Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RECYCLING OF WASTEWATER FROM THE CULTIVATION OF *Spirulina platensis* THROUGH ITS USE AS A BIOSTIMULANT FOR THE GERMINATION OF *Phacelia tanacetifolia* Benth. (Melifera) SEEDS MAINTAINED IN COLLECTIONS

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Abstract

This article presents the experimental results obtained from the application of a biostimulant based on wastewater derived from the cultivation of the cyanobacterium Spirulina platensis in various concentrations, on the germination of Phacelia tanacetifolia (Melifera) seeds, maintained under collection conditions for 1 and 4 years. The results show that the seeds treated with biostimulants have a higher germination capacity compared to those in the control group, where germination ranged from 28-40%. The highest germination rates were obtained for the 1-year-old seeds (56%) treated for 1 hour with a biostimulant at a 2% concentration, while for the 4-year-old seeds (54%), the best results were recorded at a 1% concentration with a 1-hour treatment period. The application of the biostimulant contributed to the relative elongation of the root system of the studied culture. Based on the conducted research, we can conclude that the application of the investigated biostimulant has the effect of stimulating seed germination and root elongation in seedlings, which provides grounds to consider that it demonstrates a positive effect and could be practically applied.

Key words*: wastewater, biostimulator, Spirulina platensis, Phacelia tanacetifolia (Melifera).*

INTRODUCTION

The cyanobacteria *Spirulina platensis* is one of the most effective and applicable species in agriculture due to the high content of biologically active substances with a biostimulatory effect, the high growth rate and the possibilities of industrial cultivation. Spirulina can be applied as a biostimulator in the cultivation of crops and medicinal plants starting with the vegetative stage and until the end of the generative stage, resulting in positive effects both quantitatively and qualitatively.

Research conducted by Nguyen Quang Thinh highlighted the fact that treating *Vigna mungo* L. seeds before sowing, for 3 hours, with extract obtained from *Spirulina platensis* biomass at a concentration of 1.5%, highlighted the fact that it contributed to increasing the gemination rate by 17%, root elongation by 1.48 cm, shoot length by 1.53 cm, dry matter by 13.10 mg/10 seedlings, increasing the content of proteins, gibberellic acids, α-amylase, dehydrogenase activity accompanied by a decrease in lipid peroxidation and free sugar (Thinh, 2021).

Other research has demonstrated that treating Chilli, Tomato, Carrot, Bean, Maize, Paddy and Ragi seeds, for 12 hours, with *Spirulina platensis* biomass suspended in distilled water in various concentrations had the effect of stimulating seed germination, increasing seedling vigor, creating premises for crop growth and productivity (Basavaraja et al., 2023).

Other research has highlighted the fact that applying extracts obtained from *Spirulina platensis* biomass to the treatment of radish seeds has the effect of stimulating seed germination, increasing plant length and weight, chlorophyll, and increasing the content of nutrients in seedlings (Godlewska et al., 2019).

Treating chickpea seeds (*Cicer arietinum* L.*)* with *Spirulina* biomass at doses of 2%, 4%, 6%, 8% and 10% had the effect of stimulating seed germination, shoot growth and increasing protein content (Htwe et al., 2009).

Treating onion seeds before sowing with *Spirulina* sp. extract had the effect of increasing the germination rate and germination index (Neag et al., 2022).

The application of *Spirulina platensis* extracts in optimal concentrations of 25-75%, when treating wheat and barley seeds before sowing, had the effect of stimulating germination and seedling growth and development (Akgül, 2019).

Treating *Lactuca sativa* L. seeds before sowing with *Spirulina platensis* extract in optimal concentrations, also attested a positive effect on seed germination and plant growth-development (Fusun & Riza, 2019).

A major problem resulting from the cultivation process of *Spirulina platensis* is the wastewater that represents around 98-99.50% of the total volume of the suspension, these waters contain significant amounts of biologically active substances (micro and macroelements, metabolites, hormones, etc.) and are of interest for recycling as a biostimulator of plant seed germination.

Phacelia tanacetifolia is a plant species that is part of the *Hydrophyllaceae* family originating from the Americas, being brought to Europe in the last century and acclimatized for use as a fodder and pollinator plant, being increasingly used as a component of the cover crop in agriculture (Kilian, 2016). The plant is part of the top 20 flowers producing high-quality pollen for bees, being attractive to pollinating insects including bumblebees (Hayden, 2014). A plant has more than 5000 flowers and 500-1000 kg of honey is obtained from one hectare of *Phacelia tanacetifolia.* Phacelia has a positive impact on the soil due to the penetration of the crop's roots to depths of over 1 m, being considered a "green plow", and after harvesting the crop, the root residues accumulate significant amounts of organic matter and nitrogen, being considered an "organic fertilizer" and presenting considerable interest as a sidereal crop. Some research indicates that the *Phacelia tanacetifolia* variety has a yield of 25.000 kg/ha green mass and over 7.000 kg/ha dry mass (Popović et al., 2016).

