Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 36, issue 3, 2026, pp. 141 – 148

INFLUENCE OF PREGERMINATION TREATMENT WITH ATONIK ON NUTLETS IMBIBITION AND SEEDLING GROWTH OF *ECHIUM RUSSICUM* J.F. GMEL.

Alexandra-Roxana SAVA[1], Anișoara STRATU[1]*, Maria-Magdalena ZAMFIRACHE[1]

[1]Departament of Biology, Faculty of Biology, "Alexandru Ioan Cuza" University of Iași, Romania

Abstract: *Echium russicum* J.F. Gmel. (syn. *Pontechium maculatum* Böhle & Hilger), a species of community interest belonging to the Boraginaceae family, is included in Romania's Red List, being considered vulnerable with population decline. The aim of the study was to investigate the influence of pregermination treatment with the synthetic biostimulant Atonik (1‰ and 3‰ solutions) on *Echium russicum* seeds under laboratory conditions. The effects on imbibition and the growth of newly germinated seedlings were investigated analysing the following parameters: the amount of water absorbed during imbibition, emergence percentage, survival percentage, root length, shoot length, number of leaves per seedling, true leaf area, and cotyledon area; additionally, microscopic observations were conducted on the surfaces of the aerial organs of the seedlings. The results obtained highlighted positive effects of Atonik treatment on the growth of vegetative organs (statistically significant for root length and seedling length parameters at the 1‰ concentration) and on seedling survival. Morphological analysis of the leaf blades of cotyledons and true leaves revealed that they are amphistomatic (anomocytic-type stomata) and have simple, unicellular trichomes, which are more abundant on the upper epidermis.

Keywords: *Echium russicum* J.F. Gmelin (syn. *Pontechium maculatum* Böhle & Hilger), Atonik solution 1‰ and 3‰, imbibition, growth, seedlings.

INTRODUCTION

The species *Echium russicum* J.F. Gmelin (syn. *Pontechium maculatum* Böhle & Hilger) is a biennial (Grințescu, 1960), therophyte-hemi therophyte, heliophilous, sub-thermophilous, xerophilous, neutrophilous, and oligo-mesotrophic species (Sârbu *et al.*, 2013), distributed from Central and Southeastern Europe to the Caucasus, Asia Minor, and Central and Southwest Asia (POWO, 2026).

Echium russicum has been designated a species of community interest, being included in Annexes II and IV of the Habitats Directive (92/43/EEC) on the conservation of natural habitats and of wild fauna and flora (as revised in 2007), as well as in Annex I to Resolution No. 6 (1998) of the Bern Convention (as revised in 2011) (EUNIS, 17/03/2021). This species has been recorded at 132 Sites of Community Importance in Europe, 45 of which are in Romania (European Environment Agency). In many European countries, this species is included in national red lists: the Czech Republic (Grulich, 2012), Poland (Kaźmierczakowa *et al.,* 2016), Slovakia (Turis *et al.,* 2014), Bulgaria (Petrova and Vladimirov, 2009), and Romania (Oprea, 2005). In Romania, the species is considered vulnerable (Oprea, 2005), with population declines caused by various factors (Sava *et al.,* 2019; Chirilă *et al.,* 2025).

This species shows potential as melliferous, ornamental, and medicinal plant (Grințescu, 1960; Chwil and Weryszko-Chmielewska, 2007; Bădărău, 2013; Nowak *et al.,* 2020). Plants of this species synthesize secondary metabolites (flavonoids, phenolic acids, allantoin, shikonin) with potential pharmacological activities (Dresler *et al.,* 2015; 2017). Nowak *et al.* (2020) reported that soaking seeds of this taxon in a 1.8 mM gibberellin (GA3) solution for 24 hours had positive effects on their germination.

Studies in the scientific literature have reported positive effects of using the compound Atonik (applied to seeds and/or foliarly) on the germination, growth, and development of cultivated plant species (Djanaguiraman *et al.,* 2005; Kocira *et al.,* 2017; Covașă *et al.,* 2019; Amin *et al.,* 2019; Abood and Mirare, 2024). Atonik is a synthetic biostimulant of Japanese origin containing three phenolic compounds: sodium para-nitrophenolate (0.3%), sodium ortho-nitrophenolate (0.2%) and sodium 5-nitroguaiacolate (0.1%), along with water (Kocira *et al.,* 2017). This study aims to evaluate the imbibition process of *Echium russicum* nutlets (seeds) and the growth of newly formed seedlings following a pregermination treatment with Atonik solutions at different concentrations (1‰ and 3‰).

