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EFFECT OF *ASCOPHYLLUM NODOSUM* EXTRACT ON THE GROWTH PARAMETERS AND YIELD OF WILD ROCKET (*DIPLOTAXIS TENUIFOLIA)*

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Abstract

The aim of the research was to assess the significance of variations in yield, morphological (stem length, leaf length, mean number of leaves, root lenght) and physiological (nitrogen balance index, chlorophyll, flavonoids, anthocyanins) traits of wild rocket under application of different doses of *Ascophyllum nodosum* extract (ANE) mixed with distilled water (0, 2, 4 and 6 ml/l). The experiment was conducted in greenhouse, in pots, from the fourth week of April to the second week of July 2025. Each pot was filled with 1.4 kg/pot of homogenized Vertisol, soil with a very clayey texture and acidicic reaction. Physiological parameters were determined using a Dualex leaf clip sensor. The obtained results indicated a very noticeable influence of all applied doses of ANE on all tested plant parameters in relation to soil not treated with ANE. The most pronounced effect on plant morphological traits and yield had ANE dose at 4 ml/l, while on physiological parameters - ANE doses at 2 and 4 ml/l. Concluding, improved plant nutrition with 4 ml/l of ANE promoted the root growth, reflecting on the growth, development and yield of wild rocket plants. Further research in this area of study on larger soil areas could suggest new possibilities for *A. nodosum* application in the cultivation of rocket plants and other vegetable crops from an ecological point of view.

Introduction

Perennial wild rocket (*Diplotaxis tenuifolia* (L.) D.C.) is a plant from the cabbage family (Brassicaceae) with a specific smell and taste. Its taste is very similar to that of cultivated arugula, but is more intense. The young plants are also edible. Traditionally grown as a spontaneous plant along roadsides and in abandoned places, it was mostly domesticated in Europe and western Asia. Nowdays, it is a cultivated crop species of increasing importance with worldwide distribution.

Wild rocket can grow in any moderately fertile soil, slightly acidic to neutral, partially sunny and moist, but well-drained, with high drought resistance. This is due to the presence of a well-developed taproot, suggesting limiting irrigation. Nutritional requirements of wild rocket are modest, and can be supplied through fertigation [1].

Wild rocket is recognized for various medicinal and therapeutic properties and thus health beneficial effects in bactericidal and anti-inflammatory use. The leaves of wild rocket have notable nutritional properties related to their contents of flavonoids, fibre, calcium, iron, potassium, phosphorus, richness in vitamins such as A, B, C, and K, essential proteins, as well as a number of other microelements essential for the human health [1, 2].

The plant can be grown in open fields but more often in greenhouses, particularly in organic production [3]. Rocket grown in organic conditions without pesticides and inorganic fertilizers is an increasingly common choice for health-conscious consumers [2].

Methods in organic production imply the application of natural processes (crop rotation, intercropping, cover crops, minimal tillage) and substances (organic fertilizers, symbiotic associations, biostimulators), and limit or completely eliminate the use of synthesized agents, promoting ecosystem preservation by integrating biodiversity and biological cycles [4].

Among the innovative and promising solutions for improving the sustainability and profitability of organic agriculture, biostimulants are being considered, especially those based on seaweed extracts. The use of natural seaweeds as fertilizer enables the gradual substitution of conventional inorganic fertilizers. These fertilizers are biodegradable, non toxic and non polluting in nature, and also non hazardous to humans, animals and birds.

A wide range of beneficial effects of seaweed extracts, necessary for plant growth and development, are based on the presence of multiple growth regulators (cytokinin, auxins, gibberellins, betaines), macronutrients (Ca, K, P), and micronutrients (Fe, Cu, Zn, B, Mn, Co and Mo) [5].

