of Moldova, was used in this study as a source of biologically active compounds.

A. platensis was cultivated in an optimized mineral medium [20] supplemented with Cu nanoparticles (CuNP) and CuO nanoparticles (CuONP) (Merck KGaA, Darmstadt, Germany) of 100 nm in size, at concentrations of 10, 15, and 20 mg/L. Cultivation was carried out under autotrophic conditions, with continuous lighting and temperature conditions of 26-28°C. The cultivation cycle lasted 6 days. At the end of the cultivation period, the biomass was separated from the culture liquid by filtration.

To prepare the biostimulant extracts, 100 mg of native biomass obtained from cultivation of *A. platensis* in the presence of 100 nm CuNP and CuONP at concentrations of 10, 15, and 20 mg/L were mixed with 10 mL of distilled water. The mixtures were placed in a water bath at 60°C for 60 min. The extracts were then separated from the biomass by centrifugation and standardized to 10 mg/mL of dry substance.

In the experiments with triticale, seeds of the Kostel variety, belonging to the *Erythrospermum* type (author *Galina Lupascu, dr. hab., prof.*), were obtained from the Institute of Genetics, Physiology, and Plant Protection, USM, Moldova, were subjected to a 3-hour priming procedure using a 10 mg/L *A. platensis* extract solution. The seeds were then rinsed with distilled water, dried, and planted in soil. Before planting, the soil was homogenized, placed in experimental containers, and evenly watered. The seeds were uniformly distributed on the soil surface at a depth twice their diameter and covered with a thin layer of soil to ensure proper germination. The experimental growth cycle lasted 21 days. At the end of the experiment, triticale leaves were collected and used for biochemical assays.

Pigments were extracted from fresh triticale leaf biomass using 80% acetone. The absorbance of the extract was measured at 470 nm, 645 nm, and 663 nm using a spectrophotometer. The total chlorophyll and carotenoid content were calculated in mg/100 g of biomass. A 50% hydro-ethanolic solution was used to prepare the leaf extract for the assessment, of antioxidant activity. The reduction of the radical 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was monitored over 6 minutes, and antioxidant activity was expressed as the % ABTS inhibition.

The total phenolic content of the leaf extracts was determined using the Folin-Ciocalteu spectrophotometric method. A mixture of the leaf extract, Folin-Ciocalteu reagent, and sodium carbonate solution was prepared and incubated for 30 minutes at room temperature. The absorbance was then measured at 765 nm. The phenolic content was quantified as gallic acid equivalents per 100 g leaf material.

Malondialdehyde (MDA) content was measured using the thiobarbituric acid (TBA) assay to assess lipid peroxidation. A 150 mg sample of leaves was macerated, and 3 mL of a reagent solution containing 0.25% TBA in 10% trichloroacetic acid was added. The mixture was heated at 95°C for 30 minutes, then cooled and centrifuged. Absorbance was recorded at 532 nm and 600 nm, and MDA concentration was calculated using the extinction coefficient.

The data were statistically analyzed by calculating the arithmetic mean, standard error, and confidence interval, applying a parametric t-test with a significance threshold of $p < 0.05$.

Results and discussion

One of the goals of seed priming is to enhance plant resistance by stimulating metabolism and increasing photosynthesis. The chlorophyll content in triticale leaves was determined and analyzed (Fig. 1A). The spirulina extract, used as a positive control, resulted in a 12.3% increase ($p < 0.05$) in total chlorophyll content. Spirulina extracts cultured in the presence of CuNP at a concentration of 15 mg/L reduced the total chlorophyll content in triticale leaves with 15.08% ($p < 0.05$).

