MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Impact of UVC and Seaweed Extract on Betalain Content *in Lepidium sativum* Leaves

Zeinab Hassanin[1], A. Abdelrazek[2], Eladel Eltanahy[1], Hamed El-Shora[1]

[1]*Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt*

[2]*Physics Department, Faculty of Science, Mansoura University, Mansoura, Egypt*

**** Correspondence to:** (zezesakr @yahoo.com, Tel; 01009068108)*

Received:10/6/2024
Accepted: 27/6/2024

Abstract: The influence of both UVC radiation and water extract of *Colpomenia sinuosa* on betaxanthin and betacyanin as well as the total betalains in leaves of *Lepidium sativum*. Seeds were primed with UVC or primed in *C. sinousa* extract for different time intervals (1, 2, 3 and 4 h). The results reveal that UVC irradiation caused in an increase in betaxanthin, betacyanin after exposure for 1 and 2 h, then their content declined after 3 and 4 hours. The priming of *L. Sativum* seeds in *C. sinousa* enhanced the content of total betalains and then the enhancement was remarkable than that recorded in case of UVC. Therefore, the results recommend priming seeds of the medicinal plants in seaweed extract before cultivation for enhancing the secondary products and pigments.

keywords: *Lepedium sativum;* UVC; *Colpomenia sinuosa;* Betaxanthin; Betacyanine

Introduction

Betalains are natural pigments that are frequently present in plants including beets, cactus, and *Amaranthus sp*. Betalains composed of betacyanins (BC), which present a reddish color and betaxanthins (BX) responsible for the brownish color [1]. The sources of betacyanins are numerous and include grains of amaranth (*Amaranthus sp.*), *Chenopodium quinoa*, red and yellow beetroots (*B. vulgaris*), Gomphrena globosa, flowers and leaves [1]. *Ullucus tuberosus* and *Rivina humilis* fruits are two less popular sources [2]. Betalains in plants are derived from L-tyrosine, which is hydroxylated to give rise to L-3, 4-dihydroxyphenylalanine (L-DOPA).

The betacyanin biosynthesis is known to be induced under the influence of UV radiation, high salinity, low temperature, mechanical damage or inoculation with pathogenic fungi [3, 4]. The production of betaxanthins does not imply additional enzymes once betalamic acid is synthesized. However, the production of betacyanins implies structural modifications by the action of additional enzymes, except for the above-mentioned betanidin. The biosynthesis of betalains initiates with two different enzymes mediated reactions and final spontaneous reaction [5]. Betacyanins are distinctive, water-soluble, nitrogen-containing natural plant pigments that are formed from reddish-violet indolines and dihydropyridine [6]. Simultaneously with carotenoids, and chlorophylls, betalains are one of the most common plant pigments found in nature. These compounds share betalamic acid as the main chromogenic structural unit condensed with cyclo-DOPA, forming betanidin or glycosylated cyclo-DOPA in other betacyanins as well as different amino acids or amines in betaxanthins [6].

Garden cress *(Lepidium sativum* L.) belongs to family Cruciferae (Brassicaceae). It is a glabrous, annual, upright plant that is grown around the world as a culinary vegetable but is originated from Egypt [8]. The phytochemical analysis of *L sativum* reveled that it contained alkaloids, cardiac glycoside, cardiotonic glycosides, phenolic, flavonoids, proteins, glucosinolates, coumarins, carbohydrates and amino-acids, mucilage, saponins, sinapic acid, resins, sterols, volatile oils, tannins, triterpene and uric acid [9].

It has been reported that exposure to UV light has biological impacts on humans,

animals, as well as all microorganisms [10]. The sun emits a range of electromagnetic radiation, including ultraviolet light. Ultraviolet (UV) is classified into three categories with different wavelengths [11]. UVA 400- 315 nm ,UVB 315- 280 nm and UVC 280- 100 nm .