Due to their ability to be used in organic agriculture, extracts of the brown seaweed *Ascophyllum nodosum* (L.) are well-known and commercially present as a suitable alternative to reduce the use of chemical inputs and has the potential to increase the quantitative and qualitative yield of various culivars [6]. Aly et al. [7] observed the highest chlorophyll content, grain yield and essential oil content in parsley when using foliar application of *A. nodosum* extract. Nasiri et al. [8] reported the increased content of carvacrol in essential oil of savory under the application of *A. nodosum* extract. The extracts are also well-established plant biostimulants that improve stress tolerance and crop vigour, expressing ability to stimulate soil microbes [9].

The use of *A. nodosum* extract in agriculture, mostly in organic, significantly improved the germination rate, thus increasing the growth of lateral roots, enhancing water and nutrient use efficiencies, increasing the content of flavonoid, chlorophyll and nutrient, alleviating the effects of abiotic and biotic stresses in different crop plants [10].

This research aimed to evaluate the effect of different doses of *A. nodosum* extract (ANE) on the yield, morphological and physiological traits of wild rocket, grown on Vertisol, performed in semi-controlled greenhouse conditions.

Experimental

This study was performed under semi-controlled greenhouse conditions at the Institute of Soil Science, using plastic pots, from the fourth week of April to the second week of July 2025. Each pot was filled with 1.4 kg/pot of homogenized Vertisol soil [11], sourced from a field located in Mala Ivanča, Sopot Municipality (grid reference: 44°35' N, 20°36' E), about 35 km from Belgrade in Serbia. Our previous study [12] revealed high clay fraction, acidic reaction, high available K, very low available P, medium total N and SOM supply in Vertisol.

In every plastic pot, fifteen rocket seeds were sown on April 28. After the plants have grown 2.5-3 cm above the ground ten plants per pot were left. Plants were grown using standard growing methods (regular watering, manual weed control, and without chemical protection). The following four designed treatments were carried out in three replications: ANE (0.2%, 2 ml/l distilled water); ANE (0.4%, 4 ml/l distilled water); ANE (0.6%, 6 ml/l distilled water); Control (ANE 0 ml/l distilled water). According to the manufacturer's packaging, the extract of *A. nodosum* is a nitrogenous liquid organic fertilizer of plant origin (algae).

After sowing, soils were sprayed with appropriate doses of ANE, and when the plants grew to 2.5-3 cm above the soil and after triage, soil and foliar spraying were performed every 7 days. Regular watering was done as needed, so that the grown plants always had enough moisture. Seven days before the complete removing the plants, morphological traits (stem length, SL; leaf length, LL; mean number of leaves, MNL), and physiological parameters (nitrogen balance index, NBI; chlorophyll, Chl; flavonoids, Flv; anthocyanins, Ant) of the plants aerial biomass were measured. Physiological parameters were measured using portable Dualex optical leafclip sensor (FORCE-A, Orsay, France). After seven days, on July 14, the rocket plants were completely removed from the pots, taking care to separate the root from the soil in the safest

way possible. The root was then thoroughly cleaned of soil using distilled water and its length (root length, RL) was measured for each treatment. The yield of the rocket aerial biomass from each treatment was first measured for fresh weight, then air-dried and measured for dry weight, both parameters expressed in g/pot.
Analysis of variance (ANOVA, SPSS 22.0), followed by Duncan's multiple range tests (DMRT) at $p < 0.05$, were used to statistically analyse the data relevant to the treatment effects on all plant parameters.

Results and discussion

The values of the yield, morphological and physiological traits of wild rocket showed distinct responses under influence of treatment with different doses of *A. nodosum* extract (ANE).
Statistical analysis of the differences between the treatments for individual comparison of plant morphological traits is presented in Table 1. The obtained results indicated a statistically significant effect ($p < 0.05$) of all applied doses of ANE on plant morphological traits in relation to soil not treated with ANE. The most pronounced significant effect ($p < 0.05$) on all plant morphological traits compared to other tested treatments was determined in the treatment where ANE at dose of 4 ml/l was applied, followed by treatments sprayed with ANE at doses of 6 and 2 ml/l. Accordingly, improved plant nutrition with 4 ml/l of ANE promoted the root growth, reflecting on the growth and development of an aerial biomass of wild rocket plants (Figure 1).

