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EFFICIENCY OF *ASCOPHYLLUM NODOSUM* SEAWEED EXTRACT IN ENHANCING *LINUM GRANDIFLORUM* (L.) GROWTH AND FLOWERING

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ABSTRACT: This study was carried out to investigate the impact of foliar application of *Ascophyllum nodosum* extract (ANE) to improve the quality and performance of *Linum grandiflorum* plants. During the two succeeding growing seasons of 2021/2022 and 2022/2023, the study was conducted at the Research Nursery of Antoniadis Gardens, Ornamental Plants and Landscape Gardening Res. Dept., Horticulture Res. Inst., ARC, Alexandria, Egypt. Individual *L. grandiflorum* seedlings were planted in 25 cm-diameter plastic pots. At concentrations of 0, 2, 4, 6, 8 and 10 ml/l, ANE was added as foliar spray. The results demonstrated that the spraying with ANE extract improved the *L. grandiflorum* plant height (cm), number of branches/plant, branch diameter (cm), shoot dry weight (g), number of flowers/plant, flower diameter (cm), and root length (cm). Furthermore, the data obtained indicated that the application of ANE spray led to the highest levels of carotenoids, anthocyanin, chlorophyll a, and b, as well as carbohydrates in leaves.

Keywords: *Linum grandiflorum*, *Ascophyllum nodosum* extract, foliar spray application

INTRODUCTION

Linum grandiflorum is an annual herb belonging to the Linaceae family, famous for its vibrantly colored blossoms, producing beautiful bright red blooms. Grows in fields and gardens and goes by several other names, including red flax, flowering flax, crimson flax, and scarlet flax. Although it is native to Algeria, it is now a common natural plant in parts of North Africa and Southern Europe. Additionally, it is cultivated as an ornamental species and has been introduced to many other regions worldwide (Asad *et al.*, 2021).

Seaweeds are multicellular, macroscopic organisms that inhabit coastal and marine ecosystems. They are abundant sources of polyunsaturated fatty acids (PUFAs), polysaccharides, bioactive peptides, and enzymes (Okolie *et al.*, 2018).

According to Abdel Aziz *et al.* (2011), the application of saline water at 1000 ppm and seaweed extracts at 2.5 and 3.0 cm^3/l had a positive impact on the chemical compositions, flowering, and vegetative growth of *Amaranthus tricolor*. Seaweed provides not only growth hormones but also micro and macronutrients that enhance the growth and quality of a broad range of ornamental crops (Kularathne *et al*., 2021).

Ascophyllum nodosum is a large, common brown alga or seaweed. It has been reported by various sources that the content of nutrients found in grapevines, particularly the buildup of phenolics and anthocyanins, can be increased by applying *Ascophyllum nodosum*

(ANE) extract as a foliar spray on *Vitis vinifera* after full bloom (Frioni *et al.*, 2018). *A. nodosum* extracts have a very variable auxin content. According to earlier studies, *A. nodosum* has a high concentration of indole acetic acid (IAA). The auxin content can vary depending on the extraction and processing methods used, the location of the raw material harvest, and even seasonal variations. According to previous research, *A. nodosum* commercial extracts include several different cytokinins, the most prevalent plant growth regulators (Shukla *et al*., 2019).

The main object of this study was to investigate the effect of different rates of seaweed extract (*Ascophyllum nodosum*) on the vegetative growth, flowering and phytochemical composition of *Linum grandiflorum*, which has wider landscape value.

MATERIALS AND METHODS

This study was carried out during the two successive seasons (2021/2022 and 2022/2023) in the Research Nursery of Antoniadis Gardens, Ornamental Plants and Landscape Gardening Res. Dept., Horticulture Res. Inst., ARC, Alexandria, Egypt, to study the effect of *Ascophyllum nodosum* extract on *Linum grandiflorum*.