MATERIALS AND METHODS

Nutlets (seeds) of *Echium russicum* EA017, purchased from the company "*Jelitto Perennial Seeds*", Germany, were used as biological material. The term "seed" is not used in the strict sense throughout this study, as species of the genus *Echium* produce dry, indehiscent fruits known as nutlets, within which the seed remains intimately attached to the fruit (Roso *et al.,* 2017).

The aqueous Atonik solution was used at concentrations of 1‰ and 3‰, with the used concentrations selected based on information from the scientific literature (Djanaguiraman *et al.,* 2005; Kocira *et al.,* 2017).

To determine gravimetric imbibition, the nutlets were placed in glass containers with a volume of 20 ml of distilled water (V1) and, respectively, Atonik solutions at concentrations of 1‰ (V2) and 3‰ (V3). Over an 8-hour experimental period, the nutlets were removed from the water or Atonik solutions at hourly

***Correspondence:** Stratu Anișoara, "Alexandru Ioan Cuza" University of Iași, Faculty of Biology, Department of Biology, Bd. Carol I, Nr. 20A, UAIC, Corp B, Tel. +40232201072, e-mail: anisoara.stratu@uaic.ro

intervals, blotted dry with filter paper, weighed, and returned to the experimental liquids. Three replicates were used for each experimental treatment, with each replicate consisting of 12 nutlets; the mass of each sample was determined with a precision of 0.0001 g using an analytical balance.

The amount of water absorbed at each time interval was calculated using the formula described by Marler (2019):

$\%Wa = ((Wi - Wf) / Wf) \times 100$,

where %Wa represents the percentage of absorbed water, Wi represents the weight after water absorption and Wf represents initial weight before imbibition.

To study the growth process were used nutlet that have been subjected to an 8-hour imbibition treatment in distilled water (V1), Atonik solution at 1‰ (V2), and Atonik solution at 3‰ (V3); for the control(C), nutlets that had not undergone water imbibition were used. After the treatment, the nutlets were placed in seedling trays for germination. Three trays, each containing 12 nutlets, were used for each experimental treatment. A commercial universal potting mix served as the germination and growth substrate. This universal soil consisted of ALONET sphagnum peat substrate (60%), finely shredded and sifted leaf soil (15%), compost (10%), washed river sand (10%), and coniferous sawdust (5%), plus perlite. The soil used was loose in texture, had high water-holding capacity, and a pH ranging from 6 to 7.

The effect of treatment with 1‰ and 3‰ Atonik solution on the growth process was evaluated by calculating the emergence percentage at 7 and 14 days after the start of the experiment.

Following the initial 14 days period, the seedlings were monitored further for 47 days, with observations recorded regarding their survival and growth rates. The experiment concluded when significant changes in the analysed parameters (survival rate) were observed. The seedling containers were kept under laboratory conditions, maintaining an ambient temperature of 24°C–27°C and an average photoperiod of 15 hours of light and 9 hours of darkness. Soil moisture in the containers was monitored daily and maintained through watering with tap water. The total duration of the experiment was 61 days.

At the end of the experiment, the following morphological indicators were determined: root length, shoot length (hypocotyl + epicotyl), number of leaves per plant, true leaf area, and cotyledon leaf area. Leaf area was determined using a compact, portable AM-100 Area Meter (ADC BioScientific Ltd Analytical, Hoddesdon, UK), while lengths were measured using a ruler.

To analyse the surfaces of the vegetative organs, the plant material was preserved in 70% alcohol and subsequently stained with Ruthenium Red, following the techniques described in the literature (Tarnavschi et al., 1974; Șerbănescu et al., 1983). The resulting stained specimens were examined using an Euromex iScope series light microscope with 4x, 10x, 20x, and 40x objectives; images were captured using an Euromex sCMEX-20 camera mounted on an adapter with 0.5x magnification, utilising ImageFocusAlpha software.

The values obtained for all studied parameters are expressed as the mean ± standard deviation. Statistical differences between variables were assessed using statistical tests, specifically one-way ANOVA and the Tukey HSD post-hoc test. The analyses were performed using GraphPad Prism 10 software. The plotted data represent the mean ± standard deviation. Statistical significance is indicated by an asterisk (*) for $p < 0.05$.