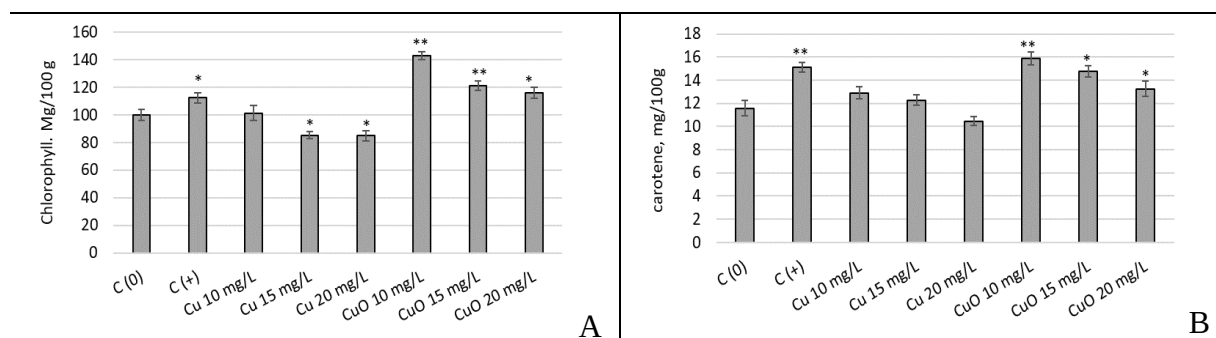


Figure 1. The effect of seed priming in triticale leaves with extracts from spirulina cultured in media supplemented with CuNP and CuONP on chlorophyll (A) and carotenoid (B) accumulation in leaves. C(0) – negative control, purified water; C(+) – positive control, extract from *A. platensis*; * $p < 0.05$; ** $p < 0.01$.

The extracts from spirulina cultured in media supplemented with CuONP significantly increased chlorophyll content in triticale leaves with 42.8% ($p < 0.01$) compared to C(0) and with 27.2% ($p < 0.05$) compared to C(+). These results highlight the impact of CuONP on the bioactive content of *A. platensis* biomass. For the extracts from spirulina cultured in the presence of CuONP at concentrations of 15 mg/L and 20 mg/L, the stimulatory effect was observed as an increase in chlorophyll content in leaves with 20.8% ($p < 0.01$) and 16.0% ($p < 0.05$), respectively, compared to the C(0) sample.

A 30.3% increase ($p < 0.01$) in carotenoid content was established in *Triticale* leaves in the samples primed with spirulina extract, the positive control variant (Fig. 1B). The extract from spirulina biomass obtained through cultivation with 10 mg/L CuNP resulted in an 11.24% increase in carotenoid content. The extracts from spirulina cultured with CuONP significantly increased carotenoid content, ranging from 37.4% ($p < 0.01$) to 14.5% ($p < 0.05$) compared to the negative control. These results suggest a synergistic effect between spirulina biomolecules and nanoparticles, influencing the composition of cyanobacterial biomass.

Similar effects on enhancing photosynthetic activity were observed in the priming of *Hibiscus sabdariffa* plants with 0.01% Al_2O_3 NPs, which led to increases in chlorophyll content with 12.90%, chlorophyll b by 16.32%, and carotenoids by 31.13% compared to the control [1]. Seed pretreatment of *Citrullus lanatus* with 20 mg/L MnONP significantly increased chlorophyll content [6]. The application of AgNP for seed priming of *Capsicum annuum* L. led to significant accumulations in chlorophyll (51-142%) and carotenoids (23-94.2%), as well as total phenol content (73%) in all plant tissues. Seed priming of *Capsicum annuum* with CuNP at concentrations 1, 10, and 20 ppm increased chlorophyll content by up to 142% and carotenoids by up to 94.2% [10]. Various concentrations of *S. platensis* extract were tested to evaluate the effects on the growth, pigments, and photosynthetic capacity of *Lupinus luteus*. Application of a 0.25% extract significantly increased chlorophyll a, b, and carotenoid content, which enhanced photosynthetic capacity and, in turn, improved plant growth and yield. In contrast, the 1.0% concentration had an inhibitory effect, reducing growth and yield parameters [21].

Seed priming of triticale with extracts from spirulina cultured in media supplemented with CuNP and CuONPs led to an increase in phenolic content in the leaves (Fig. 2A). The highest increases were observed with extracts from spirulina cultured with 10 mg/L CuNP, resulting in a 46% increase in carotenoid content ($p < 0.01$), and with CuONP, resulting in a 43.1% increase in carotenoid content ($p < 0.05$).