To ensure high growth and obtain large amounts of biomass, it is necessary to treat the seeds preventively. Thus, we aimed to evaluate the impact of the biostimulator obtained from the wastewater generated from the cultivation of the cyanobacterium *Spirulina platensis* on the germination process of *Phacelia tanacetifolia* Benth. (Melifera) seeds.

MATERIALS AND METHODS

The experiments used the strain of cyanobacterium *Spirulina platensis* selected in culture by m.c., prof. Vasile Șalaru, acad., prof. Valeriu Rudic and co-authors. The strain of *Spirulina platensis,* stored in the collection of LCȘ "Algologie Vasile Șalaru" of the Moldova State University, was cultivated on modified Zarrouk liquid nutrient medium (with the following composition (g/l): $NaHCO_3$ - 8; K_3HPO_4 - 0.5; $NaNO_3$ - 2.5; K_2SO_4 - 1; NaCl - 1; $MgSO_4$ - 0.2; $CaCl_2$ - 0.04; $FeSO_4$ - 0.01; NaEDTA - 0.08; drinking water - 1 L), the inoculum constituting 0.5 g/l (absolutely dry biomass). On the 20th day of cultivation of the cyanobacterium *Spirulina platensis,* the algal biomass was separated from the culture liquid by centrifugation (at a speed of 6000 rpm), and the culture liquid (waste water) was used to obtain the biostimulant applied in the experiments. The cyanobacterial biostimulant was obtained by thermal activation of the separated culture liquid.

The experiments used solutions with concentrations of 1-4% obtained by diluting the culture liquid generated from the cultivation of the cyanobacterium *Spirulina platensis* with distilled water. Seeds of *Phacelia tanacetifolia* Benth. (Melifera), maintained in collection conditions for 1-4 years, in the amount of 100 for each experimental batch, were exposed to the prepared solutions for 1-4 hours, and as a control the same seeds exposed to distilled water for the same time interval served. The experimental seeds of *Phacelia tanacetifolia* Benth. (Melifera) were made available by Dr. Victor Ţiţei, an employee of the Botanical Garden of the Al. Ciubotaru Institute of the State University of Moldova. To study the germination process, the seeds were placed in Petri dishes, on filter paper moistened with distilled water, under natural lighting and a temperature of 22°C. The following indicators were determined during the research:

- *Seed germination* (FG), according to the formula: FG = Nsg/Nts * 100, where Nsg - number of germinated seeds; Nts - total number of seeds;
- *Germination index* (IG), calculated on the 5th day of the experiment, according to the formula: $IG = \sum(Gt/Tt)$, where Gt - number of germinated seeds in the time period t, and Tt - number of days.
- *Relative root elongation* (ARR) calculated on the 7th day of germination, according to the

formula: ARR = (Le/Lc)*100, where: Le - root length in the experimental variant; Lc - root length in the control variant.

RESULTS AND DISCUSSIONS

It is well known that treating plant seeds before sowing increases the biological value of these seeds, plant resistance to diseases and crop yield (Siminel, 1999). Taking into account the fact that the cyanobacterium *Spirulina platensis* has a major content of biostimulating substances for seed germination and that a good part of them are found in the culture liquid, this liquid is of major interest for use as a bistimulator for seed germination of *Phacelia tanacetifolia* Benth.

Table 1. Germination capacity of *Phacelia tanacetifolia* Benth. (Melifera) seeds treated with biostimulator obtained from the culture liquid resulting from the cultivation of the cyanobacterium *Spirulina platensis*, %

Seed treatment period, hours	Experimental variants							
	1%		2%		4%		Control	
	1 - year	4 - years	1 - year	4 - years	1 - year	4 - years	1 - year	4 - years
1- a day								
1	4.0	36.0	18.0	34.0	4.0	32.0	6.0	22.0
2	6.0	36.0	6.0	38.0	18.0	20.0	4.0	16.0
4	8.0	28.0	10.0	36.0	4.0	30.0	6.0	24.0
2- a day								
1	30.0	36.0	46.0	34.0	38.0	32.0	14.0	22.0
2	28.0	36.0	32.0	38.0	40.0	20.0	26.0	16.0
4	34.0	28.0	32.0	36.0	34.0	30.0	36.0	24.0
3- a day								
1	60.0	38.0	56.0	46.0	44.0	44.0	34.0	32.0
2	32.0	50.0	38.0	44.0	40.0	28.0	32.0	28.0
4	34.0	40.0	38.0	42.0	44.0	36.0	36.0	24.0
4- a day								
1	60.0	40.0	56.0	46.0	48.0	44.0	40.0	32.0
2	40.0	54.0	42.0	44.0	44.0	30.0	36.0	28.0
4	42.0	40.0	40.0	46.0	46.0	38.0	41.0	34.0