Table 1. Effect of treatments on morphological traits of wild rocket

Treatments	Morphological traits[1]			
	SL (cm)	LL (cm)	MNL	RL (cm)
T1 - ANE (0 ml/l)	9.17 c (±1.26)	3.53 c (±0.93)	3.33 c (±0.58)	6.83 d (±2.08)
T2 - ANE (2 ml/l)	13.45 b (±3.25)	4.18 bc (±0.66)	5.77 b (±0.58)	8.63 c (±0.85)
T3 - ANE (4 ml/l)	16.33 a (±1.44)	6.50 a (±1.32)	6.53 a (±0.58)	10.83 a (±1.61)
T4 - ANE (6 ml/l)	13.53 b (±1.63)	4.33 b (±1.53)	5.89 b (±1.00)	9.67 b (±1.61)

[1]Means ± STDEV (n = 3); Duncan's test at $p \leq 0.05$ - values followed by the same letter in a column are not significantly different.

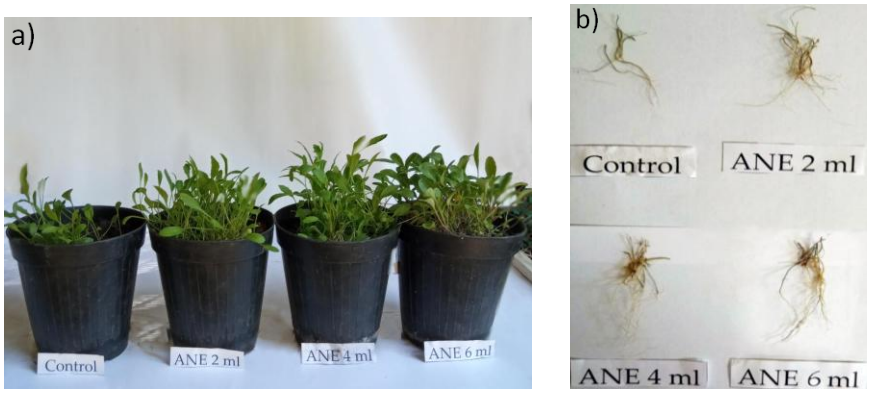

Figure 1. Growth and development of wild rocket plants: a) aerial biomass; b) root

Contrary to obtained results, Morelatto and Silva [13] determined that none of the tested doses of ANE (0, 1, 2 and 4 m/l) promoted improvements in the growth and root length of cultivated rocket. Nevertheless, other authors [8] reported significance in the response of the savory to the *A. nodosum* soil and foliar application, regarding plant height and number of lateral branches. In some studies pot foliar application and pot root application of 0.05% and 0.10% of *A. nodosum* extract ameliorated the growth performance of *Vigna aconitifolia* [14].

Statistical analysis of the differences between the treatments for individual comparison of plant physiological parameters is presented in Table 2. The obtained results indicated a statistically significant effect ($p < 0.05$) of all applied doses of ANE on all plant physiological parameters in relation to soil not treated with ANE. The most pronounced significant effect ($p < 0.05$) on all plant physiological parameters compared to T1 (0 ml/l ANE) and T4 treatments (6 ml/l ANE) was determined in the treatments where ANE doses at 2 and 4 ml/l were applied. Overall results revealed that the treatments with ANE at doses of 2 and 4 ml/l, rather than ANE at dose of 6 ml/l, had the greatest influence on physiological parameters.

A. nodosum extract is significant as having bioactive ingredients that potentially regulate the molecular, physiological, and biochemical processes of crop plants [15]. Previous research on grapes revealed that repeated foliar sprays with *A. nodosum* extracts improved vine physiological performances, leaf assimilation, and water use efficiency, preserving photosystems integrity and resilience when water deficit is moderate [6].