RESULTS AND DISCUSSION

Water absorption by imbibition

Over the one-eight-hour interval, the amount of water absorbed by the nutlets through imbibition showed different patterns depending on the experimental treatment. In treatment V1, the amount of water absorbed through imbibition showed a progressive increase up to six hours (from 5.40 g% to 13.51 g%), after which it remained constant until the eight-hour mark. In treatment V2, the amount of water absorbed remained constant up to three hours, increased up to four hours, remained constant up to five hours, and subsequently showed a progressive increase up to eight hours; compared to experimental treatments V1 and V3, the amount of water absorbed via imbibition was higher (Fig. 1). In treatment V3, the amount of water absorbed remained constant up to two hours, increased up to five hours, and subsequently remained constant until the eight-hour mark.

According to the literature, the imbibition process—an essential stage of germination—is influenced by several factors, such as the nature of the seed coat and seed storage reserves (Toma and Jităreanu 2007; Costa *et al.,* 2019), as well as the solutions used for pregermination treatments (Tian *et al.*, 2014; Costa *et al.,* 2019).

The amount of water absorbed through imbibition by *Echium russicum* nutlets was low, a fact associated with their specific morpho-structural and physiological characteristics. In Boraginaceae species, the pericarp acts as a structural barrier that limits water absorption and oxygen penetration. At *Echium plantagineum*, as well as in other members of the Boraginaceae family, water enters the pericarp through the vascular bundle zone located at the base of the nutlet, while the remainder of the pericarp is sclerified (Diane *et al.,* 2002; Molinelli *et al.,* 2020).

Data obtained for the *E. russicum* species indicate that soaking seeds in 1‰ Atonik solutions had a positive effect on imbibition-based absorption starting from the first hour of monitoring, compared to the control. The higher concentration of the Atonik solution (3‰ – V3) resulted in lower values for the quantity of liquid absorbed via imbibition compared to the control (V1), suggesting the presence of slight osmotic stress.

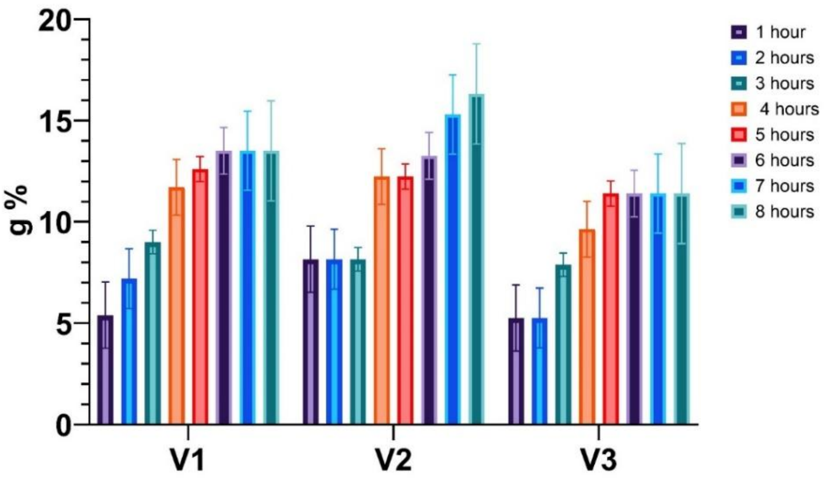

Fig. 1. Percentage of water absorbed through imbibition by *Echium russicum* nutlets immersed for eight hours in distilled water (V1) and in Atonik solutions of various concentrations: V2 – 1‰; V3 – 3‰. The plotted data represent the mean ± standard deviation.

Emergence and survival percentage of seedlings resulting from the germination process

At *Echium russicum*, growth is epigeal, just as *Echium plantagineum* (Molinelli *et al.* 2020); consequently, the hypocotyl axis undergoes the most intense elongation, the epicotyl axis remains short, and the cotyledons are raised to the soil surface.

Analysis of the data obtained from the plant material under study (*Echium russicum* specimens) revealed that, seven days after the experiment began, emergence rates ranged from 16.66% to 41.66%; higher values were recorded for the experimental treatments using 1‰ and 3‰ Atonik solution (40% and 50%, respectively) compared to the control (Fig. 2). For V1 (pregermination treatment with distilled water), the emergence rate was lower than that of the control (Fig. 2).

At 14 days after the start of the experiment, the germination percentage ranged from 38.88% (V1) to 58.33% (V3). The difference between the control and the experimental treatments where pregermination treatments were applied to the seeds (using 1‰ and 3‰ Atonik solutions and distilled water) is smaller than it was at 7 days (Fig. 2).