For the first and second seasons, *Linum grandiflorum* seeds were sown on September 1st, 2021 and 2022. The seedlings were planted in trays filled with a soil mixture that had (1 clay:1 sand) ratio by volume, and placed in partial shade. Every day, the trays received water. The trays were gradually moved from a shaded area to a sunny one for one week prior to transplantation. When the seedlings reached about 10 cm in height, they were transplanted into 30 cm diameter plastic pots filled with sandy loam soil with a pH of 7.82.

Experimental layout:

The experiment contains six treatments, with five plants in each treatment. Each treatment has three replicates which are arranged in a factorial experimental layout of a Randomized Complete Block Design (Gomez and Gomez, 1984).

One month later, plants were fertilized with 19-19-19 (NPK chemical fertilization using soluble fertilizer) at the rate of 0.5 g/l and repeated every two weeks.

Ascophyllum nodosum (ANE) extract (the commercial seaweed extract product Stimplex) was used as a foliar spray. The ANE extract was applied in concentrations of 0, 2, 4, 6, 8 and 10 ml/l. Control plants were sprayed with water, and the application was repeated every two weeks (the plants were sprayed 8 times).

A hand sprayer was used to apply all the rates, and a wetting agent tween-20 was added to each test solution. Each plant was sprayed individually until the solution reached the point of run-off, from December 6th (in both seasons), till flowering begins in March in all treatments.

Experimental data:

All data were recorded at the end of the flowering season. The following data were recorded:

- Plant height (cm).
- Stem diameter (cm) above the soil surface and at the base of the stem.
- Main branches number.
- Leaves number/plant.
- Plant fresh weight was recorded in grams for each treatment in each replicate.
- Plant dry weight was recorded for plants from each replicate that were allowed to be dried for 72 hours at 70 °C to a constant weight, then cooled inside the oven before being weighed in grams.
- Flowering date was indicated as the number of days from seeding to the first flowering stage on each plant, and the average for each treatment in each replicate was calculated.
- Number of flowers per plant was expressed as the number of flowers per plant in each treatment for each replicate.
- Flower diameter was measured in centimeters at the full opening stage; the measurements

were carried out for 10 flowers per treatment in each replicate.

- Flower length by measuring the length of the corolla tube in centimeters at the full opening stage, the measurements were carried out for 100 flowers per each treatment in each replicate.
- Flowering period as the number of days from the first bloom to appear on each plant to the last flower to appear in each treatment for each replicate was used to define the flowering period.
- Total leaf chlorophyll contents (a and b) (mg/100 g fresh weight) were determined according to Moran (1982).
- Total carotenoid content was determined according to Torres *et al.* (2014).
- Total carbohydrate content (%) in dried leaves was determined using the Herbert *et al.* (1971) method.
- Anthocyanin content in the flower was determined according to the method of Fuleki and Francis (1968).

Statistical analyses:

Data were subjected to analysis of variance (ANOVA) using the SAS program, SAS Institute (Snedecor and Cochran, 1974) and the mean values were compared using L.S.D test at 5% level (SAS Institute, 2002).

RESULTS

Vegetative growth and flowering parameters:

Table (1) illustrates that *Linum grandiflorum* plants showed a marked improvement in their vegetative growth metrics after two seasons of being treated with seaweed *Ascophillum nodosum* extract (ANE), which was applied by foliar application.

As compared to the control values, the results in Table (1) indicated that all studied vegetative growth parameters significantly increased after the application of (ANE) treatments, and the foliar spray from 2 to 10 ml/l of ANE resulted in stimulatory effects on all vegetative growth parameters. However, the highest plants were obtained after foliar application of 10 ml/l ANE by 35.89% and 28.28% as compared to control in both seasons respectively. An increase in the number of branches and branch diameter was observed at all treatments of ANE in the two seasons, respectively compared to control.

