# USE OF *CHLORELLA VULGARIS* SUSPENSION AS BIOFERTILIZER IN SUNFLOWER CULTIVATION IN THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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**ABSTRACT**
The present study evaluates the effect of *Chlorella vulgaris* suspension used as a biofertilizer in sunflower (*Helianthus annuus L.*) cultivation under field conditions in the Republic of Moldova during the 2025 agricultural year. The experiment included pre-sowing seed treatment, soil application, and foliar treatments with algal suspension, compared with a control treated with distilled water.
Morpho-physiological and biochemical parameters were analyzed, including field emergence, plant height at different ontogenetic stages, yield, and seed quality indicators. Results showed that *Chlorella vulgaris* positively influenced early plant development, increasing field emergence by 4.90% (relative growth of 5.93%).
Treated plants showed improved growth during early ontogenetic stages, while final yield increased by about 10%. Significant improvements were also observed in seed quality, particularly oleic acid content (+29.16%), oil content (+4.14%), and protein content (+2.11%).
These findings highlight the potential of Chlorella vulgaris as an effective and sustainable biofertilizer that enhances sunflower productivity and quality while supporting environmentally friendly agricultural practices.
**Keywords:** *Chlorella vulgaris*, biofertilizers, sunflower, crops.

## 1. INTRODUCTION
Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops worldwide, ranking after soybean, oil palm, and canola [1]. Its seeds are valued for their proteins, fibers, minerals, and phenolic compounds, which explains its wide cultivation across diverse agroecological regions [5].
Efficient fertilization is essential for sunflower production. Conventional agriculture mainly relies on mineral fertilizers (N, P, K), applied according to soil type and environmental conditions [8, 14]. Although these inputs sustain high yields, long-term use may cause soil degradation, reduced biological activity, lower humus content, and environmental pollution.
Biofertilizers and biostimulants are sustainable alternatives that improve nutrient availability and stimulate plant growth through biological processes [2]. Among them, microalgae have gained attention for their multifunctional role in soil–plant systems, contributing to better soil structure, nutrient cycling, water retention, and growth stimulation through bioactive compounds [4, 6, 10, 12, 15, 16]. One of the most studied microalgae is *Chlorella vulgaris*, widely used as a biostimulant due to its rapid growth, metabolic versatility, and rich biochemical composition. Its biomass provides organic matter and bioactive compounds that may enhance soil fertility and plant development [18]. Therefore, this study aimed to evaluate the effect of *Chlorella vulgaris* suspension on field emergence, growth, and selected quality parameters of sunflower seeds.

## 2. MATERIALS AND METHODS

The experiments were conducted using a *Chlorella vulgaris* strain maintained in the collection of the Scientific Research Laboratory of Algology "V. Șalaru" at Moldova State University. An algal suspension in the exponential growth phase was applied, with a concentration of 2.98 g/L absolutely dry biomass. Field trials were performed during the 2025 agricultural year, following classical experimental design methods [17], on farmland near Hîrtopul Mic, managed by SRL Schim Agromex. The experimental soils were classified as typical moderately humus-rich chernozems.

Seeds of *Helianthus annuus* L. were treated before sowing by spraying with an algal suspension, while distilled water was used for the control. Seeds were then sown at a depth of 3 cm according to standard agricultural practices. Each experimental variant covered 100 ha.

The algal suspension was applied to the soil immediately after sowing at 100 L/ha. Three additional foliar treatments were performed at key ontogenetic stages, each at 5 L/ha.

In both variants, soil fertilization was identical. In autumn 2024, pea green manure was incorporated into the soil. In spring 2025, 2000 kg/ha of solid fertilizer and 5 L/ha of liquid bioorganomineral fertilizer were applied, with chemical composition described in specialized literature [9].

Field emergence was determined under field conditions using representative sampling plots. In each experimental variant, three randomly selected plots of 1 m² were used to record the total number of sown seeds and emerged plants. Field emergence was calculated as the percentage of emerged plants relative to the total number of sown seeds.

Plant morphological changes were evaluated through field measurements at different ontogenetic stages until harvest. The main parameters assessed (in 20 replicates) were plant height, stem diameter, leaf development, and capitulum size.