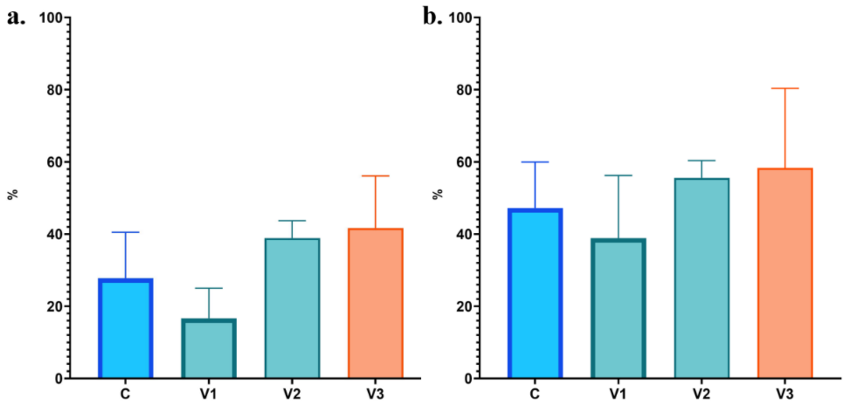

Fig. 2. Seedling emergence percentage for *Echium russicum* obtained from nutlets germination at 7 days (**a**) and 14 days (**b**) (C – non-soaked seeds; V1 – distilled water; V2 – Atonik 1‰ solution; V3 – Atonik 3‰ solution). The plotted data represent the mean ± standard deviation.

The seedling survival rate between day 14 and day 47 (61 days after the start of the experiment, when the seedlings had developed true leaves) was higher in the experimental treatments than in the control. Treatment V3 stood out with the highest survival percentage (56.01%) (Fig. 3).

The positive effects on the two analysed indicators (seedling emergence percentage and survival percentage) for *Echium russicum*, following seed pre-treatment with Atonik solution at concentrations of 1‰ (V2) and 3‰ (V3) for eight hours, are not statistically significant (Fig. 1–3).

In this context, the results obtained in the presented experiment demonstrated that the pre-germination treatment with the tested Atonik biostimulator (at concentrations of 1‰ and 3‰) stimulated the germination process and increased the survival rate of the newly formed seedlings.

According to Chen *et al.* (2012), the germination and seedling stages are considered the most vulnerable stages in the plant life cycle regarding diverse habitat conditions. For many rare plant species, the processes of germination, growth, and development can be limited by habitat conditions (Jusaitis *et al.,* 2004; Chen *et al.,* 2012).

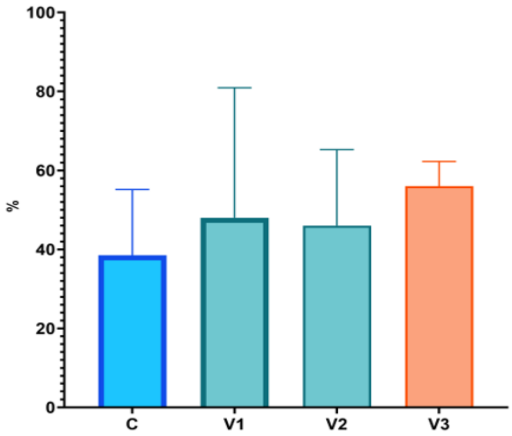

Fig. 3. Survival rate of *Echium russicum* seedlings obtained from nutlet germination after a 47-day experimental period: C – non-soaked seeds; V1 – distilled water; V2 – 1‰ Atonik solution; V3 – 3‰ Atonik solution. The plotted data represent the mean ± standard deviation.

Growth parameters of newly formed seedlings

The results obtained regarding the growth parameters analysed at *Echium russicum* seedlings are presented in Tables 1 and 2.

Root length showed higher values compared to the control at seedlings grown from seeds soaked in 1‰ and 3‰ Atonik solutions (by 63.25% and 29.33%, respectively) and in distilled water (by 36%); the difference observed was statistically significant for the experimental treatment using the 1‰ Atonik solution (V2).

The length of shoot (hypocotyl + epicotyl) was greater in the experimental treatments compared to the control: by 5.51% for V1, 14.38% for V3, and 24.70% for V2.

Seedling length exceeded that of the control by 22.18% (V1) to 45% (V2), with the difference being statistically significant for the experimental treatment using 1‰ Atonik solution (V2).

