VALORIZATION OF WASTEWATER FROM *SPIRULINA PLATENSIS* CULTIVATION AS A BIOLOGICAL STIMULANT FOR THE GERMINATION OF *GALEGA ORIENTALIS* L. SEEDS PRESERVED IN COLLECTIONS

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Abstract

The article presents the experimental results obtained from applying a biostimulant based on residual water from the cultivation of the alga Spirulina platensis on the germination of *Galega orientalis* seeds maintained in collection conditions for 2, 3, and 4 years. The results show that seeds treated with biostimulants exhibit a higher germination capacity than those in the control group, where germination ranged between 27-33%. The highest germination rates were obtained for the 4-year-old seeds (60%) and 3-year-old seeds (47%) treated with biostimulant concentrations of 2% and 4% for 2-4 hours. Moreover, the germination index is significantly higher in the treated seeds, reaching maximum values of 12 and 8.6 for the 4- and 3-year-old seeds, respectively, compared to the control group values (5,4-6,6). The relative root elongation was greater in the 2-year-old seeds treated with a 1% biostimulant, but for the older seeds (3-4 years), the 2% concentration applied for 4 hours yielded the best results. The 4% concentration showed stability, although with a smaller root elongation compared to the lower concentrations. In conclusion, the 2% biostimulant applied for 2-4 hours is the most effective for stimulating germination and root growth in the older seeds of *Galega orientalis*.

Key words*:* biostimulants, *Galega orientalis, Spirulina platensis*, wastewater.

INTRODUCTION

Cultivation of spirulina *(Spirulina platensis*) has gained increasing interest due to its nutritional value and applicability in various fields. According to recent estimates, global spirulina production is around 1,5 million tons of dry biomass, a significantly higher amount than chlorella, which stands at approximately 1 million tons of dry biomass (Market Watch, 2024; Global Algae Market Report, 2024). To meet these biomass quantities, algae are cultivated on an industrial scale, and as a result, large amounts of wastewater, primarily consisting of the cultural liquid, accumulate. Managing this wastewater requires significant resources, especially for the maintenance of treatment systems. It is important to note that this wastewater is rich in nutrients, biologically active substances, and other compounds that need recovery and utilization, presenting a real opportunity to apply the environmental protection principles of "R-R-R" (recovery - reuse - recycling). Thus, recent research has begun to explore the potential of this wastewater for reuse, recycling, and recovery, offering innovative solutions that could contribute to the ecological sustainability of spirulina cultivation.

Research conducted by Li and co-authors demonstrated that wastewater from spirulina cultivation can be successfully used as a fertilizer for various crops, including for stimulating seed germination, showing a pronounced positive effect (Li X. *et al*, 2021). In a similar study, Zhang and co-authors used wastewater from spirulina cultivation as a soil fertilizer, which improved soil quality and increased the productivity of agricultural crops (Zhang Y. *et al*, 2022). Kumar and co-authors developed an advanced technology that involves using spirulina for wastewater treatment to recover nutrients, thus reducing environmental impact and turning waste into valuable resources (Kumar P. *et al*, 2023). Based on this, we believe that wastewater from spirulina cultivation can not only be recycled, recovered, and reused, but also significantly contribute to the development of more sustainable agricultural practices.

Therefore, we aimed to utilize this wastewater as a biostimulant for the germination of *Galega orientalis* seeds stored under collection conditions.

The *Galega orientalis* Lam. Fabaceae family is known commonly as eastern galega or fodder galega - is a perennial herbaceous plant native to the North Caucasus area. The Fabaceae speces are of interest in agriculture due to its

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ability to fix atmospheric nitrogen in the soil through symbiosis with bacteria from the *Rhizobium* genus (Giller K., 2001). *Galega orientalis* develops a branched tap root system that reaches a depth of 50-135 cm, forming a dense network of roots with adventitious bristles, on which there may be up to 1500 nodules containing *Rhizobium galegae* bacteria. This species had a multipurpose utility, including the early beginning of vegetation, fast growth, optimal capacity for regeneration after mowing, so that they can be cut 2-3 times per year with high protein yield.

