JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(5), 28–39
https://doi.org/10.12911/22998993/199526
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.11.28
Accepted: 2025.02.07
Published: 2025.03.16

Morphological and physiological differences in fern *Athyrium nipponicum* exposed to drought, salinity and depolymerized sodium alginate

Anna Pietrak[1*], Piotr Salachna[1], Łukasz Łopusiewicz[2]

[1] The Faculty of Environmental Management and Agriculture, West Pomeranian University of Technology in Szczecin, Słowackiego 17, 71-434 Szczecin, Poland
[2] School of Medical and Health Sciences, University of Economics and Human Sciences in Warsaw, Okopowa 59, 01-043 Warszawa, Poland
* Corresponding author's e-mail: anna.pietrak@zut.edu.pl

ABSTRACT

A significant gap remains in the understanding of the systemic responses of ferns to multiple stress factors and the application of biostimulants. Effects of single drought stress (DS), salinity stress ($CaCl_2$), combined stress ($CaCl_2$+DS) and depolymerized sodium alginate (DA) on morphology and physiology characteristics of the fern *Athyrium nipponicum* cv. Metallicum in a pot experimental were investigated. Plants grown in a plastic tunnel were treated with $CaCl_2$ at a target concentration of 100 mmol dm^{-3} and DA at 100 mg dm^{-3} under normal irrigation and drought stress conditions. Combined stress ($CaCl_2$+DS) most severely reduced plant height, plant width, leaf number, and leaf length. Plant leaf and rhizome weights were reduced to a significantly greater degree under water deficit stress than excess soluble salts in the substrate. The plants exposed to $CaCl_2$ had elevated leaf carotenoids (by 40.9%), total polyphenols (by 22.1%) and total flavonoid (by 15.0%) content relative to control plants. The application of DA had no apparent effect on the aboveground part of the plants, while it increased the fresh and dry weights of the rhizomes by 48.1% and 51.0%, respectively. Moreover, DA application increased the chlorophyll a, chlorophyll b, and total chlorophyll contents of leaves by 53.0%, 56.3%, and 53.1%, respectively, compared to the control. Application of DA under single ($CaCl_2$+DA and DS+DA) and combined ($CaCl_2$+DS+DA) stress conditions significantly reduced the fresh and dry weights loss of rhizomes. Comparing the content of secondary metabolites in fern, rhizomes had 4.8 times more total polyphenols and 3.6 times more total flavonoids than leaves. The results suggest the potential application of DA in mitigating the adverse effects of salinity and water stress during fern cultivation.

Keywords: environmental stress, single stress, combined stresses, $CaCl_2$ stress, water stress.

INTRODUCTION

Plants are constantly exposed to various environmental stresses during growth and development. Drought and soil salinity are the most important abiotic stresses from an agronomic point of view (Nazari et al., 2023). As a result of climate change, extreme droughts of increasing duration are becoming more common, often leading to soil salinization, erosion, and desertification (Spinoni et al., 2018). It is estimated that by 2025, up to two-thirds of the Earth's surface will be under drought stress, and by 2050, more than 50% of arable land will be over-salinized (Shrivastava et al., 2015; Marien et al., 2023). Both drought and salinity stress lead to stunted plant growth, abnormal leaf color, defoliation or death of the growing tip (Fan et al., 2023), reduced biomass (Huanhe et al., 2024), impaired chlorophyll synthesis (Kiran et al., 2023), as well as production of primary metabolites (Sharif et al., 2018) and secondary metabolites (Najjaa et al., 2018). Drought and elevated levels of soluble salts are common environmental stressors that often induce similar toxic effects in plants (Garcia-Caparros et al., 2022). Solutions to counteract the effects of drought and salinity stress are being intensively sought (Shrivastava and Kumar, 2015; Nephali et al., 2020; Wahab et al., 2022).

Most works focus on evaluating single stresses on plant growth and physiology (Babaei et al., 2022; Du et al., 2023; Fan et al., 2023). The effects of multiple stressors on plants are still too rarely addressed simultaneously in the research streams of plant biology and ecology.

Biostimulants are compounds that can modify the growth and development of plants as well as increase their resistance to stresses (Nephali et al., 2020; Mystkowska et al., 2023). The use of biostimulants in crop cultivation can reduce the use of fertilizers, synthetic plant growth regulators and chemical pesticides, thereby reducing production costs. Sodium alginate, an anionic polysaccharide, is recognized as a plant biostimulant with significant application potential due to its bioactivity, biocompatibility, and biodegradability (Martínez-Cano et al., 2022; Samdurkar et al., 2024). Sodium alginate is an eco-friendly and cheap biopolymer obtained from the marine brown algae and bacteria. The degradation of sodium alginate produces derivatives that often exhibit properties distinct from those of the native compound (Rosiak et al., 2021; Salachna 2023). It has been shown that alginate oligosaccharides can positively affect plant growth (El Idrissi et al., 2023), increase plant tolerance to salinity (Salachna et al., 2018; Golkar et al., 2019), and drought stress (Zhang et al., 2024). Most research on the use of sodium alginate and its fractions has focused on seed plants. There is currently no knowledge regarding how plants from lower taxonomic groups respond to sodium alginate and oligosaccharides derived from algae.

Ferns are some of the oldest plants on Earth, dating back over 360 million years and currently number about 10,000 species (Gerrienne et al., 2016). Ornamental and edible ferns are the most important economically (Cao et al., 2017). Ornamental ferns are widely valued as popular potted plants, as well as garden and park perennials (Marimuthu et al., 2022). Many species of tropical ferns are produced as attractive cut greens (McCulloch-Jones et al., 2021). Edible ferns are offered on the market in the form of young leaves and rhizomes which are abundant in polysaccharides, proteins, vitamins, minerals, polyphenols, as well as valuable ω-3 and ω-6 fatty acids (Zhu et al., 2019). Ferns are also used in folk medicine (Muhammad et al., 2020; Moussa et al., 2024). Fern extracts exhibit various medicinal effects and contain antioxidants with potential applications in the pharmacology, food, and cosmetic industries (Rosso, 2019; Dvorakova et al., 2024). The genus *Athyrium* Roth belongs to the Dryopteridaceae family and includes 230-300 species of ferns found mainly in Asia (Wyatt et al., 2022). Many are ornamental, edible, and medicinal plants (Salehi et al., 2019). Japanese goldenrod (*Athyrium nipponicum* (Mett.) Hance) is found in the forests of Japan, Korea, and Manchuria (Wyatt et al., 2022). The species includes many ornamental varieties with different leaf colors: metallic, light purple, dark purple, or burgundy (Dehgan, 2023). The leaves change their colors depending on the stage of development, season, growing conditions, and plant age. Numerous varieties of *A. nipponicum* are among the most beautiful garden ferns and, for this reason, are increasingly sought after on the ornamental plant market.

Knowledge regarding the effects of abiotic stresses and biostimulants on the growth of ornamental garden plants within the fern group remains limited. Therefore, the present study aimed to determine the effects of drought, salinity stress, and depolymerized sodium alginate on the growth, photosynthetic pigments, and metabolite content of *A. nipponicum* cv. Metallicum. The study poses the following research hypothesis:

1) There is a differential response of plants to single drought and salinity stress versus combined stress.
2) Depolymerized sodium alginate can alleviate the deleterious effects of abiotic stresses in ferns.