The number of true leaves per seedling and the true leaf area in the variants treated with the Atonik biostimulant were greater than in the control by 6.19% and 29.75%, and by 16.5% and 29.75%, respectively.

The cotyledon leaf area was larger in seedlings derived from nutlets soaked in Atonik solution, with recorded increases of 22.07% and 36.49% compared to the control.

The results obtained indicated that the Atonik solution (at concentrations of 1‰ and 3‰), applied to *Echium russicum* nutlets prior to sowing, positively influences the germination process, seedling survival, and the growth of the resulting new plants.

As indicated in the specialized literature, the immersion of nutlets in the Atonik solution may trigger the activation of metabolic processes within the seeds, stimulate embryo division and elongation, and enhance the germination and growth of the resulting new plants (Djanaguiraman *et al.,* 2005; Al-Rawi *et al.,* 2021).

Positive effects of the Atonik biostimulant (at concentrations of 1‰ and 3‰) on seed germination, root and shoot elongation in seedlings, and the number of leaves on young plants have also been reported by other authors, such as Djanaguiraman *et al.* (2005) for cotton and tomatoes, and Prathumyot *et al.* (2025) for chili peppers.

The results obtained for the species *E. russicum* confirm and complete data from the literature regarding the positive effects of the Atonik biostimulant solution on germination and growth processes—data primarily reported for cultivated species (Djanaguiraman *et al.,* 2005; Al-Rawi *et al.,* 2021; Abood and Mirare, 2024).

Table 1.

Growth parameters of *Echium russicum* seedlings obtained from germinated nutlets in distilled water / Atonik solutions (1‰ and 3‰) (mean ± standard deviation)

Experimental treatments	Root length (mm/seedling)	Shoot length (mm/seedling)	Seedling length (mm/seedling)
C	24,85±9,02 (100)	20,85±9,51 (100)	45,71±16,53 (100)
V1	33,85±9,58 (136,21)	22±5,47 (105,51)	55,85±14,31 (122,18)
V2	40,57*±6,24 (163,25)	26±7,46 (124,70)	66,57*±6,24 (145,63)
V3	32,14±9,45 (129,33)	23,85±4,14 (114,38)	56±10,63 (122,61)
*= significant at $p < 0.05$, Tukey test Values in parentheses represent the percentage relative to the control (C)			

Table 2.
Cotyledon and true leaf area and number of true leaves of *Echium russicum* seedlings obtained by germination of nutlets immersed in water / Atonik 1‰ and 3‰ solutions (mean ± standard deviation)

Experimental treatments	Cotyledon leaf area (mm^2/seedling)	Number of true leaves/seedling	Area of true leaf (mm^2 $seedling^{-1}$)
C	16,85±6,44 (100)	2,42±0,79 (100)	20,6±7,73 (100)
V1	16,71±5,9 (99,16)	2,28±0,76 (94,21)	23,2±9,73 (112,62)
V2	20,57±5,12 (122,07)	2,57±0,53 (106,19)	24 ±10,07 (116,50)
V3	23±8,14 (136,49)	3,14±0,90 (129,75)	35±15,16 (169,90)
Values in parentheses represent the percentage relative to the control (C)			

Analysis of the vegetative organs of newly formed *Echium russicum* J.F. Gmel. seedlings

Cotyledonary leaves are the first leaves to form during a plant life; in many species, they appear as very thin leaf-like structures and have veins and stomata (Grințescu, 1985). According to the literature, the cotyledonary leaves of species in the Boraginaceae family also bear protective hairs (Barykina and Alyonkin, 2016; 2017).

Microscopic examination of the seedlings from the control and treatment groups revealed no visible differences regarding the shape of epidermal cells or the trichomes and stomata; therefore, the description below focuses on the seedlings from the control group.

On the cotyledonary leaves analysed, simple unicellular trichomes and ranunculaceous (anomocytic) stomata can be observed on both the upper and lower surfaces, the leaf being amphistomatic. The trichomes on the upper epidermis are denser and longer than those on the lower epidermis, while the stomata are more numerous on the lower surface of the cotyledonary leaf (Fig. 4, Fig. 5).