Table 2. Effect of treatments on physiological parameters in wild rocket

Treatments	Physiological parameters[1]			
	NBI	Chl (μ/cm$^{2)}$	Flav (μ/cm$^{2)}$	Ant (μ/cm$^{2)}$
T1 - ANE (0 ml/l)	19.97 c (±5.95)	17.00 c (±1.75)	0.59 c (±0.12)	0.15 c (±0.01)
T2 - ANE (2 ml/l)	32.90 a (±1.65)	23.02 a (±5.40)	0.76 a (±0.01)	0.19 a (±0.01)
T3 - ANE (4 ml/l)	33.77 a (±8.47)	23.33 a (±2.62)	0.77 a (±0.08)	0.20 a (±0.02)
T4 - ANE (6 ml/l)	26.93 b (±3.78)	20.30 b (±1.74)	0.66 b (±0.12)	0.17 b (±0.01)

[1]Means ± STDEV (n = 3); Duncan's test at $p \leq 0.05$ - values followed by the same letter in a column are not significantly different.

Differences in the values of fresh and air-dried biomass yield between the treatments indicated a statistically significant effect ($p < 0.05$) of all applied doses of ANE on plant yield in relation to soil not treated with ANE. The treatments where ANE doses at 4 ml/l, followed by treatments sprayed with ANE at doses of 6 and 2 ml/l, were applied showed the most pronounced significant effect ($p < 0.05$) on plant yield. Overall data on biomass yield were in accordance with morphological traits and physiological parameters, meaning that the highest yield was registered in pot treatment with the dose of ANE at 4 ml/l (Table 3).

Table 3. Effect of treatments on yield of wild rocket

Treatments	Yield (g/pot)[1]	
	Fresh biomass	Air-dried biomass
T1 - *A. nodosum* (0 ml/l)	3.62 c (±0.10)	0.48 c (±0.05)
T2 - *A. nodosum* (2 ml/l)	7.95 b (±0.16)	0.97 b (±0.03)
T3 - *A. nodosum* (4 ml/l)	9.15 a (±0.04)	1.17 a (±0.02)
T4 - *A. nodosum* (6 ml/l)	9.13 a (±0.03)	1.02 b (±0.03)

[1]Means ± STDEV (n = 3); Duncan's test at $p \leq 0.05$ - values followed by the same letter in a column are not significantly different.

The use of *A. nodosum*-based biostimulant in improving plant growth and yield was reported previously [8, 15, 16, 17]. In evaluating the effect of *A. nodosum* extract on the growth and yield of savory it was reported that the soil or foliar application of seaweed has significant effects on increasing the dry weight of plant [8]. Analyzing the effect of soil and foliar application of ANE in improving the growth of fennel plants grown under salinity stress, significant increase in the fennel yield and yield components was determined [15]. Studying the role of ANE in improving the productivity of *Zea mays* under phosphorus-limited conditions

and in nutrient-deficient soils, Shukla and Prithiviraj [16] reported an increase in the agricultural productivity of this plant. Also, Shukla et al. [17] emphasize multiple and beneficial effects of *A. nodosum* extracts on plants growth and their defense responses.

Conclusion

The results of the paper indicate that the most pronounced effect on morphological and physiological parameters had the treatment where ANE at dose of 4 ml/l was applied, followed by treatments sprayed with ANE at doses of 2 and 6 ml/l. All applied doses of ANE (2, 4, and 6 ml/l) showed a significant effect on all tested rocket parameters in relation to soil not treated with ANE. Overall data on biomass yield were in accordance with morphological and physiological parameters, meaning that the highest yield was registered in treatments with the dose of ANE at 4 ml/l, which could be proposed in the wild rocket cultivation in greenhouse pots conditions. Nevertheless, further research in this area of study on larger soil areas is desirable.

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