Galega orientalis has been researched in several university and scientific centers as fodder crops, medicinal plant, honey plant, energy crops (Skerman P.J. *et al*, 1988; Koleva I.I., 2002; Dubrovskis V. *et al*, 2008; Adamovics A. *et al*, 2011; Avetisyan A.T., 2013; Domash V.I. *et al*, 2013; Povilaitis V. *et al*, 2016; Skórko-Sajko H. *et al*, 2016; Meripold H. *et al*, 2017; Darmohray L.M. *et al*, 2018, 2021; Shymanska O. *et al*, 2018; Aizman R.I. *et al*, 2019; Cherniavskih V.I. *et al*, 2020; Khasanov E. *et al*, 2020; Starkovskiy B. *et al*, 2020; Vergun O. et al, 2020; Hunady I. *et al*, 2021; Rakhmetov J. *et al*, 2021; Żarczyński P.J. *et al*, 2021; Ignaczak S *et al*, 2022; Biligetu B. *et al*, 2024; Moiseeva E.A. *et al*, 2024).

At the "Alexandru Ciubotaru" National Botanical Garden (Institute) of Moldova State University, the species *Galega orientalis* it has been researched the '80s of the past century, being identified valuable forms and created local cultivars, evaluated the quality of green mass, hay and haylage, also the quality indices of biomass for renewable energy production (Teleuţă A. & Ţîţei V. 2011, 2012; Teleuţă A. *et al*, 2015; Coşman S. *et al*, 2017; Ţîţei V. & Coşman S., 2019; Cerempei V. *et al*, 2023; Ţîţei V., 2024).

To preserve the gene pool of *Galega orientalis* Lam., its seeds are stored under collection conditions for several years, a process that affects their germination. Therefore, it is important to develop new, affordable, and efficient biostimulants to stimulate the germination of dormant plant seeds.

MATERIALS AND METHODS

In the experiments, the *Spirulina platensis* cyanobacteria strain, selected in culture by corresponding member Prof. Vasile Șalaru, academician Prof. Valeriu Rudic, and co-authors, was used. The *Spirulina platensis* strain, stored in the Sceintific Laboratory "Algology Vasile Șalaru" collection of the Moldova State University, was cultivated on modified Zarrouk liquid 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; potable water – 1 L. The inoculum was 0,5 g/L (absolute dry biomass). On the 20th day of cultivating the *Spirulina platensis* cyanobacteria, the algal biomass was separated from the cultural liquid by centrifugation (at 6000 rpm), and the cultural liquid (wastewater) was used to obtain the biostimulant applied in the experiments. The cyanobacterial biostimulant was obtained by thermal activation of the separated cultural liquid.

Solutions with concentrations of 1-4% were used in the experiments, obtained by diluting the cultural liquid from *Spirulina platensis* cultivation with distilled water. Seeds of *Galega orientalis* L., maintained in collection conditions for 2-4 years, numbering 100 for each experimental group, were exposed to the prepared solutions for 1-4 hours, while the control group consisted of the same seeds exposed to distilled water for the same duration.

The experimental *Galega orientalis* cultivar 'Sofia' seeds were provided by "Alexandru Ciubotaru" National Botanical Garden (Institute) of the Moldova State University.

To study the germination process, the seeds were placed in Petri dishes on filter paper moistened with distilled water, under natural light and at a temperature of 22°C. In the research, the following indicators were determined:

Germination capacity of the seeds (GC), according to the formula: GC = Nsg/Nts * 100, where Nsg = number of germinated seeds; Nts = total number of seeds.

Germination index (GI) calculated on the 5th day of the experiment, according to the formula: GI = ∑(Gt/Tt), where Gt = number of seeds germinated at time t, and Tt = number of days.

Relative root elongation (RRE), calculated on the 7th day of germination, according to the formula: RRE = (Le/Lc) * 100, where Le = root length in the experimental group, and Lc = root length in the control group.

RESULTS AND DISCUSSIONS

Stimulating the germination of seeds maintained in collections is crucial for both scientific research in the field and for individuals interested in agriculture, such as breeders. To ensure effective stimulation, it is essential that the products used are of biological origin and, at the same time, cost-effective. The valorization of wastewater from the cultivation of spirulina presents a significant opportunity for obtaining cheap, accessible, and efficient biostimulants.

As a result of our research, we found that the use of the experimental cyanobacterial biostimulant significantly enhanced the germination process of *Galega orientalis* 'Sofia' seeds maintained in collection conditions. This was demonstrated by the increased germination capacity of the seeds (*Table 1)*.