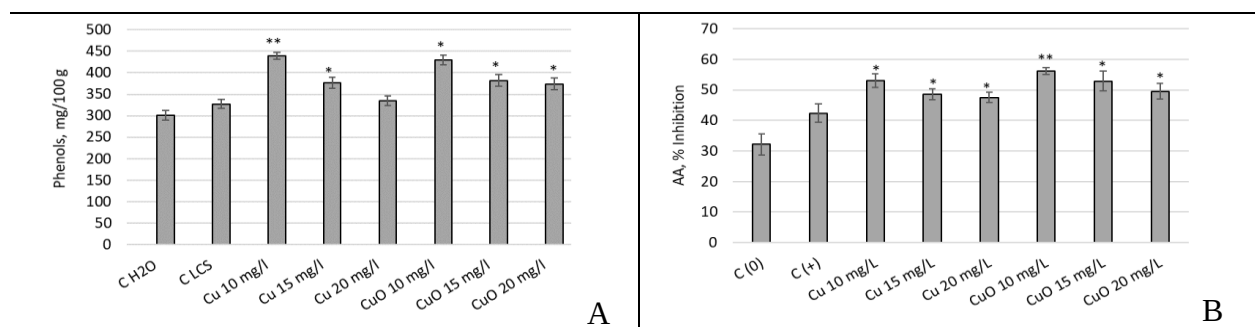


Figure 2. The effect of seed priming in triticale leaves with extracts from spirulina cultured in media supplemented with CuNP and CuONP on phenolic accumulation (A) in leaves and antioxidant activity (B). C(0) – negative control, purified water; C(+) – positive control, extract from *A. platensis*; * $p < 0.05$; ** $p < 0.01$.

In all experimental variants, an increase in antioxidant activity was observed (Fig. 2B). Seed pretreatment with spirulina extract led to a 32% increase in antioxidant capacity. Extracts from spirulina biomass cultured with CuNP increased the antioxidant activity in triticale leaves with 65.3% ($p < 0.05$) to 48% ($p < 0.05$) compared to C(0) and with 25.2% ($p < 0.01$) to 12.1% compared to C(+). Extracts from spirulina biomass obtained from CuONP experiments resulted in an increase in antioxidant activity with 54.2% ($p < 0.01$) to 74.7% ($p < 0.01$) compared to C(0) and with 16.8% ($p < 0.05$) to 32.3% ($p < 0.01$) compared to C(+).

Phenols are compounds that significantly contribute to plant stress tolerance. It was determined that MnONP at a concentration of 80 mg/L significantly increased phenolic content in *Citrullus lanatus* plants but significantly reduced antioxidant activity [6]. CuNP enhanced total antioxidant activity, with increases of 14.06%, 82.07%, and 143.01% observed at 20, 60, and 80 ppm, respectively [10].

An indicator of crop resilience is the maintenance of cellular membrane integrity, monitored by determining malondialdehyde levels (Fig. 3). In samples obtained through seed priming of triticale with spirulina extracts cultured in media supplemented with 20 mg/L CuNP, MDA levels were 24.5% ($p < 0.05$) lower compared to the negative control.

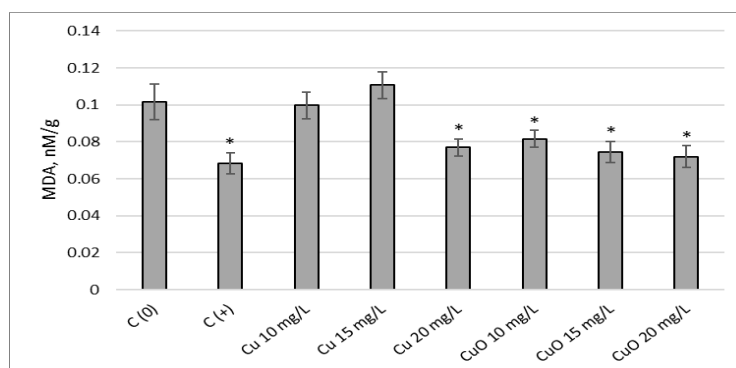


Figure 3. The effect of seed priming in triticale leaves with extracts from spirulina cultured in media supplemented with CuNP and CuONP on malondialdehyde content in the leaves. C(0) – negative control, purified water; C(+) – positive control, extract from *A. platensis*; * $p < 0.05$.