MATERIAL AND METHODS

Plant material and growing conditions

3-year-old plants of *A. nipponicum* cv. Metallicum with an average height of ~25 cm and a width of ~30 cm were planted on 14.04.2021 into round PVC pots 18.5 cm high, 21 cm wide, and with a capacity of 5 dm^3 (Maxiplast, Poland) filled with TS1 peat substrate (Klasmann-Deilmann, Germany) with a pH of 6.0, mixed with Complex fertilizer (Yara International ASA, Norway) with a composition of N 12%, P_2O_5 11%, K_2O 18%, MgO 2.7%, S 8%, B 0.015%, Fe 0.2%, Mn 0.02% and Zn 0.02% at a dose of 1.5 g dm^{-3} of substrate. Pots with plants were set in an unheated plastic tunnel with shades installed (~25% of the ambient sunlight), nine pots per 1 m^2. Plants were watered with a tap water drip system with an EC of ~0.63

$mS\cdot cm^{-1}$. Plants were grown under natural daylength. The average, maximum, and minimum air temperatures in the tunnel during the experiment were 20.7 °C, 36.0 °C, and 12.9 °C, respectively, and were regulated by a temperature-control system using a Type Z-AGRO 24-2 climate computer (Agrosur, Poland). Relative humidity was monitored and ranged between 65 and 85%.

Experimental design

The experiment was set up in a randomized block design. The experiment included eight treatments (Table 1), comprising a total 144 potted plants, with 18 potted plants each treatment.

In total, 72 potted plants were assigned randomly to each of four DA treatments. Plants were sprayed seven times with a depolymerized alginate (DA) solution at 100 $mg\cdot dm^{-3}$, starting June 1st, 2021, every 3 days, and each plant received ~20 ml of solution each time. Control plants were sprayed with water. The DA selected for the study with a molecular weight of 42,000 $g\cdot mol^{-1}$ was obtained by acid hydrolysis (Salachna, 2023).

The plants subjected to salinity stress (72 potted plants) were drenched with $CaCl_2$ solution (Chempur, Poland) at a concentration of 10 $mmol\cdot dm^{-3}$ (9.07.2021), 30 $mmol\cdot dm^{-3}$ (12.07.2021), 60 $mmol\cdot dm^{-3}$ (14.07.2021) and 100 $mmol\cdot dm^{-3}$ (16.07 and 21.07. 2021), to avoid osmotic shock, using 100 ml of solution per plant each time. The increasing concentration of $CaCl_2$ was established based on previous studies (Etehadnia et al., 2010). The plants not treated with $CaCl_2$ were watered with water. To prevent solution and water leaching, a plastic dish was inserted under each pot.

The plants subjected to drought stress from 21.07.2021 to 15.09.2021 were watered manually every 7 days with 100 ml of water per pot (72 potted plants). The plants not subjected to drought stress (72 potted plants) were watered with a drip system every 3 days, providing ~500 ml of water per pot each time. The substrate water potential read from the readings of tensiometers placed in the pots was approximately -400 hPa under water deficit conditions and approximately -100 hPa under drip irrigation conditions.

Determination of morphometric parameters

After the end of the drought stress (15.09.2021), morphological parameters were assessed, including plant height (from the substrate surface to the tallest part of the plant), plant width (measured at the widest part), number of leaves per plant, length of the longest leaf, as well as the fresh weight of leaves and rhizomes per plant. Plant rhizomes were washed free of substrate. The collected plant material was dried at 25 °C in a ventilated and shaded place for 3 weeks, after which the dry weight of leaves and rhizomes was determined. The dried leaves and rhizomes were ground in an electric grinder for further analysis.

Determination of photosynthetic pigments

The assessment of assimilation pigment content in leaves was conducted following the procedure proposed by Lichtenthaler and Wellburn (1983), with minor modifications, as described by Grzeszczuk et al. (2018). Pigments were extracted using 80% acetone (Merck, Germany). The samples underwent ultrasonic bath treatment for five minutes and were subsequently centrifuged at 13,000 × g for ten minutes. The absorbance of the resulting solutions was measured at 441, 646, 652, and 663 nm, and the concentrations of chlorophyll a, chlorophyll b, total chlorophyll, as well as carotenoids were calculated.

Table 1. Description of the treatments applied

Treatments	Description
Control	Cultivation under normal growing conditions
$CaCl_2$	Salinity stress induced by calcium chloride anhydrous
DS	Drought stress
$CaCl_2$+DS	Salinity stress + drought stress
DA	Depolymerized alginate
$CaCl_2$+DA	Salinity stress + depolymerized alginate
DS+DA	Drought stress + depolymerized alginate
$CaCl_2$+DS+DA	Salinity stress + drought stress + depolymerized alginate

Determination of reducing sugars content, total free amino acids content, total polyphenols and total flavonoids content

The content of primary and secondary metabolites in leaf and rhizome samples was analyzed using an extract preparation method described by Grzeszczuk et al. (2018). Plant material (0.5 g) was combined with a solution consisting of 80% methanol (Chempur, Piekary Śląskie) and deionized water in a 7:3 ratio (*v*/*v*). The mixture was sonicated for 15 minutes in an ultrasonic bath (Elmasonic S30H, Elma Schmidbauer GmbH, Germany). Subsequently, the mixture was centrifuged for 5 minutes at 5000 × g (Centrifuge 5418, Eppendorf, Poland) and filtered through nylon membrane filters (0.22 μm, Merck, Germany). The resulting extracts were stored in a freezer at -20 °C. Three independent test samples were prepared from each extract, and all measurements were performed using a spectrophotometer (Synergy LX, USA).

The content of reducing sugars was determined colorimetrically using the reaction with 3,5-dinitrosalicylic acid (DNS method) as described by Łopusiewicz et al. (2019). The extracted samples were combined with 0.05 M acetate buffer (pH 4.8) (Merck, Germany) and DNS reagent (Merck, Germany). The mixture was then heated at 96 °C for 5 minutes using an Eppendorf Thermomixer Compact 5350 (Eppendorf AG, Germany). Subsequently, the samples were cooled to room temperature, and absorbance was measured at 540 nm.

The content of free amino acids was determined using the colorimetric reaction of amino acids with ninhydrin (Merck, Germany), as described by Łopusiewicz et al. (2019). The extract was mixed with Cd-ninhydrin reagent and heated for 5 minutes in a heat block at 84 °C. The mixtures were then cooled on ice, transferred to a 96-well plate, and the absorbance was measured at 507 nm.

The total polyphenol content was determined using the Folin-Ciocalteu reagent (Chempur, Poland), while the total flavonoid content was assessed according to the methodology described by Tong et al. (2019). For polyphenol determination, distilled water, Folin-Ciocalteu reagent, and Na_2CO_3 (Sigma-Aldrich, Germany) were added to the extract. The samples were incubated in the dark at 40 °C for 30 minutes, and the absorbance was measured at 765 nm. For flavonoid determination, H_2O, 5% $NaNO_2$, 10% $AlCl_3$, and 1 M NaOH (Merck, Germany) were added to the extract. Finally, distilled water was added, and the absorbance was measured at 510 nm. Each analysis was performed in triplicate.