The results of the analysis of the germination capacity of *Phacelia tanacetifolia* Benth. (Melifera) seeds indicate that the biostimulator obtained from the wastewater generated from the cultivation of the cyanobacterium Spirulina platensis has a stimulating effect on the germination of the analyzed seeds. The most significant results were obtained in the variants of 1-2% biostimulator with exposure to the treatment for 1 hour in the case of seeds maintained in collections for 1 year where 60-56% of all analyzed seeds germinated. Keeping *Phacelia tanacetifolia* Benth. (Melifera) seeds in collection conditions for 4 years has the effect of reducing their germination capacity. In this case too, the treatment of the seeds with the investigated biostimulator resulted in the germination of a larger number of seeds (up to 54%) and in the maximum control variant 34% of the total analyzed seeds germinated. In the case of older seeds, as in the case of newer ones, the most suitable variant proved to be 1% with exposure to the treatment for 2 hours (Table 1). *Spirulina* is rich in metabolites with bioregulatory functions (Mehmood et al., 2024). A good part of the metabolites of the cyanobacterium *S. platensis* are released into the cultivation medium, which gives it a pronounced biostimulatory effect on the germination of *Phacelia tanacetifolia* Benth. (Melifera) seeds investigated by us.

Table 2. Germination index values of *Phacelia tanacetifolia* Benth. (Melifera) seeds treated with biostimulator obtained from the culture liquid resulting from the cultivation of the cyanobacterium *Spirulina platensis*

Seed treatment period, hours	**Experimental variants**							
	1%		**2%**		**4%**		**Control**	
	1 - year	**4 - years**	**1 - year**	**4 - years**	**1 - year**	**4 - years**	**1 - year**	**4 - years**
1	11.2	8.0	9.2	9.2	9.6	8.8	8.0	6.4
2	8.0	10.8	8.4	8.8	8.8	6.0	7.2	5.6
4	8.4	8.0	8.0	9.2	9.2	7.6	8.2	6.8

The germination index values of *Phacelia tanacetifolia* Benth. (Melifera) seeds also indicate that treating the seeds with the biostimulator studied has positive effects. The highest values were observed in the variants of 1-2% biostimulator with exposure to the treatment for 1 hour. The seeds of *Phacelia tanacetifolia* Benth. (Melifera) treated with the biostimulator studied showed much higher germination index values compared to those in the control variant (Table 2). Similar results were also obtained in the case of treating the seeds of *Galega orientalis* L. maintained under collection conditions with the cultural liquid generated from the cultivation of the cyanobacterium *Spirulina platensis* (Dobrojan et at., 2024) (Table 3).

Table 3. Relative root elongation of *Phacelia tanacetifolia* Benth. (Melifera) treated with biostimulator from the culture liquid of the cyanobacterium *Spirulina platensis*, %

Seed treatment period, hours	Experimental variants					
	1%		2%		4%	
	1 - year	4 - years	1 - year	4 - years	1 - year	4 - years
1	127.45	94.94	152.94	84.81	141.17	82.28
2	78.92	73.08	99.21	82.05	105.97	101.28
4	72.34	134.32	87.23	100.0	102.13	126.86

The highest values of relative root elongation were attested in the case of young 1-year-old seeds with exposure to the treatment for 1 hour in the treatment variant with 2% biostimulant (152.94%), followed by the concentration of 4% (141.17%) and 1% (127.45%). In the case of older seeds, the highest results of this indicator were attested in the variant with 4% exposure to the treatment for 1 hour and 1% with exposure to the treatment for 4 hours. The increase in the values of this indicator creates the possibility to consider that the treated seedlings will have a better capacity to absorb water and nutrients from the soil. This can contribute to a more vigorous and healthy growth of the plants. Longer and better developed roots allow a more stable anchorage in the soil and a greater resistance to stress conditions, such as drought or soil conditions.