Seaweeds are marine macroalgae which play a significant role in marine biological resources. They contain major and minor mineral elements, fatty acids, vitamins, cytokinins, auxins and abscisic acid [12]. Seaweeds fall under the three subclasses: brown (Phaeophyta), red (Rhodophyta) and green (Chlorophyta) algae [13]. Macroalgae extracts are currently being utilized as foliar sprays or presoaking for stimulating the growth and production of plants, notably crops, due to the availability of several growth regulators, trace elements, vitamins, and amino acids [14]. The use of seaweeds as plant bio-stimulants is now one of the most promising applications. This effect can be explained by the presence of plant growth–promoting molecules like indoleacetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), cytokinins, and polyamines in algal extracts [15]. Furthermore, seaweed extract was found to boost the activity of antioxidative and nitrogen metabolizing enzymes [16]. Also, [17] reported that seaweed extracts significantly upregulated the genes involved in carbon fixation resulting in enhanced photosynthetic efficiency. Consequently, seaweeds have been recognized as a major source of macro- and micronutrients necessary for regular metabolism and proper plant growth.

2. Materials and methods

2.1 The experimental plant

The experimental plant involved in the present investigation was *Lepidium sativum* (Family: Brassicaceae). Pure seeds were punchased from Egyptian Ministry of Agriculture.

2.2 Primig of seeds with UVC

Seeds of *L. sativum* plants were exposed to UVC irradiation for different time intervals (0, 1, 2, 3 and 4 h) from the lamp according to [18] using a system consists of fluorescent lamp (Type-C with λ from 2000 to 2800 Å) and its power equal to 15 watt. Also, the system was covered with aluminum foil to illuminate the samples from all sides.

2.3 Collection of seaweed

The seaweed used in the study was *C. sinuosa* collection site was a long Hurghada shores, Red sea coast of Egypt during autumn 2022 . To prevent the seaweed from drying out, the samples were transported to the lab in plastic bags filled with seawater. The seaweed was recognized in accordance with [19]. The gathered seaweed was washed with sterile seawater to get rid of the sand and salt. To get rid of any adhering epiphytes or depressed marine microbes, the sample was also carefully cleaned with a soft brush. After that, the dried seaweed was shade-dried, pulverized in a commercial grinder, and stored at 4oC for further use in analysis.

2.4. Preparation of seaweed material

A sample (5g) of *C. sinuosa* powder was extracted with 250 ml distilled water at 35oC with shaking water bath. After 72h the sampled was cooled down to the room temperature and centrifugated at 5000 rpm for 10 min. The supernatant was used as seaweed extract [20].

2.5. Priming of L. *sativum* seeds in *C. sinuosa* extraction

Seeds were primed in *C. sinuosa* with agitation for various time intervals (1, 2, 3 and 4h) at 35^{o}c and then collected and washed with distilled water

2.6. Growth of *L. saivum* seeds

The primed seeds of *L. sativum* with UVC or *C. sinuosa* were germinated in petri dishes for 7 days. The 7- day old seedling were transported to trays containing Hoagland's solution [21] and left to grow for 21 days in trays. Hoagland's solution composed of: 5mM KNO_3, 5mM Ca $(NO_3)_2$, 50 µM H_3BO_3, 1 mM kH_2PO_4, 4.5µM $MnCl_2$, 1mM MgSO4, 0.3µM $CuSO_4$, 3, 8 µM $ZnSO_4$, 0.1 mM $(NH_4)_6$ Mo_7O_{24} and 10 µM Fe EDTA at 30 ^{O}C in an incubator with 12h/12h light/dark cycles. The trays were covered from both sides with a sheet of aluminum foil and current air was pumped throughout the experimental period for 21 days and plant leaves were collected and used for analysis.

2. 7 Estimation of betalains

The content of betaxanthins and betacyanins in the *L. sativum* extract was determined

spectrophotometrically at 538 nm and 480 nm, respectively according to the method of [22].