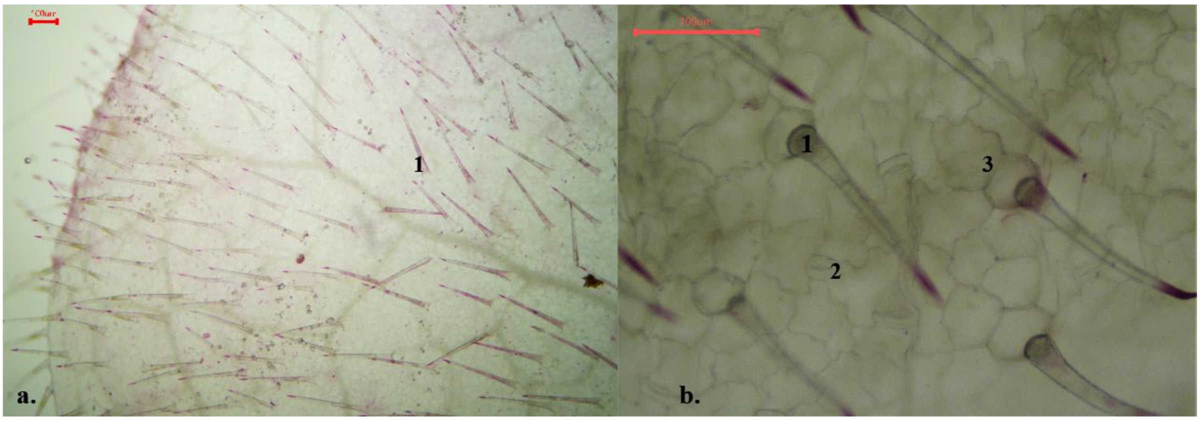

Fig. 4. Upper leaf epidermis of *Echium russicum* seedlings. Control: **a.** general view (4x objective); **b.** detail showing stomata and trichomes (20x objective) (1 – trichome; 2 – stoma; 3– epidermal cell).

At the true leaves, the midrib is prominent on the lower surface of the leaf blade, while a narrow groove is visible on the upper surface. Over the midrib of the true leaf, the epidermis consists of cells slightly elongated in a radial direction; across the rest of the blade surface, the epidermal cells are isodiametric. Same as the cotyledon, trichomes are more abundant on the upper surface of the leaf blade and sparser on the lower surface. The epidermal layers are composed of isodiametric or slightly tangentially elongated cells with undulate anticlinal walls. Stomata are observed here and there in both epidermal layers, the leaf blade being amphistomatic (Fig. 6, Fig. 7).

Thick, protective, cystolithic trichomes are also observed on both epidermal surfaces, supported by a multicellular base composed of epidermal cells.

The trichomes on the lower epidermis are simple and unicellular, whereas those on the upper epidermis—found on both the leaf blade and the cotyledon include both simple hairs and those with a basal rosette.

Numerous long, unicellular trichomes can be observed on the aerial parts. The epidermal cells are elongated, and stomata can be seen here and there (Fig. 8).

The results of observations made on *Echium russicum* seedlings confirm and complete data from the scientific literature regarding various Boraginaceae species (Barykina and Alyonkin, 2016; 2017; 2018) concerning the presence of stomata on the cotyledonary and true leaf blades, the stomatal type, and the presence and type of protective hairs.

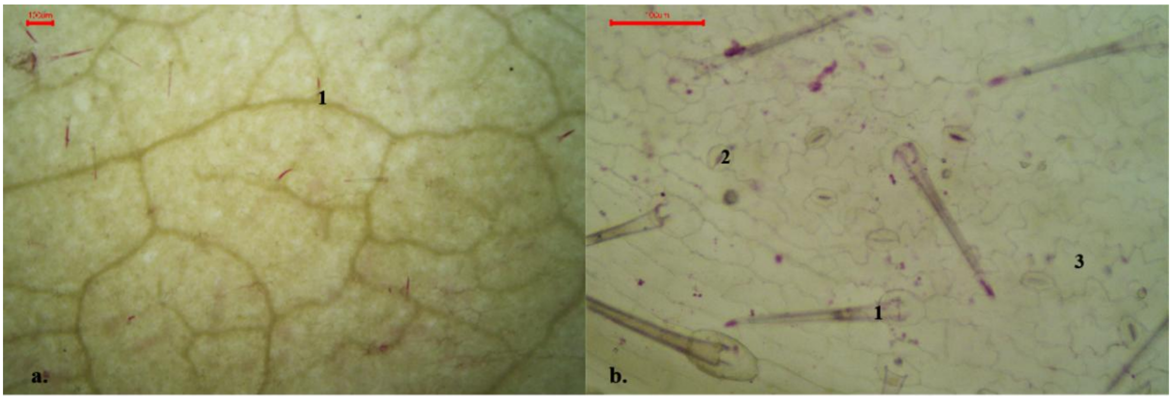

Fig. 5. Epidermis of the lower surface of the cotyledonary leaf in *Echium russicum* seedlings – control: **a.** general view (4x objective); **b.** detail showing stomata and trichomes (20x objective) (1 – trichome; 2 – stoma; 3– epidermal cell).