Statistical analysis

The results were statistically verified with ANOVA using TIBCO Statistica™ Professional 13.3.0 software (TIBCO Software, USA). Tukey's multiple comparison tests assessed the significance of mean value variation at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Morphological parameters

From the data presented in Table 2 and Figures 1–3, it can be seen that both stress factors and depolymerized alginate (DA) had a significant effect on the morphological characteristics of the ferns. Potent growth inhibition was observed in the plants growing under combined stress conditions ($CaCl_2$+DS). These plants were the lowest, had the smallest width, produced the fewest leaves, and were the shortest among all treatments. The plants at $CaCl_2$, DS and $CaCl_2$+DS showing foliar damage (Fig. 3). In the case of leaf biomass, both stress factors and depolymerized alginate reduced it, with significant differences from the control shown in all variants with drought (DS; DS+DA and $CaCl_2$+DS+DA). Similarly, drought showed a negative effect on rhizome biomass in this trait. Drought-stressed ferns (DS) produced rhizomes with the lowest fresh and dry weight among all treatments. The results confirm previously observed adverse effects of drought on fern growth (Xia et al., 2013; Salachna et al., 2021). Most ferns grow in shaded, humid habitats and exhibit low tolerance to drought stress. In the plants under the conditions of water deficit in the substrate, hydrostatic pressure decreases, as a result of which cell elongation growth is inhibited and dehydration occurs. These changes led to a reduction in the volume of plant cells and, as a result, a reduction in growth and biomass gain (Coussement et al., 2021). Research by other authors (Wang et al., 2019) shows that there are fern genotypes that can tolerate substrate water deficiency to some extent. As climate change indicates that droughts will become more

Table 2. Effects of salinity stress, drought stress and depolymerized alginate on morphometric parameters of fern *A. nipponicum* cv. Metallicum

Treatment	Plant height (cm)	Plant width (cm)	Number of leaves	Leaf length (cm)
Control	41.5 ± 4.60 a	63.3 ± 5.25 a	52.8 ± 6.70 a	48.2 ± 2.79 a
$CaCl_2$	28.0 ± 0.82 d	60.5 ± 2.08 ab	51.0 ± 8.41 a	38.0 ± 3.58 ab
DS	32.0 ± 3.74 bd	60.0 ± 2.16 ab	40.5 ± 2.08 ab	42.9 ± 4.55 ab
$CaCl_2$+DS	26.5 ± 0.58 d	48.5 ± 4.80 c	37.5 ± 6.03 b	35.8 ± 3.52 b
DA	36.5 ± 0.58 abc	50.5 ± 3.49 bc	46.5 ± 4.93 ab	40.9 ± 4.01 ab
$CaCl_2$+DA	37.9 ± 1.31 ab	58.1 ± 1.77 abc	43.8 ± 3.30 ab	41.5 ± 4.65 ab
DS+DA	37.1 ± 5.04 ab	56.1 ± 3.84 abc	42.5 ± 6.45 ab	39.7 ± 6.52 ab
$CaCl_2$+DS+DA	29.9 ± 1.75 d	49.0 ± 8.98 c	45.8 ± 0.96 ab	37.3 ± 4.35 b

Note: means ± S.D. (n = 18) within a column followed by the same letter(s) are not significantly different according Tukey`s multiple range test at $p \leq 0.05$.

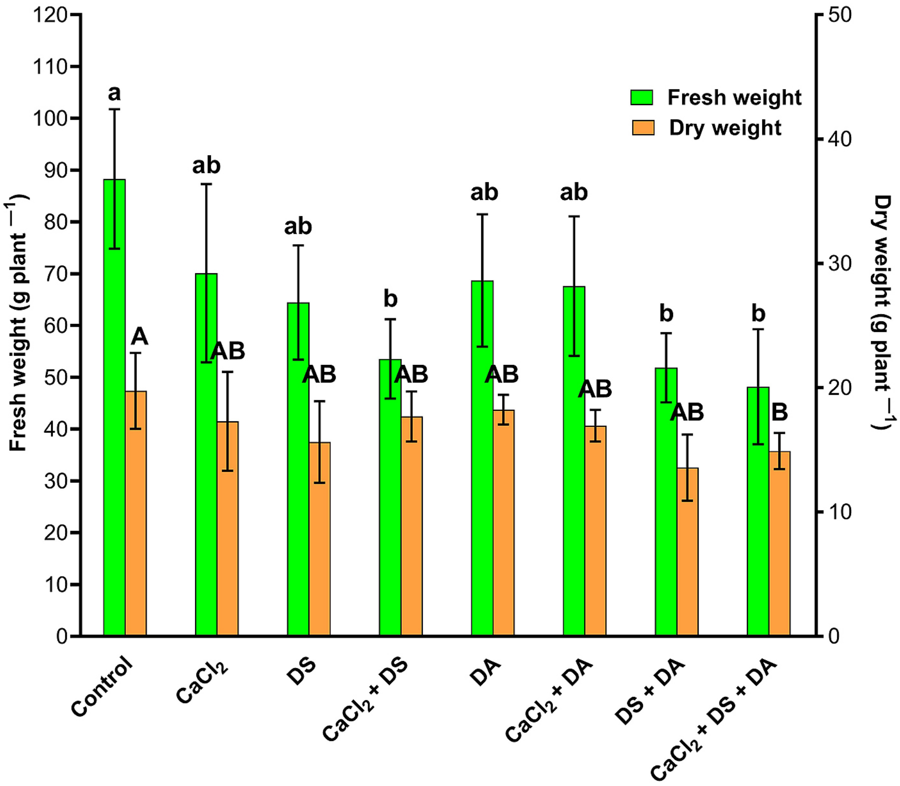


Figure 1. Effects of salinity stress, drought stress and depolymerized alginate on fresh weight and dry weight *A. nipponicum* cv. Metallicum leaves. Vertical bars indicate S.D. (n = 18). Bars marked by letter(s) show significant differences for fresh weight (lower-case letters) and for dry weight (capital letters) at $p \leq 0.05$ according Tukey`s multiple range test

severe, more research is needed to understand the adaptive mechanism of fern species and cultivars tolerating drought stress.

Stresses in plants caused by unfavorable environmental factors often overlap, compounding their harmful effects. The obtained results confirm that the growth of ferns was most severely reduced by combined stress (drought and salinity). Salinity alone ($CaCl_2$) reduced the height of ferns and slightly reduced the fresh and dry weight of leaves (Fig. 1) and rhizomes (Fig. 2). A similar negative effect of salinity stress on biomass was shown in *A. nipponicum* cv. Red Beauty (Pietrak et al., 2023). The observed adverse effect of $CaCl_2$ on the height of ferns may have been mainly due to ionic imbalance