In samples treated with extracts from spirulina cultured in media supplemented with CuONP at concentrations of 10, 15, and 20 mg/L, MDA levels were reduced with 19.9% ($p < 0.05$) to 29.4% ($p < 0.05$) compared to C(0). These results are similar to those obtained with the extract from spirulina cultured without nanoparticles, which reduced MDA accumulation by 32.2% ($p < 0.05$). The effect of nanoparticles on MDA levels was also observed in foliar exposure to AgNP in *Mentha spicata* [14]. Seed priming of *Hibiscus sabdariffa* with 0.01% Al₂O₃ NPs resulted in an 18.74% reduction in MDA content, suggesting a protective effect of these nanoparticles against oxidative stress [1].

Therefore, the results obtained using extracts from the cyanobacterium *Arthrospira platensis* cultured in media supplemented with Cu and CuO nanoparticles as active agents for seed priming of triticale demonstrate that the stimulatory effect can be achieved even without direct contact with the nanoparticles.

Conclusions

The study demonstrated that seed priming of triticale with extracts from the cyanobacterium *Arthrospira platensis* cultured in media supplemented with CuNP and CuONP yields significant benefits for plant health and productivity. These treatments increase chlorophyll, carotenoid, and phenolic content, and enhance the antioxidant activity of triticale without direct contact with the nanoparticles. Extracts from spirulina cultured in the presence of CuNP and CuONP significantly reduced MDA levels, indicating a protective effect against oxidative stress. This approach offers a sustainable method for improving crop performance without the risks associated with the direct use of nanoparticles.

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References

1. Abdel Latef, A. A. H., Zaid, A., Abu Alhmad, M. F., & Abdelfattah, K. E. The impact of priming with Al₂O₃ nanoparticles on growth, pigments, osmolytes, and antioxidant enzymes of Egyptian Roselle (*Hibiscus sabdariffa* L.) cultivation. *Agronomy*. 2020;10(5):681.
2. Bulgariu, L., et al. Microalgae – An innovative resource for agriculture. *Sustainability*. 2019;11,1-15. <https://doi.org/10.3390/su11010205>
3. Donia, D.T., Carbone, M. Seed Priming with Zinc Oxide Nanoparticles to Enhance Crop Tolerance to Environmental Stresses. *Int. J. Mol. Sci.* 2023; 24:17612. <https://doi.org/10.3390/ijms242417612>
4. El Arroussi, H., Benhima, R., El Mernissi, N., Bennis, I., and Wahby, I. *Dunaliella salina* exopolysaccharides: A promising biostimulant for salt stress tolerance in tomato and pepper plants. *Journal of Applied Phycology*. 2018;30(5):2929–2941. <https://doi.org/10.1007/s10811-017-1382-1>
5. Gemies, L.S.M., Elsadany, A.Y. Maximizing growth and productivity of onion (*Allium cepa* L.) by *Spirulina platensis* extract and nitrogen-fixing endophyte *Pseudomonas stutzeri*. *Arch. Microbiol.* 2021, 203:169–181.
6. Kasote, D.M., Lee, J.H.J., Jayaprakasha, G.K., & Patil, B.S. Manganese oxide nanoparticles as safer seed priming agent to improve chlorophyll and antioxidant profiles in watermelon seedlings. *Nanomaterials*. 2021;11(4):1016. <https://doi.org/10.3390/nano11041016>
7. Khan, F., Pandey, P., Upadhyay, T.K. Applications of Nanotechnology-Based Agrochemicals in Food Security and Sustainable Agriculture: An Overview. *Agriculture*. 2022; 12:1672. <https://doi.org/10.3390/agriculture12101672>