The obtained data in Table (1) revealed that the highest number of branches scored with the treatment of 10 ml/l ANE doses during two seasons (8.78 and 9.11 respectively). Also, the maximum values of branch diameter (4.45 and 4.70 cm) were obtained in the application of 10 ml/l of ANE in both seasons; respectively.

Also, the increments of leaves number and shoot dry weight were in parallel to the increase of ANE level to reach the maximum increase at the high level in both seasons. The highest value was obtained with 10 ml/l of ANE.

Significant differences were observed in the number of flowers/plant, diameter and dry weight of flowers in *Linum grandiflorum*. Table (2) demonstrates that the data on flowering parameters, including the average maximum flower number of 50.33 and 52.33 per plant, flower diameter of 41.26 and 40.70 mm per plant, and flower dry weight for 10 flowers of 0.99 and 1.10 g respectively, all of which were recorded under a 10 ml/l of ANE foliar spray.

In all previous measurements, the treatment of 10 ml/l, was followed by the increase in the treatment of 8 ml/l with no significant difference between each other.

Data illustrated in Table (2) show that all treatments of ANE scored a significant increase in root length in comparing to the control. The longest root was obtained by foliar application of 10 ml/l ANE by 42.70 % and 21.12 % in the two seasons respectively. Followed by the treatment of 8 ml/l, there was an increase in root length without a significant difference.

Chemical analysis:

Based on data from various biochemical studies presented in Table (3), it was indicated

Table 1. Means of plant height (cm), number of branches/plant, branch diameter (cm), shoot dry weight (g) and numbers of leaves/plant of *Linum grandiflorum*, as influenced by *Ascophyllum nodosum* extract (ANE), in the two seasons of 2021/2022 and 2022/2023.

Treatments (ml/l)	Plant height (cm)		Number of branches/plant		Branch diameter (cm)		Shoot dry weight (g)		Number of leaves/ plant	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
ANE 0 (ml/l)	54.78	59.33	3.67	4.00	1.46	1.61	3.21	3.02	455.33	484.67
ANE 2 (ml/l)	56.44	62.00	4.33	4.89	2.04	2.10	3.39	3.60	510.56	513.17
ANE 4 (ml/l)	62.50	68.33	5.11	5.89	2.43	2.86	4.37	4.06	611.78	630.67
ANE 6 (ml/l)	69.33	72.55	7.00	6.67	3.61	3.57	5.48	5.41	693.11	715.67
ANE 8 (ml/l)	70.11	74.34	8.00	8.89	4.25	4.19	6.11	6.30	725.67	734.50
ANE 10 (ml/l)	74.44	76.11	8.78	9.11	4.45	4.70	6.22	6.78	737.84	744.17
L.S.D. at 0.05	2.59**	2.42**	1.23**	0.81**	0.44**	0.36**	0.57**	0.50**	7.76**	5.77**

L.S.D. = Least significant difference at 0.05 level of probability
**** = highly significant at 0.01 level of probability**

Table 2. Means of flower number/plant, flower diameter (cm), flowers dry W/10 flowers (g) and root length of *Linum grandiflorum*, as influenced by *Ascophyllum nodosum* extract (ANE), in the two seasons of 2021/2022 and 2022/2023.

Treatments (ml/l)	Flower No.		Flower diameter (cm)		Flowers dry weight /10 flowers (g)		Root length (cm)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
ANE 0 (ml/l)	22.22	19.45	39.91	41.30	0.94	0.95	14.33	14.77
ANE 2 (ml/l)	33.78	34.33	40.81	41.31	0.93	1.00	14.15	14.83
ANE 4 (ml/l)	37.89	40.67	40.93	41.27	0.98	1.01	15.94	15.11
ANE 6 (ml/l)	42.00	43.78	40.99	40.95	0.96	1.03	17.56	15.87
ANE 8 (ml/l)	49.11	51.67	41.17	40.82	1.00	1.03	19.86	16.82
ANE 10 (ml/l)	50.33	52.33	41.26	40.70	0.99	1.10	20.45	17.89
L.S.D. at 0.05	3.69**	2.57**	0.74**	N.S.	N.S.	0.08**	0.88**	0.46**