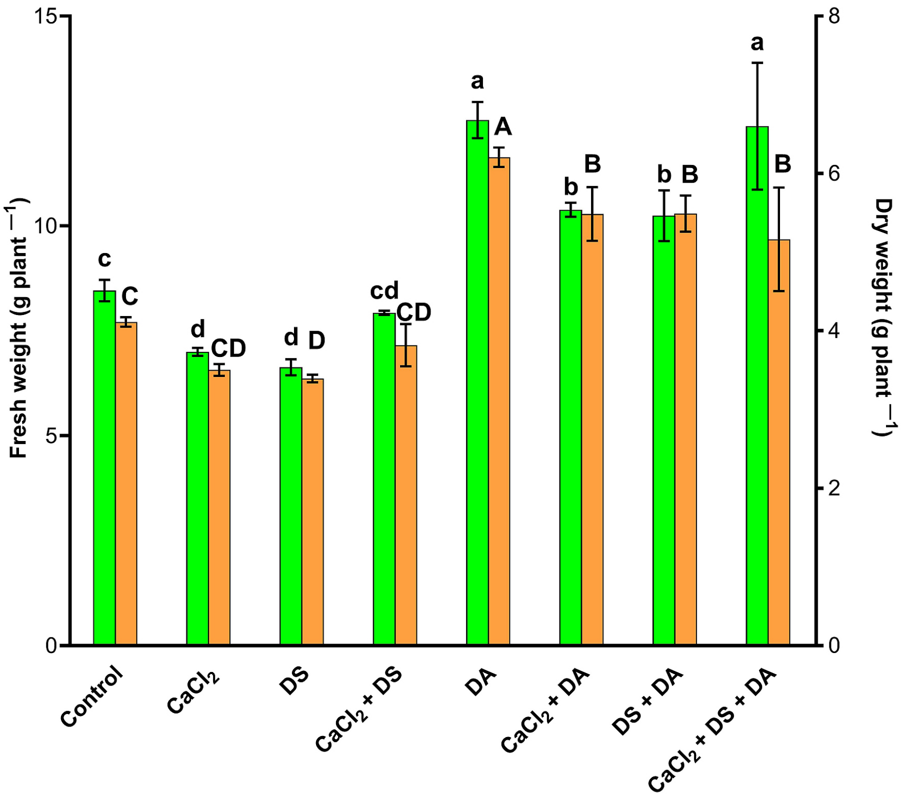


Figure 2. Effects of salinity stress, drought stress and depolymerized alginate on fresh weight and dry weight *A. nipponicum* cv. Metallicum rhizomes. Vertical bars indicate S.D. (n = 18). Bars marked by letter(s) show significant differences for fresh weight (lower-case letters) and for dry weight (capital letters) at $p \leq 0.05$ according Tukey`s multiple range test

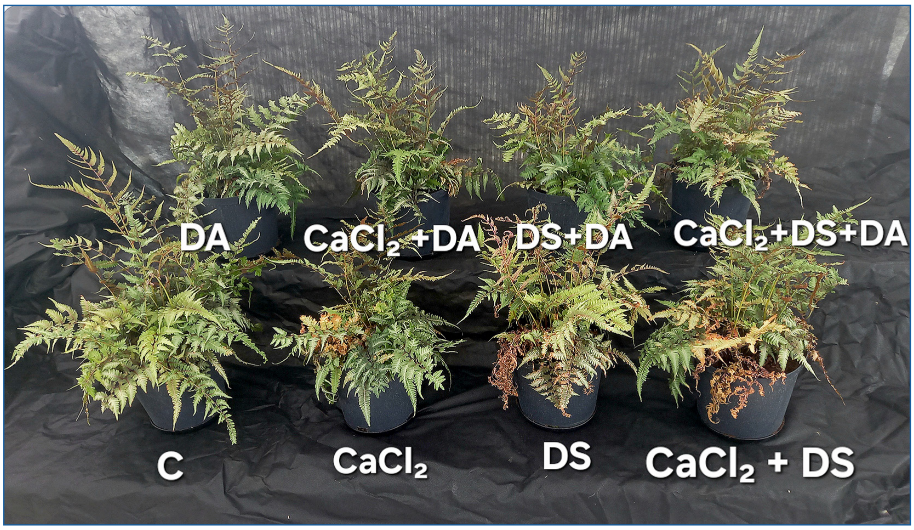


Figure 3. Effect of drought, salinity and depolymerized sodium alginate on visual quality of *Athyrium nipponicum* cv. Metallicum

and reduced water availability resulting from ions lowering the osmotic potential of the soil solution, leading to reduced growth (Wahab et al., 2022). In the literature, most studies on the effects of salt on plants focus on using NaCl (Grzeszczuk et al., 2018; Du et al., 2023; Fun et al., 2023). In the conducted study, $CaCl_2$ was chosen, a concentration higher than NaCl in soil and groundwater in many areas of the world, as a source of salt stress.

The application of DA in the cultivation of ferns had no apparent effect on the aboveground part of the plants, i.e., height, number of leaves, leaf length, and fresh and dry weight of leaves. DA had a different effect on the underground part of the ferns. The rhizomes of the DA-treated plants had the highest fresh and dry weights. Compared to the control, the fresh and dry weight of rhizomes after DA application increased by 48.1% and 51.0%, respectively. Moreover, when DA was applied under stress conditions ($CaCl_2$+DA; DS+DA; $CaCl_2$+DS+DA), the fresh and dry weights of rhizomes were higher than in the control and in the treatments without DA (Fig. 2). The plants under stress conditions and treated DA did not exhibit foliar damage (Fig. 3). The results demonstrate the biostimulatory effect of DA on the weight gain of fern rhizomes and, in part, confirm the previous reports that sodium alginate fractions and derivatives mitigate the effects of stress on plants (Du et al., 2023; Zhang et al. 2023). It is noteworthy that plant responses to the biostimulant depends on the species or cultivar, concentration and application method, substrate fertility, and environmental conditions, such as light, temperature and humidity.

Physiological parameters

As shown in Table 3, the applied stress factors and DA stimulated the content of assimilation pigments in fern leaves. The leaves of the plants treated with DA and $CaCl_2$ + DA had the most chlorophyll a, 56.3% and 53.1% more than the control, respectively. The leaves of DA-treated plants had the most chlorophyll b and total chlorophyll (an increase of 53.0% and 48.2%, respectively). Increased chlorophyll content in leaves due to DA application may have improved photosynthetic efficiency, increasing rhizosphere biomass production under optimal and stress conditions. Research results confirm the positive effect of sodium alginate and its derivatives on chlorophyll biosynthesis in plants (Gamel et al., 2023; Aly et al., 2024). It should be noted that the plants characterized by efficient chlorophyll metabolism and photosynthesis can better adapt to adverse environmental conditions (Yong et al., 2024).

Under the influence of stress factors and DA, fern leaves showed a significant increase in carotenoid content. The ferns treated with $CaCl_2$ had the highest carotenoid content, 40.6% more than the control (Table 3). Similarly, He et al. (2020) showed an increased content of carotenoids, including lutein and zeaxanthin, in maize under $CaCl_2$ stress. In addition, they showed that $CaCl_2$ promoted the expression of all essential carotenogenic genes and thus increased carotenoid formation. Increased carotenoid biosynthesis in response to salinity stress may be part of the plant defense mechanism that allows plants to minimize the effects of stress and maintain homeostasis (Babaei et al., 2022). As non-enzymatic antioxidants, carotenoids may help protect cells from reactive oxygen species and minimize the harmful effects of stress (Li et al., 2024).