8. Kim, S. J., Ko, E. J., Hong, J. K., and Jeun, Y. C. Ultrastructures of colletotrichum orbiculare in cucumber leaves expressing systemic acquired resistance mediated by chlorella fusca. *Plant Pathol. J.* 2018;34 (2):113. <https://doi.org/10.5423/PPJ.OA.09.2017.0204>
9. Kusvuran, S. Microalgae (*Chlorella vulgaris* beijeirinck) alleviates drought stress of broccoli plants by improving nutrient uptake, secondary metabolites, and antioxidative defense system. *Hortic. Plant J.* 2021; 7(3):221-231. <https://doi.org/10.1016/j.hpj.2021.03.007>
10. Mawale KS, Nandini B, Giridhar P. Copper and Silver Nanoparticle Seed Priming and Foliar Spray Modulate Plant Growth and Thrips Infestation in *Capsicum* spp. *ACS Omega.* 2024;9(3):3430-3444. <https://doi.org/10.1021/acsomega.3c06961>. PMID: 38284086; PMCID: PMC10809252
11. Mutale-Joan, C., Redouane, B., Najib, E., Yassine, K., Lyamlouli, K., Laila, S., et al. Screening of microalgae liquid extracts for their bio stimulant properties on plant growth, nutrient uptake and metabolite profile of *Solanum lycopersicum* L. *Sci. Rep.* 2020;10(1):1-12. <https://doi.org/10.1038/s41598-020-59840-4>
12. Paramo LA, Feregrino-Pérez AA, Guevara R, Mendoza S, Esquivel K. Nanoparticles in Agroindustry: Applications, Toxicity, Challenges, and Trends. *Nanomaterials (Basel).* 2020;10(9):1654. <https://doi.org/10.3390/nano10091654>. PMID: 32842495; PMCID: PMC7558820.
13. Parmar P, Kumar R, Neha Y and Srivatsan V Microalgae as next generation plant growth additives: Functions, applications, challenges and circular bioeconomy based solutions. *Front. Plant Sci.* 2023;14:1073546. <https://doi.org/10.3389/fpls.2023.1073546>
14. Peshkova, A., Ziniovcsaia, I., Cepoi, L., Rudi, L., Chiriatic, T., Yushin, N., & Sohatsky, A. Features of copper and gold nanoparticle translocation in *Petroselinum crispum* segments. *Nanomaterials.* 2023;13(1754). <https://doi.org/10.3390/nano13111754>
15. Peshkova, A., Ziniovcsaia, I., Cepoi, L., Rudi, L., Chiriatic, T., Yushin, N., Anh, T. T., Manh Dung, H., & Corcimaru, S. Effects of gold nanoparticles on *Mentha spicata* L., soil microbiota, and human health risks: Impact of exposure routes. *Nanomaterials.* 2024;14:955. <https://doi.org/10.3390/nano14110955>
16. Prisa, D.; Spagnuolo, D. Plant Production with Microalgal Biostimulants. *Horticulturae.* 2023; 9(7):829. <https://doi.org/10.3390/horticulturae9070829>
17. Puglisi, I., Barone, V., Fragalà, F., Stevanato, P., Baglieri, A., and Vitale, A. Effect of microalgal extracts from *Chlorella vulgaris* and *Scenedesmus quadricauda* on germination of *Beta vulgaris* seeds. *Plants.* 2020;9(6):675. <https://doi.org/10.3390/plants9060675>
18. Raliya, R., Nair, R., Chavalmane, S., Wang, W.-N., and Biswas, P. Synergistic effect of titanium dioxide and zinc oxide nanoparticles on soybean plant growth under soil moisture stress. *Journal of Nanoparticle Research.* 2016;18(1):1-13. <https://doi.org/10.1007/s11051-015-3325-5>.
19. Ronga, D.; Biazzi, E.; Parati, K.; Carminati, D.; Carminati, E.; Tava, A. Microalgal biostimulants and biofertilisers in crop productions. *Agronomy.* 2019;9:192. <https://doi.org/10.3390/agronomy9040192>.
20. Rudi, L., Chiriatic, T., Cepoi, L., Miscu, V., Ziniovcsaia, I., Valuta, A., & Rudic, V. Biomass production and pigment content in *Arthrospira platensis* by adding AuNP (PEG) and AgNP (PEG) at different growth phases of the cultivation cycle. *The Annals of Oradea University, Biology Fascicle.* 2021;28(2):143-151. <http://www.bioresearch.ro/revistaen.html>
21. Rupawalla, T., et al. Priming of spinach seeds with microalgae extracts enhances germination and seedling growth. *J Appl Phycol.* 2022;34:2015–2026. <https://doi.org/10.1007/s10811-022-02770-7>
22. Shedeed, Z. A., Gheda, S., Elsanadily, S., Alharbi, K., & Osman, M. E. H. *Spirulina platensis* biofertilization for enhancing growth, photosynthetic capacity and yield of *Lupinus luteus*. *Agriculture.* 2020; 12(6):781. <https://doi.org/10.3390/agriculture12060781>