L.S.D. = Least significant difference at 0.05 level of probability
**** = highly significant at 0.01 level of probability, N.S.= not significant**

Table 3. Means of chlorophyll a, b, carotenoids in leaves (mg/100 g fresh weight) and anthocyanin in flowers (mg/ml) and total carbohydrates (%) of *Linum grandiflorum*, as influenced by *Ascophyllum nodosum* extract (ANE), in the two seasons of 2021/2022 and 2022/2023.

Treatments (ml/l)	Chl. a (mg/100 g f.w.)		Chl. b (mg/100 g f.w.)		Carotenoids (mg/100 g f.w.)		Anthocyanin in flowers (mg/ml)		Carbohydrates (%)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
ANE 0 (ml/l)	1.00	1.33	0.33	0.42	7.83	7.86	47.17	48.96	6.71	7.76
ANE 2 (ml/l)	1.06	1.46	0.38	0.46	8.41	8.266	49.82	52.23	6.95	7.63
ANE 4 (ml/l)	1.21	1.48	0.42	0.48	9.61	8.91	51.85	53.73	8.65	8.58
ANE 6 (ml/l)	1.32	1.62	0.45	0.54	8.41	9.13	53.13	55.34	8.48	8.85
ANE 8 (ml/l)	1.45	1.77	0.48	0.58	9.50	10.50	53.45	54.81	8.36	8.68
ANE 10 (ml/l)	1.64	2.01	0.54	0.64	9.41	11.83	52.18	52.81	7.16	8.76
L.S.D. at 0.05	0.11**	0.12**	0.04**	N.S.	1.04**	1.71**	2.41**	2.15**	0.75**	1.06**

L.S.D. = Least significant difference at 0.05 level of probability
**** = highly significant at 0.01 level of probability, N.S.= not significant**

that the highest increase in chlorophyll a and chlorophyll b content was recorded with the treatment of ANE at 10 ml/l as foliar spray. A significant increase in chlorophyll a was happened during both seasons due to the application of ANE at 10 ml/l by 64% and 51.12 % for chlorophyll a in comparison with the untreated plants. Similarly, a significant increase in chlorophyll b by 63.63% was recorded under the same treatment in the 1st season and by 52.38% in the 2nd season compared to the control. On the other hand, the carotenoid contents in flowers was increased with increasing the level of ANE. The maximum increase was observed at ANE levels of 4 and 8 ml/l during the first season, and 10 and 8 ml/l during the second one. It is clear that the concentration of 8 and 10 ml/l possesses the highest values of anthocyanin content in the two seasons.

All concentrations of 4, 6 and 8 ml/l seaweed extract increased significantly carbohydrate contents in leaves especially the highest levels in both seasons with no significant differences between them.

DISCUSSION

This research has shown that *Ascophyllum nodosum* extract as a foliar application can enhance plant vegetative growth parameters, i.e., plant height, leaves number, leaf dry matter, branch number and diameter. However, numerous studies have demonstrated the positive effects of *Ascophyllum nodosum* extract on plant vegetative growth. Studies discovered that the extract increased plant vitality by improving vegetative growth, which includes root and shoot biomass, better leaf area, and overall plant vigor. There have been reports that it improves crop yields and development by enhancing nutrient availability and absorption. The extract also improves photosynthetic pigments, phenolic compounds, and antioxidant activity, all of which are essential for plant growth in the face of abiotic stress. Studies has shown that it can improve the yield and quality of various plants as tomatoes and okra by enhancing their biochemical and physiological properties. It has been demonstrated that foliar spraying *Ascophyllum nodosum* extract increases plant growth performance and increases drought stress resistance. Based on these results, *Ascophyllum nodosum* extract may be a viable bio stimulant for agricultural, helping plants to develop more robustly and more resistant to stress. According to Ali *et al.* (2016) and Ali *et al.* (2022). Anthocyanin activity and chlorophyll abundance were both enhanced by a 0.3% ANE spray (Ali *et al.*, 2022).