3. Results and Discussion

3.1 Effect of UVC radiation on betaxanthin content of *L. sativum* leaves

The effect of UVC on betaxanthin content in *L*. sativum leaves was investigated by exposure of the seeds to UVC for different time intervals (1, 2, 3 and 4 h).

The results in Figure 1 indicate that the optimal time for betaxanthin production was 2 h after which the content declined with increasing the exposure time to 3 and 4 hours.

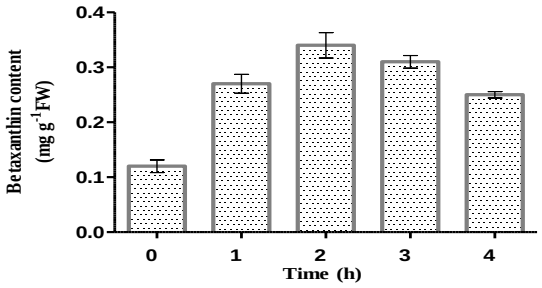

Fig. 1: Effect of UVC radiation on betaxanthin content of *L. sativum* leaves.

3.2. Effect of *C. sinuosa* extract on betaxanthin content of *L. sativum* leaves

The effect of *C. sinuosa* extract on betaxanthin content in *L. sativum* leaves was investigated by priming the seeds with UVC at different time intervals (1, 2, 3 and 4 h). The results in Figure 2 indicate that the optimal time for betaxanthin production was 3h after which the content declined at the 4th hour exposure to UVC. Generally, the exposure to UVC increased remarkably the betaxanthin content compared to the control.

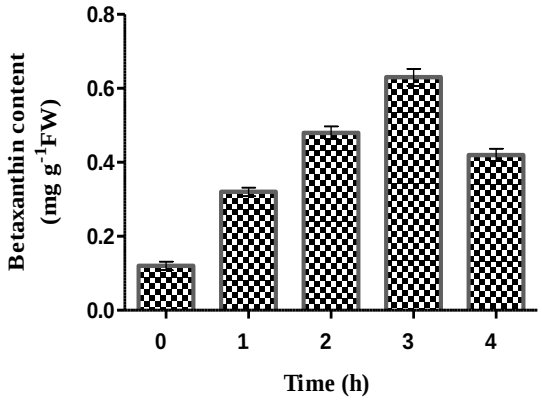

Fig.2: Effect of *C. sinuosa* extract on betaxanthin content of *L. sativum* leaves.

3.3. Effect of UVC radiation on betacyanin content of *L. sativum* leaves

The effect of UVC on betacyanin content in *L. sativum* leaves was investigated by priming the seeds with UVC at different time intervals (1, 2, 3 and 4 h). The results in Figure 3 reveal that betacyanin content increased gradually with increasing the time of exposure up to 2 h after which the content decreased to 0.39 and 0.34 mg g^{-1}FW at the 3rd and 4th hours, respectively.

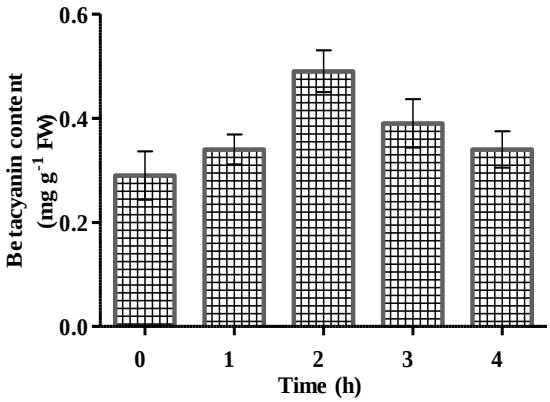

Fig. 3: Effect of UVC radiation on betacyanin content of *L. sativum* leaves.