The data presented in Figure 4 indicate significant changes in the content of reducing sugars and total free amino acids in the leaves and rhizomes of *A. nipponicum* cv. Metallicum, depending on the treatments applied. The plants growing under drought conditions (DS) had the highest amount of reducing sugars in their

Table 3. Effects of salinity stress, drought stress and depolymerized alginate on photosynthetic pigments of *A. nipponicum* cv. Metallicum

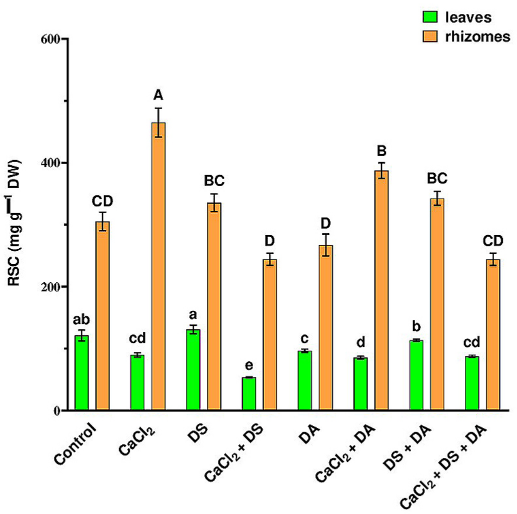
Treatment	Chlorophyll a ($mg \cdot g^{-1}$ DW)	Chlorophyll b ($mg \cdot g^{-1}$ DW)	Total chlorophyll ($mg \cdot g^{-1}$ DW)	Carotenoids (mg 100 g^{-1} DW)
Control	0.32 ± 0.00 d	0.17 ± 0.03 d	0.56 ± 0.01 d	15.90 ± 0.29 d
$CaCl_2$	0.48 ± 0.01 ab	0.22 ± 0.01 bc	0.79 ± 0.03 abc	22.35 ± 0.74 a
DS	0.44 ± 0.00 bc	0.20 ± 0.01 c	0.72 ± 0.01 c	20.63 ± 0.18 abc
$CaCl_2$+DS	0.47 ± 0.01 ab	0.24 ± 0.01 ab	0.80 ± 0.03 ab	21.79 ± 0.59 ab
DA	0.50 ± 0.02 a	0.26 ± 0.01 a	0.84 ± 0.05 a	21.80 ± 0.88 ab
$CaCl_2$+DA	0.49 ± 0.03 a	0.24 ± 0.01 ab	0.83 ± 0.04 ab	20.72 ± 1.11 abc
DS+DA	0.42 ± 0.03 c	0.21 ± 0.02 c	0.71 ± 0.05 c	19.89 ± 1.16 c
$CaCl_2$+DS+DA	0.44 ± 0.01 bc	0.21 ± 0.01 c	0.73 ± 0.03 c	20.57 ± 0.30 b

Note: means ± S.D. (n = 3) within a column followed by the same letter(s) are not significantly different according Tukey`s multiple range test at $p \leq 0.05$.

leaves. The most total free amino acids in leaves were found in control plants and $CaCl_2$+DA and DS+DA treatments. The lowest content of reducing sugars and amino acids in the leaves was observed in the plants subjected to combined stress ($CaCl_2$+DS). Analyzing the composition of underground organs showed that the rhizomes of plants subjected to salinity stress ($CaCl_2$) had the most reducing sugars, and the rhizomes of control plants had the most total free amino acids. The elevated levels of reducing sugars in fern leaves and rhizomes shown in these studies depending on the source of stress may be due to the protective role of soluble sugars in the osmotic regulation of ferns exposed to drought or salinity (Voytena et al., 2015; Wang et al., 2019).

The data presented in Figure 5 show that both stress factors and DA significantly affected the content of secondary metabolites in *A. nipponicum* cv. Metallicum. The content of total polyphenols and total flavonoids in leaves decreased due to stress and DA. A similar trend was observed for rhizomes, except for the plants exposed to salinity stress ($CaCl_2$), which showed increased total polyphenols and total flavonoids content by 22.1% and 15.0%, respectively, compared to the control. The leaves of ferns subjected to combined stress ($CaCl_2$ + drought stress) had the lowest levels of total polyphenols and total flavonoids. In contrast, the lowest levels of secondary metabolites in the rhizomes were observed in the DA-treated plants. Increased biosynthesis of polyphenolic compounds under drought stress was shown in earlier studies in the edible ferns *Athyrium multidentatum* (Doll.) Ching and *Matteuccia struthiopteris* (L.) Todar. The demonstrated increased accumulation of polyphenols and flavonoids in drought-exposed ferns may indicate the involvement of this compounds in plant adaptation processes to water deficit conditions (Bagniewska-Zadworna et al., 2008). Secondary metabolites constitute a vast and diverse group of chemical compounds, often characterized by unique biological activity. While they do not play a direct role in primary metabolism, they are crucial for plant resistance mechanisms against various stresses. (Tuladhar et al., 2021; Zawadzińska et al., 2021; Ahlawat et al., 2024). Surprisingly, the stimulating effect of DA on rhizome biomass in fern was accompanied by a clear drop in the levels of total polyphenols and total flavonoids. The decrease content of secondary metabolites may be a result of a trade-off between the production of plant biomass and secondary metabolism (Xu et al. 2024).

Comparing the average content of secondary metabolites in the organs of *A. nipponicum* cv. Metallicum showed that the fern rhizomes contained, on average, 4.8 times more total polyphenols and 3.6 times more total flavonoids than the leaves. In most studies on the evaluation of polyphenols of ferns, the raw material is the leaves (Langhansova et al., 2021). Flavonoids, especially flavonols, are the largest group of polyphenolic compounds in



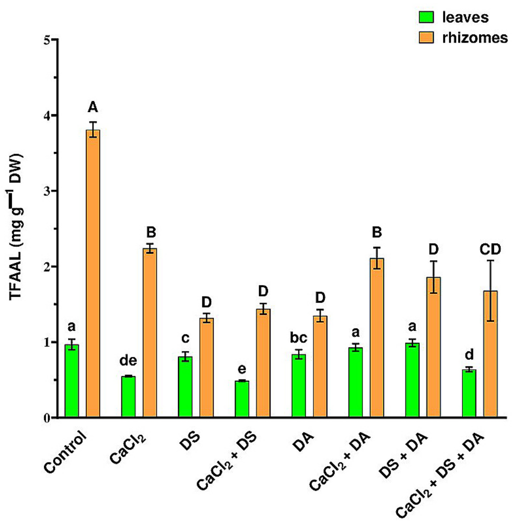


Figure 4. Effects of salinity stress, drought stress and depolymerized alginate on reducing sugar content (RSC) and total free amino acids level (TFAAL) of *A. nipponicum* cv. Metallicum. Vertical bars indicate S.D. (n = 3). Bars marked by letter(s) show significant differences for leaves (lower-case letters) and for rhizomes (capital letters) at $p \leq 0.05$ according Tukey`s multiple range test