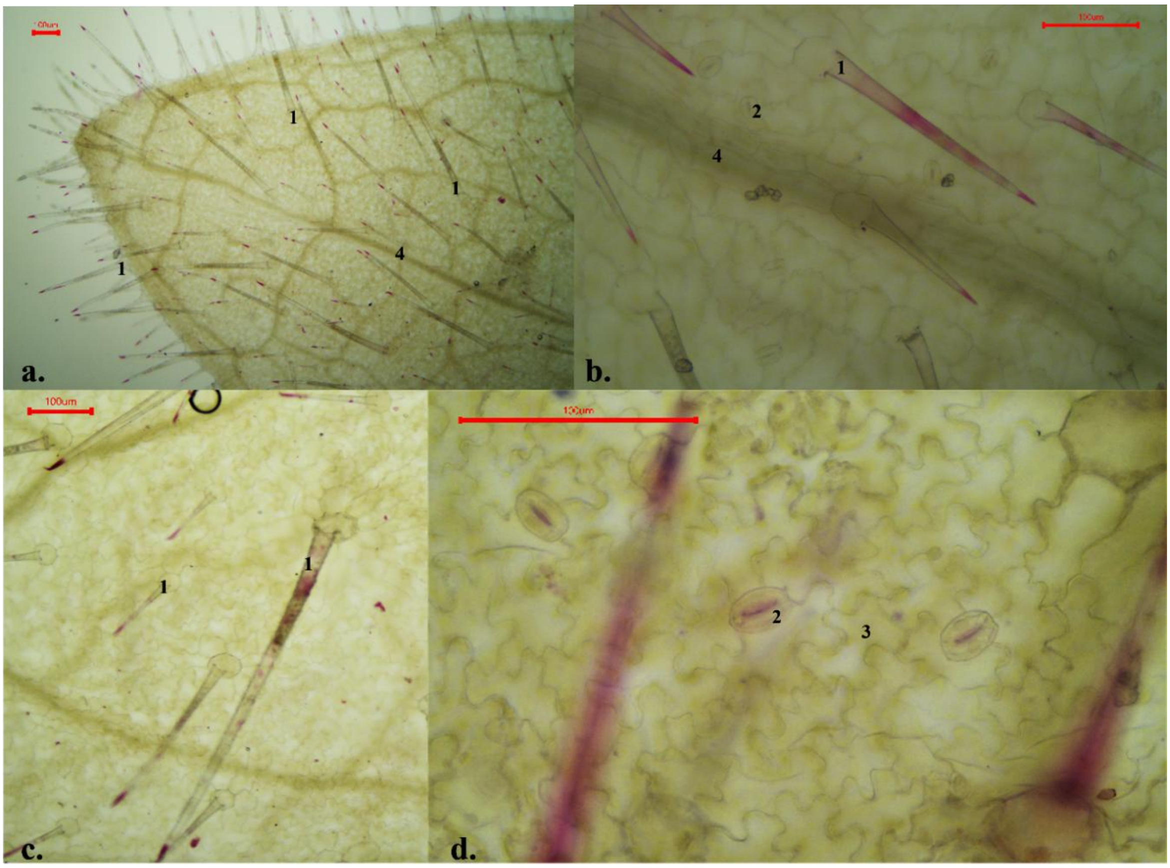

Fig. 6. Epidermis of the adaxial surface of the leaf blade of *Echium russicum* seedlings. Control: **a.** general view (4x objective); **b.** detail showing stomata and trichomes (20x objective); **c.** detail of trichomes (20x objective); **d.** detail showing epidermal cells and stomata (40x objective); (1 – trichome; 2 – stoma; 3– epidermal cell; 4 – midrib).