Seed quality was determined using the Spectra Star XT Analyzer. Data processing included calculation of the arithmetic mean (M), standard error (m), differences compared with the control, relative increase, Student's t-test, degrees of freedom (df), probability of differences (p) and effect size (Cohen's d). All determinations were performed in 5 replicates under comparable conditions for both experimental variants to ensure the reliability and relevance of the results.

## 3. RESULTS AND DISCUSSION

The results obtained in this study are aligned with the main objective of evaluating the effect of *Chlorella vulgaris* on the early stages of development of *Helianthus annuus* L., particularly on seed field emergence and initial plant establishment under field conditions.

As shown in Table 1, the application of *Chlorella vulgaris* suspension increased the field emergence of sunflower seeds compared with the control. The difference was +4.90%, corresponding to a relative increase of 5.93%. Statistical analysis showed a Student's t-test value of 8.77, with df = 398 and $p < 0.0001$, indicating a highly significant difference between variants. The effect size, expressed as Cohen's d, reached 0.88, reflecting a strong biological effect.

These results demonstrate that *Chlorella vulgaris* suspension positively influenced seed field emergence and improved crop establishment at early growth stages. The data confirm the biostimulant role of algal biomass in enhancing the initial development of sunflower plants.

The morphological analysis of sunflower plant height (Table 2) indicates that the application of *Chlorella vulgaris* had a positive effect on plant growth, particularly in the early ontogenetic stages.

**Table 1.** Influence of *Chlorella vulgaris* suspension on sunflower field emergence, %

| Experimental variants | Field emergence (%) (M±m) | Difference from control (%) | Relative growth (%) | t | df | p | Cohen's d |
|---|---|---|---|---|---|---|---|
| Control | 82,60±0,40 | - | - | - | - | - | - |
| Algal treatment | 87,50±0,39 | +4,90 | 5,93 | 8,77 | 398 | <0,0001 | 0,88 |

**Table 2.** Influence of *Chlorella vulgaris* suspension on sunflower plant height, cm

| Growth stage | Control (M±m) | Algal treatment (M±m) | Difference (cm) | t | df | p | Cohen's d |
|---|---|---|---|---|---|---|---|
| Complete emergence | 5,01±0,03 | 5,50±0,02 | 0,49 | 13,59 | 398 | 0,0000 | 1,36 |
| Two pairs of leaves | 11,05±0,50 | 13,08±0,71 | 2,03 | 2,34 | 398 | 0,0199 | 0,23 |
| Bud stage | 65,20±2,13 | 68,45±2,45 | 3,25 | 1,00 | 398 | 0,3174 | 0,10 |
| Flower | 152,35±10,21 | 157,42±11,21 | 5,07 | 0,33 | 398 | 0,7383 | 0,03 |
| Full maturity | 165,60±10,14 | 168,30±10,45 | 2,70 | 0,19 | 398 | 0,8530 | 0,02 |

During complete emergence, plant height in the treated variant (5.50 ± 0.02 cm) was significantly higher than in the control (5.01 ± 0.03 cm), with a difference of 0.49 cm ($t = 13.59$, $p < 0.0001$, Cohen's $d = 1.36$), indicating a strong effect. At the stage of two pairs of true leaves, treated plants also showed significantly greater height, with a difference of 2.03 cm ($p = 0.0199$), although the effect size was relatively small (Cohen's $d = 0.23$). In the subsequent stages (bud formation, flowering, and full maturity), the treated variant maintained higher average values, but differences were not statistically significant ($p > 0.05$), and effect sizes were low. This suggests that the stimulatory effect of *Chlorella vulgaris* suspension was more pronounced during early plant development and diminished at later stages, likely under the influence of abiotic factors such as reduced soil moisture.

The application of *Chlorella vulgaris* had a beneficial effect on yield, which increased by approximately 10% (Fig. 1), and modified the biochemical composition of sunflower seeds (Table 3). The most representative changes were recorded in palmitic acid (+3.04%), oleic acid (+29.16%), linoleic acid (+3.16%), protein (+2.11%), and oil content (+4.14%).

These results indicate that the application of *Chlorella vulgaris* suspension improves seed biochemical quality, with positive implications for the production of high-quality sunflower oil.