Seaweed extract (*Ascophylum nodosum*) contains cytokinins that stimulate endogenous synthesis (Wally *et al*., 2013), and cytokinins shield chloroplasts, which in turn affect the quantity of chlorophyll. Bio-stimulant extracts from *Aschophylum nodosum*, considerably increase chlorophyll levels (Zavaleta-Mancera *et al.*, 2007; Thirumaran *et al*., 2009 and Shehata *et al.*, 2016).

After treating strawberry cells with 10 ml of extract (ANE), Spinelli *et al.* (2010) discovered that anthocyanins prevent oxidative damage. This finding is supported by Alam *et al.* (2013) who found that strawberries treated with 2 g/l ANE had higher amounts of anthocyanin. Because of their ability to neutralize superoxide radicals, anthocyanins mitigate the negative consequences of abiotic stress.

Cell development, as well as endogenous amounts of growth promoters, macro- and micronutrients, carbohydrates, and hormone compounds (particularly cytokinins), may have been enhanced by seaweed extract application. According to Khan *et al.* (2012) and Belal *et al.* (2023), this most likely caused the increase in fruit size and weight.

Belal *et al.* (2023) reported that the increase in vegetative parameters after seaweed extract (SWE; *Ascophyllum* extract) application might be attributed to the bioactive organic compounds in SWE such as cytokinins, auxins, gibberellins, amino acids, nutrients, polysaccharides, alginates, vitamins, and enzymes. These compounds act

as elicitors for the synthesis of polyamines, a group of growth regulators that promote cell division and elongation. This results in an increase in shoot length and leaf area. These findings align with those of Mahmoud *et al.* (2016) on apple found that foliar application of SWE greatly boosted the number, area of leaf, and shoot length as vegetative growth parameters. Also, in specific experiments with tomato plants, it was found that higher concentrations of *Ascophyllum nodosum* extract spray resulted in a significant increase in plant height, leaf number and branch development compared to control plants (Ali *et al*., 2016 and Ali *et al.*, 2021). They added that the extract contains natural growth-promoting compounds such as cytokinins and auxins that stimulate cell division and lateral bud development, resulting in increased leaf number and branch formation. According to Yuqi and Mattson (2015), foliar application with *Ascophyllum nodosum* up to 5 ml/l, led to a significant rise in the shoot fresh, dry weight, and root index of petunia and tomato with increasing concentration of foliar spray rate. Also, the application of *Ascophyllum nodosum* extract has been reported to have positive effects on flowering in plants (Carmody *et al.*, 2020; Shukla *et al.*, 2019). It has been demonstrated that ANE bio-stimulants increase fruit sets, encourage early flowering, and improve fruit uniformity. Additionally, ANE treatment has been found to delay senescence, which can result in a prolonged flowering period (Carmody *et al.*, 2020).

While the exact ways in which ANE influences flowering remain a mystery, it is thought to be connected to the presence of bioactive chemicals like auxins and cytokinins in the extract. These compounds can influence various physiological processes in plants, including flowering.

Seaweed extracts enhanced flowering and yield in cucumbers. A higher number of pistillate flowers led to a bigger number of fruits, which improved yield per plant and total production, ultimately increasing cucumber yield (Sarhan and Ismael, 2014). Seaweed extracts' capacity to promote early flowering may be responsible for their positive impact on yield productivity. Studies indicate that seaweed extracts (such as *Ascophyllum nodosum*) can hasten flower initiation and flowering in plants (Aliko *et al.*, 2017). Similarly, there was an increase in flowering, early fruit ripening, and total tomato yield after plants were treated with seaweed extracts. (Crouch, 1990). Additionally, the vase life of pot marigold flowers was greatly enhanced by seaweed extracts as a foliar application (Emam, 2016).