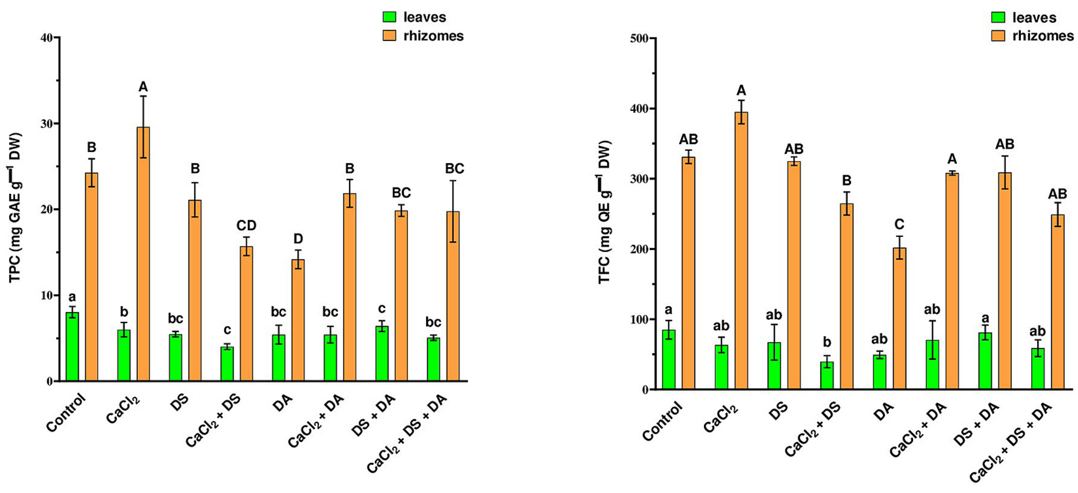


Figure 5. Effect of single and multiple stresses and depolymerized sodium alginate (DA) on total polyphenols content (TPC) and total flavonoids content (TFC) of *A. nipponicum* cv. Metallicum. Vertical bars indicate S.D. (n = 3). Bars marked by letter(s) show significant differences for leaves (lower-case letters) and for rhizomes (capital letters) at $p \leq 0.05$ according Tukey`s multiple range test

many fern species and are particularly interesting due to their antioxidant properties (Vetter, 2018). The flavonoids found in ferns may be used as natural compounds to develop new drugs to prevent and treat various diseases. These can also become leading ingredients in cosmetics and dietary supplements (Cao et al., 2017; Szypuła and Pietrosiuk, 2021).

CONCLUSIONS

In summary, the morphological response of *A. nipponicum* cv. Metallicum to combined stress ($CaCl_2$ + drought stress) was more pronounced compared to its response to single stress factors. When analyzing biomass losses and changes in the levels of total polyphenols and total flavonoids it was found that plants tolerated salinity stress better than drought stress. The DA biostimulant mitigated the deleterious effects of salinity and drought on plants by increasing leaf chlorophyll content and stimulating biomass growth of rhizomes. Future research on the evaluation of *A. nipponicum* as a source of biologically active compounds should prioritize the rhizomes, as they contain significantly higher levels of secondary metabolites compared to the leaves. The findings presented in this study contribute to the current body of knowledge on the use of eco-friendly DA as alleviators of abiotic stress in plants and have potential applications in the cultivation of other perennial species.

REFERENCES

1. Ahlawat, Y.K., Singh, M., Manorama, K., Lakra, N., Zaid, A., & Zulfiqar, F. (2024). Plant phenolics: Neglected secondary metabolites in plant stress tolerance. *Brazilian Journal of Botany, 47*(3), 703–721. https://doi.org/10.1007/s40415-023-00949-x
2. Aly, A.A., Eliwa, N.E., & Safwat, G. (2024). Role of gamma-irradiated sodium alginate on growth, physiological and active components of iceberg lettuce (*Lactuca sativa*) plant. *BMC Plant Biology, 24*(1), 185. https://doi.org/10.1186/s12870-024-04853-8
3. Babaei, M., Shabani, L., & Hashemi-Shahraki, S. (2022). Improving the effects of salt stress by β-carotene and gallic acid using increasing antioxidant activity and regulating ion uptake in *Lepidium sativum* L. *Botanical Studies, 63*(1), 22. https://doi.org/10.1186/s40529-022-00352-x
4. Bagniewska-Zadworna, A., Zenkteler, E., Karolewski, P., & Zadworny, M. (2008). Phenolic compound localisation in *Polypodium vulgare* L. rhizomes after mannitol-induced dehydration and controlled desiccation. *Plant Cell Reports, 27*(7), 1251–1259. https://doi.org/10.1007/s00299-008-0548-3
5. Cao, H., Chai, T.-T., Wang, X., Morais-Braga, M. F. B., Yang, J.-H., Wong, F.-C., Wang, R., Yao, H., Cao, J., Cornara, L., Burlando, B., Wang, Y., Xiao, J., & Coutinho, H. D. M. (2017). Phytochemicals from fern species: Potential for medicine applications. *Phytochemistry Reviews, 16*(3), 379–440. https://doi.org/10.1007/s11101-016-9488-7
6. Coussement, J.R., Villers, S.L.Y., Nelissen, H., Inzé, D., & Steppe, K. (2021). Turgor-time controls grass leaf elongation rate and duration under drought stress. *Plant, Cell & Environment, 44*(5),