As observed from the data presented in Table 1, in general, the seeds treated with biostimulants show a higher germination capacity compared to the control group. The highest germination capacity is noted in the seeds of *Galega orientalis* 'Sofia'., maintained in collection conditions for 4 years (60%) and 3 years (47%), compared to the control variants, where the germination capacity of these seeds reached a maximum of 27-33%. The most beneficial biostimulant concentrations were 2% and 4%, with exposure periods of 2 and 4 hours. The seeds of *Galega orientalis* 'Sofia'. stored in collection conditions for 2 years exhibited a reduced germination capacity, reaching a maximum of 9% in the variant treated with 2% biostimulant for 1 hour, while in the control variant, this value reached a maximum of 4% (*Table 1).* Accordingly, we conclude that the application of the biostimulant obtained from the recycling of wastewater from spirulina cultivation shows a pronounced germination effect on the seeds of *Galega orientalis* 'Sofia'. maintained in collection conditions The germination index values of *Galega orientalis* 'Sofia' seeds are significantly higher in the treated seed variants with biostimulant. The most notable results are observed in seeds maintained in collection conditions for 4 years (12) and 3 years (8,6), when treated with a 2% biostimulant and exposed for 2 hours. In the control variant, the highest values of this index were recorded for seeds of 3 and 4 years (germination index ranging from 5,4 to 6,6). These results support the efficacy of the obtained biostimulant for the germination of seeds maintained in collection conditions for 3-4 years *(Table 2).*

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Table 1.

Germination capacity of *Galega orientalis* 'Sofia' seeds treated with biostimulant obtained from the cultural liquid resulting from the cultivation of cyanobacterium *Spirulina platensis*, %

Seed treatment period, hours	Experimental variants											
	1%			2%			4%			Control		
	2 years	3 years	4 years	2 years	3 years	4 years	2 years	3 years	4 years	2 years	3 years	4 years
1st day												
1	4,00	20,00	30,00	2,00	20,00	33,00	0,00	17,00	27,00	0,00	3,00	7,00
2	0,00	20,00	20,00	2,00	23,00	20,00	0,00	13,00	27,00	0,00	10,00	10,00
4	0,00	17,00	10,00	2,00	13,00	23,00	0,00	13,00	20,00	0,00	3,00	10,00
2nd day												
1	6,00	27,00	37,00	6,00	27,00	34,00	4,00	27,00	30,00	0,00	27,00	7,00
2	2,00	23,00	40,00	4,00	40,00	60,00	2,00	33,00	37,00	2,00	20,00	23,00
4	2,00	37,00	43,00	2,00	27,00	37,00	2,00	47,00	43,00	0,00	30,00	17,00
3rd day												
1	6,00	27,00	37,00	6,00	30,00	34,00	6,00	33,00	30,00	4,00	33,00	7,00
2	2,00	23,00	40,00	8,00	43,00	60,00	2,00	43,00	37,00	2,00	27,00	27,00
4	4,00	37,00	43,00	4,00	30,00	37,00	2,00	50,00	43,00	2,00	33,00	20,00
4th day												
1	6,00	27,00	37,00	9,00	30,00	34,00	6,00	33,00	30,00	4,00	33,00	7,00
2	4,00	23,00	40,00	8,00	43,00	60,00	2,00	43,00	37,00	2,00	27,00	27,00
4	4,00	37,00	43,00	4,00	30,00	37,00	6,00	50,00	43,00	2,00	33,00	20,00
5th day												
1	6,00	27,00	37,00	9,00	30,00	34,00	6,00	33,00	30,00	4,00	33,00	7,00
2	4,00	23,00	40,00	8,00	43,00	60,00	2,00	43,00	37,00	2,00	27,00	27,00

Table 2.

Germination index values of *Galega orientalis* 'Sofia 'seeds treated with biostimulant obtained from the cultural liquid resulting from the cultivation of cyanobacterium *Spirulina platensis*

Seed treatment period, hours	Experimental variants											
	1%			2%			4%			Control		
	2 years	3 years	4 years	2 years	3 years	4 years	2 years	3 years	4 years	2 years	3 years	4 years
1	1,20	5,4	7,4	1,80	6,0	6,8	1,20	6,6	6,0	0,80	6,6	1,4
2	0,80	4,6	8,0	1,60	8,6	12,0	0,40	8,6	7,4	0,40	5,4	5,4
4	0,80	7,5	8,6	0,80	6,0	7,4	1,20	10,0	8,6	0,40	6,6	4,0

Table 3.

Relative root elongation of *Galega orientalis* L. treated with biostimulant from the cultural liquid of the cyanobacterium *Spirulina platensis*, %

Seed treatment period, hours	Experimental variants								
	1%			2%			4%		
	2 years	3 years	4 years	2 years	3 years	4 years	2 years	3 years	4 years
1	158,0	153,33	83,33	150,0	115,78	126,43	125,0	117,64	129,31
2	100,0	100,00	90,00	75,0	126,32	229,88	100,0	129,41	126,44
4	200,0	133,33	97,14	175,00	136,83	240,00	125,0	117,67	114,94

The results of the relative root elongation of *Galega orientalis* 'Sofia' plants indicate that for a 1% biostimulant concentration, root elongation is maximal in seeds stored for 2 years, with a gradual decrease observed in seeds stored for 3 and 4 years. For a 2% biostimulant concentration, a more pronounced elongation is seen in seeds of 3 and 4 years, especially for longer exposure periods (4 hours), suggesting increased efficacy on older seeds. The 4% biostimulant concentration shows stable values, with no major variations between seed groups, but root elongation is less than at lower concentrations for extended treatment periods. Thus, we conclude that the 2% biostimulant applied for 2-4 hours has the most effective impact on root elongation, particularly for older seeds (*Table 3)*.