3.4. Effect of *C. sinuosa* extract on betacyanin content of *L.sativum* leaves.

The effect of *C. sinuosa* extract on betacyanin content in L. *sativum* leaves was investigated by priming seeds with UVC at different time intervals (1, 2, 3 and 4 h). The results in Figure 4 indicate that the optimal time for betacyanin production was 3h and the content declined at the 4th hour of exposure to UVC. Generally, the exposure to UVC increased remarkably the betacyanin content compared to the control.

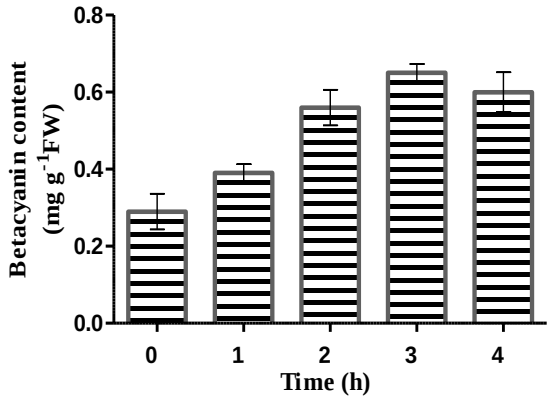

Fig. 4: Effect of *C. sinuosa* extract on betacyanin content of *L. sativum* leaves.

3.5. Effect of UVC radiation on total betalains content of *L. sativum* leaves

The total content of betalains was investigated in *L. sativum* leaves after exposure to UVC for 1, 2, 3 and 4h. The result in Figure 5 indicate that the exposure of the seeds for 2 h resulted in high content of betalains followed by reduction after exposure to UVC for 3h and 4h to reach 0.70 and 0.59 to mg g^{-1} FW, respectively.

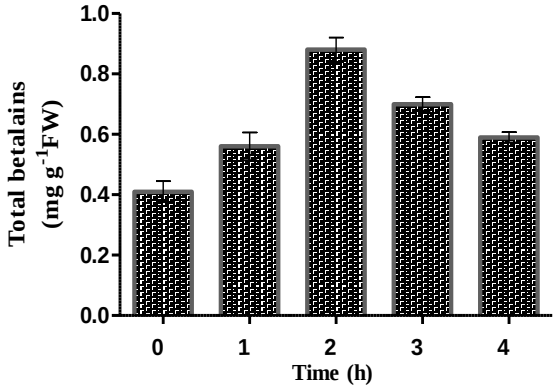

Fig.5: Effect of UVC radiation on total betalains content of *L. sativum* leaves.

3.6. Effect of *C. sinuosa* extract on total betalains content of *L. sativum* leaves

The effect of *C .sinuosa* extract on total betalains content of *L. sativum* leaves was investigated after priming for 1, 2, 3 and 4h. The results in Figure 6 indicate that total betalains after priming with *C. sinousa* increased with increasing the time of treatment. It should be stressed that the total betalains after priming with *C. sinousa* was higher than that exposed to UVC

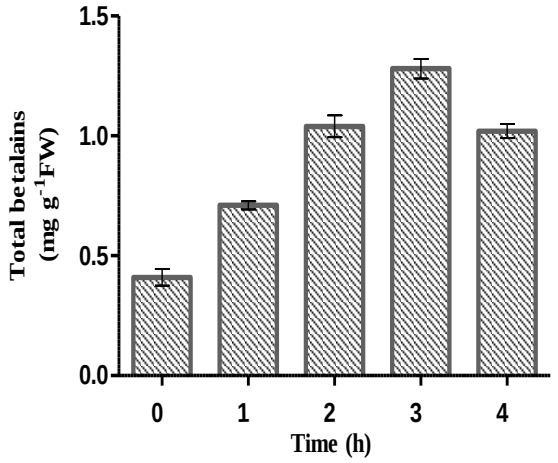

Fig.6: Effect of *C.sinuosa* extract on total betalains content of *L. sativum* leaves.