1361–1378. https://doi.org/10.1111/pce.13989

7. Dehgan, B. (2023). Lycophyes and Monilophytes (ferns). In *Garden Plants Taxonomy: Volume 1: Ferns, Gymnosperms, and Angiosperms (Monocots)* (pp. 13-56). Springer International Publishing: Cham, Switzerland. https://doi.org/10.1007/978-3-031-11561-5_2
8. Du, Y.-W., Liu, L., Feng, N.-J., Zheng, D.-F., Liu, M.-L., Zhou, H., Deng, P., Wang, Y., & Zhao, H.-M. (2023). Combined transcriptomic and metabolomic analysis of alginate oligosaccharides alleviating salt stress in rice seedlings. *BMC Plant Biology, 23*(1), 455. https://doi.org/10.1186/s12870-023-04470-x
9. Dvorakova, M., Soudek, P., Pavicic, A., & Langhansova, L. (2024). The traditional utilization, biological activity and chemical composition of edible fern species. *Journal of Ethnopharmacology, 324,* 117818. https://doi.org/10.1016/j.jep.2024.117818
10. El Idrissi, A., Dardari, O., Metomo, F.N.N.N., Essamlali, Y., Akil, A., Amadine, O., Aboulhrouz, S., & Zahouily, M. (2023). Effect of sodium alginate-based superabsorbent hydrogel on tomato growth under different water deficit conditions. *International Journal of Biological Macromolecules, 253,* 127229. https://doi.org/10.1016/j.ijbiomac.2023.127229
11. Etehadnia, M., Schoenau, J., Waterer, D., & Karen, T. (2010). The effect of $CaCl_2$ and NaCl salt acclimation in stress tolerance and its potential role in ABA and scion/rootstock-mediated salt stress responses. *Plant Stress, 4*, 72–81.
12. Fan, M., Zhang, E., Zhang, X., Liu, Q., & Guo, F. (2023). Silicon improves the growth of *Glycyrrhiza uralensis* Fisch. under salt and/or drought stress by regulating respiration metabolism. *Plant Growth Regulation, 101*(3), 743–767. https://doi.org/10.1007/s10725-023-01054-5
13. Gamel, R.M., Elsayed, N., Haroun, S.A., Hegazy, A.O., Khalil, R., El-Desouky, H. S., Zewail, R.M.Y., Helal, M., Sami, R., Harara, S., & Albakry, A. F. (2023). Sodium alginate stimulates growth and metabolism in shoots of *Corchorus olitorius*, *Vicia faba*, and *Zea mays* through the vegetative stages. *Journal of Biobased Materials and Bioenergy, 17*(1), 1–13. https://doi.org/10.1166/jbmb.2023.2254
14. García-Caparrós, P., Vogelsang, L., Persicke, M., Wirtz, M., Kumar, V., & Dietz, K.-J. (2022). Differential sensitivity of metabolic pathways in sugar beet roots to combined salt, heat, and light stress. *Physiologia Plantarum, 174*(5), e13786. https://doi.org/10.1111/ppl.13786
15. Gerrienne, P., Servais, T., & Vecoli, M. (2016). Plant evolution and terrestrialization during Palaeozoic times—The phylogenetic context. *Review of Palaeobotany and Palynology, 227,* 4–18. https://doi.org/10.1016/j.revpalbo.2016.01.004
16. Golkar, P., Taghizadeh, M., & Noormohammadi, A. (2019). Effects of sodium alginate elicitation on secondary metabolites and antioxidant activity of safflower genotypes under *in vitro* salinity stress. *In Vitro Cellular & Developmental Biology - Plant, 55*(5), 527–538. https://doi.org/10.1007/s11627-019-10008-4
17. Grzeszczuk, M., Salachna, P., & Meller, E. (2018). Changes in photosynthetic pigments, total phenolic content, and antioxidant activity of *Salvia coccinea* Buc'hoz Ex Etl. Induced by exogenous salicylic acid and soil salinity. *Molecules, 23*(6). https://doi.org/10.3390/molecules23061296
18. He, W., Wang, Y., Luo, H., Li, D., Liu, C., Song, J., Zhang, Z., Liu, C., & Niu, L. (2020). Effect of NaCl stress and supplemental $CaCl_2$ on carotenoid accumulation in germinated yellow maize kernels. *Food Chemistry, 309,* 125779. https://doi.org/10.1016/j.foodchem.2019.125779
19. Huanhe, W., Xiaoyu, G., Xiang, Z., Wang, Z., Xubin, Z., Yinglong, C., Zhongyang, H., Guisheng, Z., Tianyao, M., & Qigen, D. (2024). Grain yield, biomass accumulation, and leaf photosynthetic characteristics of rice under combined salinity-drought stress. *Rice Science, 31*(1), 118–128. https://doi.org/10.1016/j.rsci.2023.06.006
20. Kıran, S., & Baysal Furtana, G. (2023). Responses of Eggplant seedlings to combined effects of drought and salinity stress: Effects on photosynthetic pigments and enzymatic and non-enzymatic antioxidants. *Gesunde Pflanzen, 75*(6), 2579–2590. https://doi.org/10.1007/s10343-023-00901-9
21. Langhansova, L., Pumprova, K., Haisel, D., Ekrt, L., Pavicic, A., Zajíčková, M., Vanek, T., & Dvorakova, M. (2021). European ferns as rich sources of antioxidants in the human diet. *Food Chemistry, 356,* 129637. https://doi.org/10.1016/j.foodchem.2021.129637
22. Li, C., Wang, C., Cheng, Z., Li, Y., & Li, W. (2024). Carotenoid biosynthesis genes LcLCYB, LcLCYE, and LcBCH from wolfberry confer increased carotenoid content and improved salt tolerance in tobacco. *Scientific Reports, 14*(1). https://doi.org/10.1038/s41598-024-60848-3
23. Lichtenthaler, H. K., & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions, 11*(5), 591–592. https://doi.org/10.1042/bst0110591
24. Łopusiewicz, Ł., Drozłowska, E., Siedlecka, P., Mężyńska, M., Bartkowiak, A., Sienkiewicz, M., Zielińska-Bliźniewska, H., & Kwiatkowski, P. (2019). Development, characterization, and bioactivity of non-dairy kefir-like fermented beverage based on flaxseed oil cake. *Foods, 8*(11), 544. https://doi.org/10.3390/foods8110544
25. Marien, L., Crabit, A., Dewandel, B., Ladouche, B.,

Fleury, P., Follain, S., Cavero, J., Berteloot, V., & Colin, F. (2023). Salinity spatial patterns in Mediterranean coastal areas: The legacy of historical water infrastructures. *Science of The Total Environment, 899,* 165730. https://doi.org/10.1016/j.scitotenv.2023.165730

26. Marimuthu, J., Fernández, H., Kumar, A., & Thangaiah, S. (2022). Biotechnology, propagation, medicinal uses and environmental regulation. In J. Marimmuthu et al. (Eds.), *Ferns.* Springer Nature Singapore. https://doi.org/10.1007/978-981-16-6170-9
27. Martínez-Cano, B., Mendoza-Meneses, C.J., García-Trejo, J.F., Macías-Bobadilla, G., Aguirre-Becerra, H., Soto-Zarazúa, G.M., & Feregrino-Pérez, A.A. (2022). Review and perspectives of the use of alginate as a polymer matrix for microorganisms applied in agro-industry. *Molecules, 27*(13). https://doi.org/10.3390/molecules27134248
28. McCulloch-Jones, E., Kraaij, T., Crouch, N., & Fritz, H. (2021). The effect of horticultural trade on establishment success in alien terrestrial true ferns (Polypodiophyta). *Biological Invasions, 23*(11), 3583–3596. https://doi.org/10.1007/s10530-021-02599-0
29. Moussa, A.Y., Luo, J., & Xu, B. (2024). Insights into chemical diversity and potential health-promoting effects of ferns. *Plants, 13*(18). https://doi.org/10.3390/plants13182668
30. Muhammad, M., Ismail, Z. S., Schneider, H., & Hawkins, J.A. (2020). Medicinal use of ferns: An ethnobotanical review. *Sains Malaysiana, 49*(5), 1003–1014. https://doi.org/10.17576/jsm-2020-4905-05
31. Mystkowska, I., Zarzecka, K., Ginter, A., Gugała, M., & Sikorska, A. (2023). Total and protein nitrogen content in potato tubers under the influence of various care and nutrition methods with the use of biostimulants. *Journal of Ecological Engineering*, *24*(2), 247–255. https://doi.org/10.12911/22998993/157019
32. Najjaa, H., Boubakri, A., Arfa, A. B., Zouari, N., & Neffati, M. (2018). Salinity and drought stresses improve antioxidant potential of *Allium roseum* L., an edible medicinal plant. *Acta physiologiae plantarum, 40*(11), 195. https://doi.org/10.1007/s11738-018-2774-9
33. Nazari, M., Ghasemi-Soloklui, A.A., Kordrostami, M., & Latef, A.A.H.A. (2023). Deciphering the response of medicinal plants to abiotic stressors: A focus on drought and salinity. *Plant Stress*, 100255. https://doi.org/10.1016/j.stress.2023.100255
34. Nephali, L., Piater, L.A., Dubery, I.A., Patterson, V., Huyser, J., Burgess, K., & Tugizimana, F. (2020). Biostimulants for plant growth and mitigation of abiotic stresses: A metabolomics perspective. *Metabolites, 10*(12), 505. https://doi.org/10.3390/metabo10120505
35. Pietrak, A., Salachna, P., & Łopusiewicz, Ł. (2023). Changes in growth, ionic status, metabolites content and antioxidant activity of two ferns exposed to shade, full sunlight, and salinity. *International Journal of Molecular Sciences*, *24*(1), 296. https://doi.org/10.3390/ijms24010296
36. Rosiak, P., Latanska, I., Paul, P., Sujka, W., & Kolesinska, B. (2021). Modification of alginates to modulate their physic-chemical properties and obtain biomaterials with different functional properties. *Molecules, 26*(23), 7264. https://doi.org/10.3390/molecules26237264
37. Rosso, J.Q.D. (2019). *Polypodium leucotomos* extract (PLE): New study gives evidence-based insight into "ain't nothing like the real thing". *The Journal of Clinical and Aesthetic Dermatology, 12*(8), 45-46.
38. Salachna, P. (2023). Depolymerised sodium alginate as a eco-friendly biostimulant for improving herb yield and nutrient accumulation in *Hyssop*. *Journal of Ecological Engineering, 24*(9), 105–111. https://doi.org/10.12911/22998993/168553
39. Salachna, P., Grzeszczuk, M., Meller, E., & Soból, M. (2018). Oligo-alginate with low molecular mass improves growth and physiological activity of *Eucomis autumnalis* under salinity stress. *Molecules, 23*(4), 812. https://doi.org/10.3390/molecules23040812
40. Salachna, P., Siemińska, I., Pietrak, A., Zawadzińska, A., Piechocki, R., & Dymek, R. (2021). Response of hardy ferns to drought stress. *Biology and Life Sciences Forum, 3*(1). https://doi.org/10.3390/IECAG2021-09723
41. Salehi, B., Ezzat, S.M., Tsouh Fokou, P. V., Albayrak, S., Vlaisavljevic, S., Sharifi-Rad, M., Bhatt, I.D., Sharifi-Rad, M., Belwal, T., Ayatollahi, S.A., Kobarfard, F., Ata, A., Baghalpour, N., Martorell, M., Setzer, W.N., & Sharifi-Rad, J. (2019). *Athyrium* plants—Review on phytopharmacy properties. *Journal of Traditional and Complementary Medicine, 9*(3), 201–205. https://doi.org/10.1016/j.jtcme.2018.09.001
42. Samdurkar, A. N., Choudhary, A. D., Varshney, L., & Badere, R. S. (2024). Application of 28.1 kDa alginate oligosaccharide improves the yield and quality of grain in Rice. *ChemistrySelect, 9*(38), e202400742. https://doi.org/10.1002/slct.202400742
43. Sharif, P., Seyedsalehi, M., Paladino, O., Van Damme, P., Sillanpää, M., & Sharifi, A. A. (2018). Effect of drought and salinity stresses on morphological and physiological characteristics of canola. *International Journal of Environmental Science and Technology, 15,* 1859-1866. https://doi.org/10.1007/s13762-017-1508-7
44. Shrivastava, P., & Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for