RESULTS AND DISCUSSIONS

Seeds of *Galega orientalis* generally, feature a water-impermeable coating, which contributes to maintaining a dormant state. To initiate germination, the seed coat must be treated, with classic methods including scarification or chemical treatments (Laman N.A. *et al*, 2004). Additionally, research in this area highlights that prolonged seed storage reduces their ability to absorb water and delays germination. This condition, known as dormancy, is commonly observed in species with a thick or hard seed coat (Solberg S.Ø. *et al*, 2020). Long-term storage of *Galega orientalis* seeds results in a reduction in seed coat thickness. Although germination is slower in prolonged storage variants, this reduction in seed coat thickness allows biostimulatory substances to penetrate more easily to the embryo. Our results show that the highest germination rates were observed in seeds stored for longer periods, such as 3-4 years, across all experimental variants *(Table 1)*. This is attributed to both the easier access of water to the seed cotyledon and the more efficient penetration of germination biostimulants.

Numerous studies have demonstrated that treating plant seeds with microalgae accelerates the germination process and significantly increases the percentage of seeds germinated across various plant species (Runshi X. *et al*, 2022; Dobrojan S. *et al*, 2023). This is because algae contain phytohormones and growth regulators, such as cytokinins, auxins, gibberellins, betaines, abscisic acid, and brassinosteroids, as well as matrix and reserve polysaccharides (alginate, carrageenan, agar, ulvan, mucopolysaccharides, oligosaccharides, fucoidan, laminaran, starch, and fluoride). These compounds have a biostimulatory effect on seed germination in various plants (Khan W. *et al*, 2009; Hong Y.P. *et al*, 1995; López B.C., 2001; Stirk W.A. *et al*, 2014). Many of these biostimulatory substances are eliminated or accumulated in the residual water (cultural liquid) resulting from the cultivation of *Spirulina platensis*. Consequently, in our experiments, the biostimulant obtained from the residual water generated by cultivating *Spirulina platensis* had a pronounced germinative effect on seeds of *Galega orientalis* L. stored under collection conditions for 3-4 years *(Tables 1-2)*.

Plant hormones and other biologically active substances produced by *Spirulina platensis*, when used in optimal concentrations, promote root development and elongation (Thet N.H. *et al*, 2009).

Our research found that *Galega orientalis* 'Sofia' seeds, stored in collections and treated with the biostimulant, showed a 14-140% increase in root length compared to untreated variants. These results suggest that the residual waters from cultivating *Spirulina platensis* are rich in phytohormones and other biologically active substances that stimulate the germination of *Galega orientalis* seeds stored under collection conditions and present significant interest for applying circular economy principles.

CONCLUSIONS

Our study results demonstrated the effectiveness of applying a biostimulant based on residual water from the cultivation of *Spirulina platensis* on the germination of *Galega orientalis* 'Sofia'. seeds stored under collection conditions for 2, 3, and 4 years. Seeds treated with biostimulants showed significantly higher germinative capacity compared to the control group, where germination ranged from 27% to 33%.

The highest germination rates were observed in 4-year-old seeds (60%) and 3-year-old seeds (47%) treated with biostimulant concentrations of 2% and 4%, applied for 2-4 hours.

The germination index was significantly higher in treated seeds, reaching maximum values of 12 and 8,6 for 4-year-old and 3-year-old seeds, respectively, compared to the control group values (5,4-6,6). Regarding root elongation, 2-year-old seeds treated with a 1% biostimulant showed the greatest relative root elongation. For older seeds (3-4 years), the 2% concentration applied for 4 hours was most effective in stimulating root elongation, while the 4% concentration was stable but resulted in less elongation compared to the lower concentrations. Thus, the 2% biostimulant applied for 2-4 hours appears optimal for stimulating germination and root development in older *Galega orientalis* 'Sofia' L. seeds, positively impacting seed germination and plant growth under collection conditions. These results suggest significant potential for applying circular economy principles in managing residual water and improving agricultural production.

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