Discussion

Plant secondary metabolites are a large number of compounds that do not directly involve in plant growth or development but are required for plant survival. Plant secondary products are frequently utilized as food additives, nutraceuticals, and medications. Since they are scarce, it is becoming more crucial to use an alternate method, such tissue culture, to produce these needed molecules on a big scale [23]

UVC-light is known since long time to exert direct and indirect inhibitory and damaging effects on living cells and is therefore commonly used for disinfection purpose. Abiotic variables that negatively impact plant growth and production, such as salinity, dryness, and UV radiation, have a significant impact on crop plants and reduce agricultural yield [24].

Betalains represent one of the four classes of vegetable pigments on the market along with carotenoids and chlorophylls [25]. In the present work the total betalains increased in *L. sativum* leaves after priming seeds with UVC for 2 h then declined by prolonged time. The increase in betalains after seeds priming for short time (2 h) by UVC is consistent with the results reported by [26]. The increase in betalain concentration in *L. sativum* leaves in the present work in response to UVC stress has also been reported previously in *Rivina humilis* berries. [27] suggested that betalains may have a role in scavenging reactive oxygen species (ROS) because they were observed to accumulate more in *Rivina* berries treated with salicylic acid and chitosan. Increased betalains because of UVC treatment in the present work suggests a protecting function in leaves for these pigments against UVC. Indeed, it has been reported that betalains act as protectors and ROS scavengers in ice plants [28, 29, 30]. The presence of growth regulators in the seaweed extracts from C. sinuosa may be the reason for the stimulation of betalain biosynthesis in *L. sativum.* Various amino acids, minerals, vitamins, polysaccharides, polyamines, and growth regulators that affect cellular metabolism are found in seaweeds [31, 32,33].

Algal biostimulants are bioactive substances that are environmentally benign and sustainable that stimulate the growth and development of plants. [34]. Bio-stimulants incorporate several substances such as amides, proline, glycine, betaine, and α-amino butyric acid. [35]. Furthermore, seaweed's phenolic and flavonoid chemicals scavenge plants that release reactive oxygen species (ROS

Plant organelles that operate well are directly benefited by the timely removal of ROS, especially those that synthesize chlorophyll and betalains. [36]. It has been demonstrated that treating wheat with extracts from *Spirulina* and *Chlorella* promotes plant growth and photosynthesis [37].

Generally algal extracts are believed to act as bio-stimulants, providing protective support to bean growth and this was attributed to the abundance of metabolites particularly growth hormones which can induce the biosynthetic pathway of betalains [38].

4. References

1 Rocha, F., Marques, C. S., de Sousa, L. S., Minim, V. P., dos Santos Pires, A. C., Minim, L. A., & Vidigal, M. C. (2022). Betalains nanodispersions: Effects on betalains stability and on rheological properties of Greek yogurt. Food Research International, **159**: 111583.

2 Akbar Hussain, E., Sadiq, Z., Zia-Ul-Haq, M., Akbar Hussain, E., Sadiq, Z., & Zia-Ul-Haq, M. (2018). Sources of betalains. Betalains: biomolecular aspects, 15-32.

3 Wang, C.Q: Zhao, J.Q: Chen, M. & Wang, B.S. (2006). Identification of betacyanin and effects of environmental factors on its accumulation in halophyte Suaeda salsa L. *J. Plant Physiol. Mol. Biol*., **32**: 195–201

4 Hayakawa, K. & Agarie, S. (2010). Physiological roles of betacyanin in a halophyte, Suaeda japonica Makino. Plant Prod. Sci., **13**: 351–359.

5 Hatlestad, G. J., Sunnadeniya, R. M., Akhavan, N. A., Gonzalez, A., Goldman, I. L., McGrath, J. M. and Lloyd, A. M. (2012). The beet R locus encodes a new cytochrome P450 required for red betalain production. Nat. Genet., **44(7)**: 816-820.

6 Kumorkiewicz-Jamro, A; Świergosz, T; Sutor, K; Spórna-Kucab, A., & Wybraniec, S. (2021). Multi-colored shades of betalains: Recent advances in betacyanin chemistry. Natural Product Reports, **38(12)**: 2315-2346.