its alleviation. *Saudi Journal of biological sciences*, *22*(2), 123-131. https://doi.org/10.1016/j.sjbs.2014.12.001

45. Spinoni, J., Vogt, J.V., Naumann, G., Barbosa, P., & Dosio, A. (2018). Will drought events become more frequent and severe in Europe? *International Journal of Climatology, 38*(4), 1718-1736. https://doi.org/10.1002/joc.5291
46. Szypuła, W. J., & Pietrosiuk, A. (2021). Biology, phytochemistry, pharmacology, and biotechnology of European ferns, club mosses, and horsetails: A Review. In H.M. Ekiert, K.G. Ramawat, & J. Arora (Eds.), *Medicinal Plants: Domestication, biotechnology and regional importance* (pp. 605–660). Springer International Publishing. https://doi.org/10.1007/978-3-030-74779-4_19
47. Tong, T., Liu, Y.-J., Kang, J., Zhang, C.-M., & Kang, S.-G. (2019). Antioxidant activity and main chemical components of a novel fermented tea. *Molecules, 24*(16). https://doi.org/10.3390/molecules24162917
48. Tuladhar, P., Sasidharan, S., & Saudagar, P. (2021). Role of phenols and polyphenols in plant defense response to biotic and abiotic stresses. In *Biocontrol agents and secondary metabolites* (pp. 419–441). Elsevier. https://doi.org/10.1016/B978-0-12-822919-4.00017-X
49. Vetter, J. (2018). Secondary metabolites of ferns. In H. Fernández (Ed.), *Current advances in fern research* (pp. 305–327). Springer International Publishing. https://doi.org/10.1007/978-3-319-75103-0_15
50. Voytena, A.P.L., Minardi, B.D., Barufi, J.B., Santos, M., & Randi, Á. M. (2015). *Pleopeltis pleopeltifolia* (Polypodiopsida, Polypodiaceae), a poikilochlorophyllous desiccation-tolerant fern: Anatomical, biochemical and physiological responses during water stress. *Australian Journal of Botany, 62*(8), 647-656. https://doi.org/10.1071/BT14166
51. Wahab, A., Abdi, G., Saleem, M.H., Ali, B., Ullah, S., Shah, W., Mumtaz, S., Yasin, G., Muresan, C.C., & Marc, R.A. (2022). Plants' physio-biochemical and phyto-hormonal responses to alleviate the adverse effects of drought stress: A comprehensive review. *Plants, 11*(13), 1620. https://doi.org/10.3390/plants11131620
52. Wang, Y., Gao, S., He, X., Li, Y., Li, P., Zhang, Y., & Chen, W. (2019). Growth, secondary metabolites and enzyme activity responses of two edible fern species to drought stress and rehydration in Northeast China. *Agronomy, 9*(3), 137. https://doi.org/10.3390/agronomy9030137
53. Wyatt, R., & Harris, R.N. (2022). More on the spread of nonnative ferns in Georgia and the Southeastern United States. *American Fern Journal, 112*(1), 36–49. https://doi.org/10.1640/0002-8444-112.1.36
54. Xia, X., Cao, J., Zheng, Y., Wang, Q., & Xiao, J. (2014). Flavonoid concentrations and bioactivity of flavonoid extracts from 19 species of ferns from China. *Industrial Crops and Products, 58,* 91–98. https://doi.org/10.1016/j.indcrop.2014.04.005
55. Xu, Z., Xiao, Y., Guo, J., Lv, Z., & Chen, W. (2024). Relevance and regulation of alternative splicing in plant secondary metabolism: current understanding and future directions. *Horticulture Research*, *11*(8), uhae173.
56. Yong, S., Chen, Q., Xu, F., Fu, H., Liang, G., & Guo, Q. (2024). Exploring the interplay between angiosperm chlorophyll metabolism and environmental factors. *Planta, 260*(1), 25. https://doi.org/10.1007/s00425-024-04437-8
57. Zawadzińska, A., Salachna, P., Nowak, J. S., Kowalczyk, W., Piechocki, R., Łopusiewicz, Ł., & Pietrak, A. (2021). Compost based on pulp and paper mill sludge, fruit-vegetable waste, mushroom spent substrate and rye straw improves yield and nutritional value of tomato. *Agronomy*, *12*(1), 13. https://doi.org/10.3390/agronomy12010013
58. Zhang, W., Zheng, L., Lang, D., Zhang, X., Ma, X., Li, X., & Zhang, X. (2023). Eco-friendly bio-encapsulation from sodium alginate-trehalose-kaolin and its performance evaluation in improving plant growth under salt or/and drought conditions. *International Journal of Biological Macromolecules, 225,* 123–134. https://doi.org/10.1016/j.ijbiomac.2022.12.009
59. Zhang, Y., Zhan, J., Ma, C., Liu, W., Huang, H., Yu, H., Christie, P., Li, T., & Wu, L. (2024). Root-associated bacterial microbiome shaped by root selective effects benefits phytostabilization by *Athyrium wardii* (Hook.). *Ecotoxicology and Environmental Safety, 269,* 115739. https://doi.org/10.1016/j.ecoenv.2023.115739
60. Zhu, Q.-F., & Zhao, Q.-S. (2019). Chemical constituents and biological activities of lycophytes and ferns. *Chinese Journal of Natural Medicines, 17*(12), 887–891. https://doi.org/10.1016/S1875-5364(19)30108-6