Research conducted by Botnarenco et al. (2011) on the germination of seeds of approximately 70 taxa of medicinal plants has shown that storing seeds for a period of 4-6 years has a negative impact on the germination quality. In most species, an extension of the germination duration from 6 to 12 days was found. However, in the case of some medicinal plant species studied (*Althaea officinalis* L., *Amaranthus cruentus* L., *Carthamus tinctorius* L., *Coriandrum sativum* L., *Cephalophora aromatica* Gr., *Cynara scolymus* L., *Hyssopus officinalis* L., *Potentilla erecta* L.), storing seeds for 4-6 years did not significantly influence the germination capacity (Botnarenco et al., 2011). Our results showed that storing seeds of *Phacelia tanacetifolia* Benth. (honeybee) for 4 years caused a quantitative decrease in the number of germinated seeds and, implicitly, in the germination quality.

The biostimulatory effects on seed germination and plant health improvement resulting from the use of wastewater or cyanobacterial biomass have been demonstrated in numerous specialized studies.

The application of cyanobacterial filtrates from *Nostoc calcicola* and *Anabaena flos-aquae* for seed treatment of wheat, soybean, and clover varieties led to an increase in seed germination rate of up to 97.49% compared to the control group, with seedlings exhibiting visibly improved health status. Clover seedlings recorded the highest increase in shoot length (100%) compared to all other treatments applied to the tested crops. Meanwhile, soybean seedlings treated with *Nostoc* filtrate showed a 96.67% increase in root length relative to the other experimental variants. Overall, the treatment of wheat, soybean, and clover seeds with cyanobacterial filtrates had a significant positive effect, enhancing germination and promoting healthy plant development across all analyzed crops (El-Shahat Wahdan, 2007).

Investigations conducted by Lakshmi and Annamalai (2008) have demonstrated that wastewater (filtrates) obtained from the cultivation of the cyanobacteria *Anabaena ambigua* Rao and *Oscillatoria foreaui* exerts a pronounced biostimulatory effect on the plant *Withania somnifera* Dunal.

This effect was evidenced by enhanced plant growth on clay-sandy soils, as indicated by increases in the length and diameter of lateral roots, as well as total biomass. These results are attributed to the assimilation of biologically

active compounds present in the wastewater derived from cyanobacterial cultivation. (Lakshmi & Annamalai, 2008).

The application of combined filtrates (wastewaters) obtained from the cultivation of the cyanobacteria *Anabaena variabilis, Aulosira fertilissima, Nostoc muscorum*, and *Tolypothrix tenuis* on BG-11 nutrient medium had a significant impact on wheat seed germination. In the variant treated with wastewater, the germination rate reached 95%, compared to only 58.33% in the untreated control. Additionally, notable improvements were observed in germination speed and the seedling vigor index (Kumar & Kaur, 2014).

Research conducted by Zosim et al. (2021) has demonstrated that the filtrate obtained from the cultivation of the cyanobacterium *Spirulina platensis* contains compounds with biostimulatory effects on seed germination, such as amino acids (4.12-30.14 mg/ml), gibberellic acid (25.00-110.00 mg/ml), and indoleacetic acid (6.78-8.23 µg/l). The application of this filtrate, diluted with distilled water, to *Mentha spicata* seeds resulted in an increase in germination rate to 88%, compared to 68% in untreated seeds. A significant enhancement in seedling growth was also observed, with stem and root lengths being 2.12 and 2.15 times greater, respectively, than those of seedlings germinated without the addition of these biostimulants (Zosim et al., 2021).

In our research, the variants treated with the cyanobacterial biostimulant obtained by recycling wastewater from the cultivation of *Spirulina platensis* exhibited a quantitative increase in the number of germinated seeds, as well as a relative elongation of plant roots.

These results confirm the potential of cyanobacterial filtrates as an effective source of bioactive compounds with stimulatory effects on seed germination and early plant development.

CONCLUSIONS

The treatment of *Phacelia tanacetifolia* Benth. (Melifera) seeds with a biostimulator obtained from the wastewater resulting from the cultivation of the cyanobacterium *Spirulina platensis* has a positive effect on the germination capacity, especially in the variants of 1-2% biostimulator with exposure to the treatment for 1 hour. Older seeds (stored for 4 years) showed a reduction in the germination capacity, but the application of the biostimulator significantly improved the germination rate compared to the control variant. The germination index values confirm the stimulatory effect of the culture liquid, being significantly higher in the treated seeds compared to the untreated ones. The increase in the relative elongation of the roots following the treatment suggests an improved development of the root system, which may contribute to a more vigorous growth of the plants and a better adaptation to difficult environmental conditions.

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