7 Sarker, U., & Oba, S. (2020). Leaf pigmentation, its profiles and radical scavenging activity in selected Amaranthus tricolor leafy vegetables. Scientific Reports, **10(1):**1-10.

8 Diwakar, B. T., Dutta, P. K., Lokesh, B. R., & Naidu, K. A. (2010). Physicochemical properties of garden cress (Lepidium sativum L.) seed oil. *J. Am. Oil Chemi'. Soci*, **87(5):** 539-548.

9 Shaikh, S. Y., Shaikh, A. T., & Shaikh, M. A. (2023). Diuretic activity of various herbs in India: A mini review. Borneo *Journal of Pharmacy*, **6(4):** 349-359.

10 Agrawal, S. (2016). Electromagnatic radiation through mobile towers-understanding the debate and policy (Doctoral dissertation, IIPA, New Delhi).

11 Li, L., Zhang, X., Zhou, J., Zhang, L., Xue, J., & Tao, W. (2022). Non-Invasive thermal therapy for tissue engineering and regenerative medicine. Small, **18 (36):** 2107705.

12 Irkin, L. C., & Yayintas, Ö. (2018). Pharmacological properties and therapeutic benefits of seaweeds (a review). *Int. J. Trend Sci. Res. Dev*, **2**: 1126-1131

13 El Semary, N. A. (2021). Seaweeds for Sustainable Development. Sustainable Solutions for Environmental Pollution, Volume **1**: Waste Management and Value-Added Products, 1: 449-470.

14 Khalik, K. A., & Osman, G. (2017). Genetic analysis of Plectranthus L. (Lamiaceae) in Saudi Arabia based on RAPD and ISSR markers. Pak. *J. Bot*, **49(3):**1073-1084.

15 Prasad K, Das AK, Oza MD, Brahmbhatt H, Siddhanta AK, Meena R, Eswaran K, Rajyaguru M, & Ghosh PK (2010). Detection and quantification of some plant growth regulators in a seaweed based foliar spray employing a mass spectrometric technique sans

chromatographic separation. *J Agric Food Chem* **58**: 4594–4601.

16 Fan D, Hodges, D.M., Critchley AT& Prithiviraj, B (2013).A commercial extract of brown macroalga (Ascophyllum nodosum) affects yield and the nutritional quality of spinach in vitro. Commun Soil Sci Plant Anal **44**: 1873–1884.

17 Jannin, L, Arkoun ,M; Etienne, P; Laine, P; Goux D, Garnica, M; Fuentes, M; San Francisco, S; Baigorri, R; Cruz, F; Houdusse ,F; Garcia-Mina, J; Yvin J & Ourry A (2013). Brassica napus growth is promoted by Ascophyllum nodosum (L.) Le Jol. Seaweed extract: microarray analysis and physiological characterization of N, C, and S metabolisms. *J Plant Growth Regul*, **32**:31–52.

18 El-bediwi, A.B., Hasanin, S., Abdelrazek, A., and El-Shora, H.M. (2018). Effect of ultraviolet on morphological and secondary metabolites content of garden cress. *Int. J. Sci Res. Sci. Eng. Technol*., **4**:187-194.

19 Aleem A. A. (1993). The marine algae of Alexandria, Egypt, Univ. Alexandria.

20 Kokilam, G., Vasuki, S., & Sajitha, N. (2013). Biochemical composition, alginic acid yield and antioxidant activity of brown seaweeds from Mandapam region, Gulf of Mannar. *J. Appl. Pharm. Sci,* **3(11):** 099-104.

21 Hoagland, D. R., & Arnon, D. I. (1950). The water-culture method for growing plants without soil. Circular. California Agricul. Experi. Station, 347(2nd edit).medical content for plant. *Egypt .J. Chem*, ***65*(10)**: 315-320.