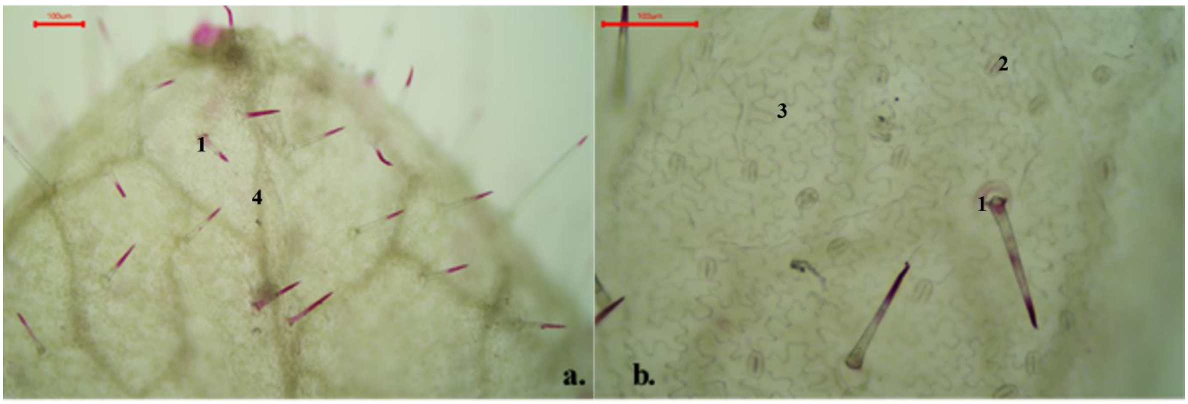

Fig. 7. Epidermis of the abaxial surface of the leaf blade of *Echium russicum* seedlings. Control: **a.** general view (10x objective); **b.** detail showing stomata and trichomes (20x objective) (1 – trichome; 2 – stoma; 3– epidermal cell; 4 – midrib).

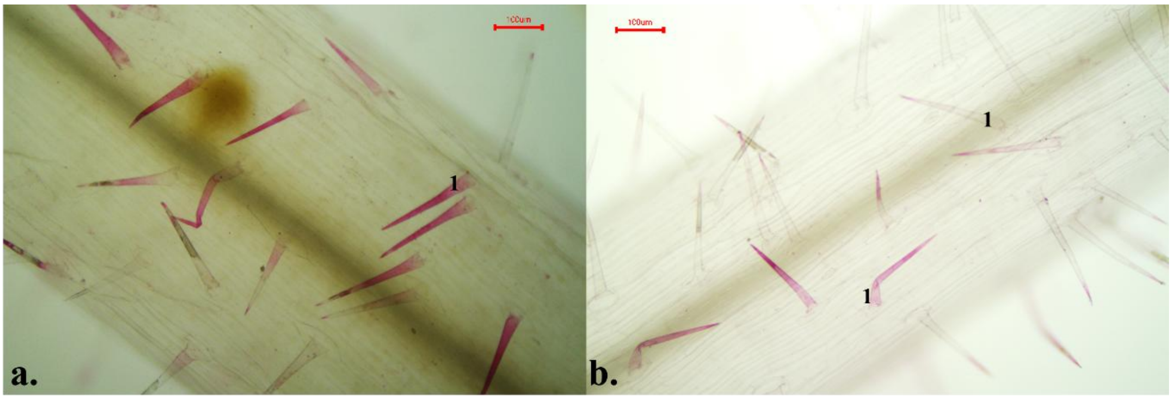

Fig. 8. Epidermis with trichomes (1) from the aerial part of *Echium russicum* seedlings, control (**a., b.**; 10x objective).

CONCLUSIONS

Research on the imbibition and germination of *Echium russicum* nutlets, as well as the growth of the resulting seedlings, has demonstrated the stimulatory effect of pre-germination treatment with the compound Atonik.

The results demonstrated that the seed preparation technique involving pregermination treatment with a biostimulant solution, already established in agricultural practice, can also be applied to the species *Echium russicum* to produce specimens with enhanced vigor, regarding introduction or reintroduction into specific natural habitats or their conservation in *ex-situ* collections of scientific interest.

The reported results confirm earlier observations regarding the beneficial effect of the biostimulant Atonik on other species and complete existing literature with new data on the potential management of the growth and cultivation of the taxon of community interest *Echium russicum*.

AUTHORS CONTRIBUTIONS

Conceptualization: A.S. and S.A.R.; methodology, A.S.; data collection S.A.R. and A.S.; data validation, A.S., S.A.R. and Z.M.M.; data processing A.S. and S.A.R.; writing—original draft preparation A.S., S.A.R. and Z.M.M and writing—review and editing A.S., S.A.R. and Z.M.M.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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