**Table 3.** Effect of applying *Chlorella vulgaris* suspension on sunflower seed quality, %

| Indicator | Control (M±m) | Algae (M±m) | Absolute difference | Relative difference (%) | t | df | p | Cohen's d |
|---|---|---|---|---|---|---|---|---|
| Moisture | 5,77±0,17 | 5,68±0,02 | -0,09 | -1,56 | -0,51 | 4 | 0,6363 | -0,42 |
| Palmitic acid | 7,89±0,30 | 8,13±0,39 | 0,24 | 3,04 | 0,49 | 4 | 0,6512 | 0,40 |
| Stearic acid | 7,93±0,17 | 7,90±0,28 | -0,03 | -0,38 | -0,09 | 4 | 0,9314 | -0,07 |
| Oleic acid | 17,49±0,31 | 22,59±0,21 | 5,10 | 29,16 | 13,62 | 4 | 0,0002 | 11,12 |
| Acid linoleic | 68,28±2,40 | 70,44±2,39 | 2,16 | 3,16 | 0,64 | 4 | 0,5583 | 0,52 |
| Protein | 14,66±0,06 | 14,97±0,12 | 0,31 | 2,11 | 2,31 | 4 | 0,0820 | 1,89 |
| Oil | 42,56±0,19 | 44,32±0,20 | 1,76 | 4,14 | 6,38 | 4 | 0,0031 | 5,21 |

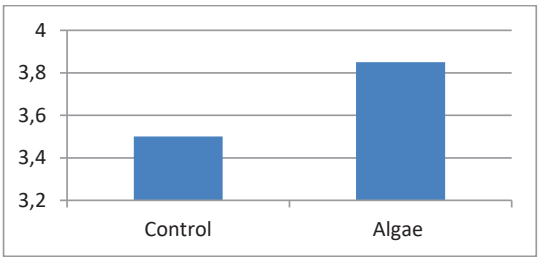


**Figure1.** Influence of *Chlorella vulgaris* suspension application on sunflower yield, t/ha

According to numerous studies, *Chlorella vulgaris* contains various biologically active compounds, including proteins, enzymes, antioxidants, and phytohormones, which stimulate plant growth, regulate nutrient uptake, enhance plant immunity, and improve tolerance to stress factors [3]. Previous studies reported that its application to *Lactuca sativa* seeds stimulated germination and increased plant growth and biomass [7]. Likewise, in tomato cultivation, *Chlorella vulgaris* treatment promoted plant height and improved biochemical traits, particularly chlorophyll a, b, and carotenoid contents [11]. The results obtained in the present study are consistent with these findings, confirming the positive biostimulant effect of *Chlorella vulgaris*. Similar to previous reports, our research demonstrated enhanced seed germination, improved plant growth, increased yield, and better seed quality, highlighting the broad applicability of this microalga in sustainable crop production.

## 5. CONCLUSIONS

The results obtained in this study demonstrate that the application of *Chlorella vulgaris* suspension as a biofertilizer has a positive effect on sunflower cultivation under the conditions of the Republic of Moldova. The treatment increased field emergence by 4.90%, indicating improved seed germination and better crop establishment during the early growth stages.

The influence of the algal suspension on plant growth was more pronounced during the initial ontogenetic phases, when significant increases in plant height were observed. In later developmental stages, although treated plants maintained slightly higher values, the differences were not statistically significant, suggesting that environmental factors may have influenced growth dynamics.

The beneficial impact of *Chlorella vulgaris* on sunflower yield highlights the practical importance of this treatment in agricultural production systems. Furthermore, important improvements in seed quality were recorded, particularly in oleic acid, protein, and oil contents, which are essential parameters for the nutritional and industrial value of sunflower.

Overall, the findings confirm that *Chlorella vulgaris* suspension can be successfully used as a sustainable biofertilizer, contributing to enhanced productivity and quality of sunflower crops. Its use represents a promising approach for reducing dependence on chemical fertilizers and promoting environmentally friendly agricultural practices.

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## DECLARATIONS

The authors declare that they have no conflict of interest.

*Author Contributions*: The authors contributed equally to the research presented in the article.

*Data Availability Statement:* The datasets generated during the current study are available from the corresponding author upon reasonable request.
*Ethics Statement:* Not applicable,
*Originality Statement:* The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

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