22 Stintzing, F.C., Schieber, A. & Carle, R. (2003). Evaluation of colour properties and chemical quality parameters of cactus juices. Eur. Food Res. Technol., **216**: 303–311.

23 Gamba, M., Raguindin, P. F., Asllanaj, E., Merlo, F., Glisic, M., Minder, B. & Muka, T. (2021). Bioactive compounds and nutritional composition of Swiss chard (Beta vulgaris L. var. cicla and flavescens): a systematic review. Crit. Rev. Food Sci., **61(20):** 3465-3480.

24 Thomas, D. T. & Puthur, J. T. (2019). Amplification of abiotic stress tolerance potential in rice seedlings with a low dose of UV-B seed priming. Funct. Plant Biol., **46(5)**: 455-466.

25 Lombardelli, C: Benucci, I: Mazzocchi, C., & Esti, M. (2021). Betalain extracts from beetroot as food colorants: Effect of temperature and UVlight on storability. Plant Foods. Hum. Nutr. **76**: 347-353.

26 Karvansara, P. R. & Razavi, S. M. (2019). Physiological and biochemical responses of sugar beet (Beta vulgaris L.) to ultraviolet radiation. *Peer J*, **7**: e6790.

27 Khan, M.I: Kumar, A. & Giridhar, P. (2016). Betalains and expression of enzymes during development and abiotic stress in Rivinia humilis L. berries. Turk. *J. Bot*., **40**: 28-36.

28 Sarker, U., & Oba, S. (2019). Antioxidant constituents of three selected red and green color Amaranthus leafy vegetable. Sci. rep., **9(1)**: 18233.

29 Jensen, K. and Koide, R.T. (2021). The protection of Salicornia rubra from ultraviolet radiation by betacyanins and phenolic compounds. Plant Environ. Interact, **2(5):** 229-234.

30 Ibdah, M., Krins, A., Seidlitz, H.K., Heller, W., Strack, D. & Vogt, T. (2002). Spectral dependence of flavonol and betacyanin accumulation in Mesembryanthemum crystallinum under enhanced ultraviolet radiation. Plant Cell Environ. **25**:1145-1154.

31 Khan, W: Rayirath, U. P: Subramanian, S: Jithesh, M. N: Rayorath, P: Hodges, D. M., & Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Reg*, **28**: 386-399

32 Rengasamy, K. R., Kulkarni, M. G., Stirk, W. A., & Van Staden, J. (2015). Eckol-a new plant growth stimulant from the brown seaweed Ecklonia maxima. *J . Appl. Phyco*., **27**: 581-587.

33 Cotas, J: Lomartire, S: Gonçalves, A. M., & Pereira, L. (2024). From Ocean to Medicine: Harnessing Seaweed's Potential for Drug Development. *International J. of Molec, Sciences,* **25(2):** 797.

34 Xu, L., & Geelen, D. (2018). Developing biostimulants from agro-food and industrial by-products. Frontiers in Plant Science, **9**: 416258.

35 Carillo, P., Ciarmiello, L. F., Woodrow, P., Corrado, G., Chiaiese, P., & Rouphael, Y. (2020). Enhancing sustainability by improving plant salt tolerance through macro-and micro-algal biostimulants. Biology**, 9(9):** 253.

36 Ammar, E. E., Aioub, A. A., Elesawy, A. E., Karkour, A. M., Mouhamed, M. S., Amer, A. A., & El-Shershaby, N. A. (2022). Algae as Bio-fertilizers: Between current situation and future prospective. *Saudi J. Biol. Sci*, **29(5):** 3083-3096.

37 Jerez-Martel, I., García-Poza, S., Rodríguez-Martel, G., Rico, M., Afonso-Olivares, C., & Gómez-Pinchetti, J. L. (2017). Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. *J. Food Quality*, 2017.

38 De Vasconcelos, A. C. F., & Chaves, L. H. G. (2019). Biostimulants and their role in improving plant growth under abiotic stresses. Biostim. Plant sci, 3-16 .