Li and Mattson (2015) found that foliar spraying on petunia with high rates of aqueous seaweed extract (ANE) up to 5 ml/l significantly increased chlorophyll content. Also, as the concentration of the foliar spray rate of *Ascophyllum nodosum* rose to 5 ml/l, the chlorophyll index (SPAD) of tomato and petunia increased significantly (Li and Mattson, 2015).

Multiple studies show that seaweed extract treatments increase chlorophyll levels in various crops including grapevine, strawberry, black gram, and onion, likely due to the cytokines, auxins, macro- and micronutrients in the seaweed (Kalaivanan and Venkatesalu, 2012; Hidangmayum and Sharma, 2017). In saline-exposed turf grass, Elansary *et al*. (2017) discovered that ANE treatments increased total non-structural carbohydrates by regulating the expression of genes involved in carbohydrate metabolism and transport and boosting photochemical efficiency. Seaweed extracts can enhance growth and development by increasing levels of endogenous plant hormones such as auxin, cytokinins, and gibberellic acid, according to a recent study by Li and Mattson (2015) observed this effect in petunia. Also, studies show that applying *Ascophyllum nodosum* extract to *Vitis vinifera* (grape) plants can increase total carbohydrate content, anthocyanin levels, and carbohydrate levels (Salvi *et al.*, 2019).

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كفاءة مستخلص الطحالب البحرية الاسكوفيلليم في تحسين نمو وإزهار كتان الزهور

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يتم استخدام نبات كتان الزهور (*Linum grandiflorum* L.) كنبات زينة للزراعة في أحواض الزهور وكنباتات أصص، كما أنه يحظى بقيمة خاصة كنبات حولي مزهر لجمال أزهاره و لونها الأحمر المميز. تُستخدم مستخلصات الأعشاب البحرية على نطاق واسع لتحسين نمو النبات وتطوره. تم تنفيذ هذا البحث بمشتل حديقة أنطونيادس، قسم بحوث الزينة وتنسيق الحدائق، معهد بحوث البساتين، مركز البحوث الزراعية بالاسكندرية، مصر خلال الموسمين٢٠٢١/٢٠٢٢ و٢٠٢٢/٢٠٢٣. هدفت هذه الدراسة إلى تقييم تأثير الرش بمستخلص *Ascophyllum nodosum* بتركيزات (٠، ٢، ٤، ٦، ٨، ١٠ مل/لتر) على النمو الخضري، الزهري والمكونات الكيميائية لنبات كتان الزهور. أدى رش النباتات بمستخلص الطحالب البحرية إلى أقصى قدر من الزيادة في معدلات النمو الخضري (ارتفاع النبات، عدد الأفرع، قطر الفرع، عدد الأوراق ، محتوى الاوراق من المادة الجافة) وقياسات الأزهار (عدد الأزهار، قطر الزهرة ، الوزن الجاف للأزهار) وكذلك طول الجذور. أظهرت النباتات المعاملة بالرش بمستخلص الطحالب البحرية (مقارنة بالنباتات غير المعالجة) قيم أعلى لعوامل النمو، صفات الأزهار، الكلوروفيل (أ) و (ب) فى الاوراق ومحتوى الأزهار من صبغة الأنثوسيانين والكاروتينويدات وكذلك محتوى الأوراق من الكربوهيدرات الكلية. من النتائج المتحصل عليها يتضح ان استخدام مستخلصات الطحالب البحرية يعزز نمو النبات و صفاته، حيث أشارت النتائج إلى أن استخدام مستخلص الطحالب البحرية بمعدل ٨ مل/لتر أعطى تأثير إيجابي على النمو الخضري والأزهار والمكونات الكيميائية لنباتات كتان الزهور.