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EVALUATION OF THE EFFECT OF A SEAWEED-BASED BIOSTIMULANT ON GERMINATION AND BIOCHEMICAL QUALITY OF THE SPECIES *Momordica charantia*

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ABSTRACT. *Momordica charantia* L. (bitter cucumber) is a species of interest due to its nutritional value and remarkable nutraceutical properties. Natural biostimulants, such as those based on seaweed extracts, can help improve plant physiological processes by stimulating germination, metabolism, and stress tolerance. This study examined the effect of the seaweed-based biostimulant Algevit (0.5%) on five genotypes of *M. charantia* compared with an untreated control condition. First, seed germination was monitored, and then biochemical changes induced by the biostimulant, including the vitamin A (β-carotene), total phenolic, and soluble protein contents were determined. The biostimulant reduced the germination time and increased all three analysed biochemical parameters compared with the control, with the most pronounced effect observed for the vitamin A (β-carotene) content and the Line 1 genotype, with an increase from 3.95 to 8.19 µg/mL, confirming the effectiveness of Algevit.

Keywords: *Ascophyllum nodosum*; biostimulants; carotenoids; germination; vitamin A.

INTRODUCTION

Rapid population growth throughout the world represents a rising problem due to the ever-increasing need for food. According to the United Nations, the global population is expected to exceed 9 billion by 2050 and peak at 9.73 billion in 2060 (Miladinov, 2023; Willett *et al.*, 2019). Most of the population relies on plant products as their main source of food. Poor agricultural practices, such as inadequate irrigation, excessive use of pesticides, and the increasing impact of climate

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change, have contributed to the degradation of agroecosystems (Becker and Fanzo, 2023).

Moreover, estimates from the Food and Agriculture Organization of the United Nations (FAO) indicate that over 40% of the global arable land is already used for agricultural production, which limits the possibility of expanding cultivated land and requires the development of sustainable strategies to intensify agricultural production (FAO, 2022).

Momordica charantia L. (2x = 2n = 22), popularly called bitter cucumber or bitter gourd, is a climbing plant belonging to the Cucurbitaceae family, used in traditional medicine and as food (Alisofi *et al.*, 2020). This plant possesses the highest nutritional value among cucurbit species and represents an important source of carbohydrates, fibre, protein, vitamins, and minerals (Gayathry and John, 2022). Bitter cucumber has a long history of use in traditional medicine to treat more than 30 conditions. Its most important nutraceutical properties are antidiabetic (hypoglycaemic) activity, anticancer properties, as well as a strong antioxidant activity (Chanda *et al*., 2019).

Natural biostimulants are of plant origin and enhance crop productivity by increasing photosynthetic efficiency, delaying senescence, and alleviating the negative effects of biotic and abiotic stress due to their richness in phytohormones, amino acids, and beneficial microorganisms (Rouphael and Colla, 2020). Germination is the physiological process in which the seed resumes metabolic activity and develops into a new plant. It is critical for ensuring crop stability; however, its effectiveness is frequently limited by unfavourable environmental conditions, seed dormancy, and abiotic stress, all of which can reduce emergence uniformity and the overall germination percentage (Fernández and Tapias, 2022; Yu *et al.*, 2025).

Carotenoids, which serve as precursors of vitamin A, absorb light primarily in the blue-green spectrum and transfer excitation energy to chlorophylls. In doing so, they protect the photosynthetic apparatus photo-oxidative damage and optimise the conversion of light energy into chemical energy (Sun *et al.*, 2022). Carotenoids also act as key precursors in the biosynthesis of abscisic acid through oxidative cleavage catalysed by 9-cis-epoxycarotenoid dioxygenase (Li *et al.*, 2025). In the humans, vitamin A is essential for visual function, cell differentiation and proliferation, and immune system regulation (Hanson *et al.*, 2018; Norsa *et al.*, 2019).

Plant polyphenols play a crucial role in scavenging reactive oxygen species and suppressing phytopathogen activity through their antioxidant properties and ability to modulate hormonal signalling. They also contribute to the regulation of plant growth and developmental processes by influencing tissue elongation, lignification, and morphogenesis (Ji *et al.*, 2024; Wang *et al.*, 2025). In humans, dietary antioxidants neutralise reactive oxygen and nitrogen species, thereby preventing lipid peroxidation and protein damage and contributing to the maintenance of cellular integrity (Kapoor et al., 2025).

Soluble proteins constitute a major fraction of cellular proteins in plants. They are involved in essential metabolic processes such as enzymatic catalysis, molecular transport, and the regulation of

growth and development. They also play an important role in in plant responses to biotic and abiotic stress, a process closely regulated by abscisic acid, a key phytohormone that modulates the synthesis and accumulation of proteins associated with stress adaptation (Mo *et al.*, 2024; Hao *et al.*, 2025).

The aim of the present study was to evaluate the physiological responses of selected bitter cucumber genotypes adapted to the temperate continental climate of Romania to the application of the commercial biostimulant Algevit, in order to assess its compatibility and effectiveness in relation to the investigated plant material.

MATERIALS AND METHODS

Materials

The materials used in the present study included seeds and leaves of *M. charantia*, Algevit, the Folin–Ciocalteu reagent (Sigma-Aldrich), sodium carbonate (Na_2CO_3) (Sigma-Aldrich), gallic acid ($C_7H_6O_5$) (Merck), Coomassie Brilliant Blue G-250 ($C_{47}H_{48}N_3NaO_7S_2$) (Merck), phosphoric acid (H_3PO_4) (Merck), ethanol (C_2H_5OH) (Chemicals), bovine serum albumin (Merck), benzothiadiazole ($C_7H_5N_3S$) (Merck), hexane (C_6H_{14}) (Merck), potassium hydroxide (KOH) (Sigma-Aldrich), β-carotene ($C_{40}H_{56}$) (Merck), and Tris buffer (pH 8) (Merck).

Algevit is a commercial plant biostimulant that contains an extract of the seaweed *Ascophyllum nodosum* (30%), potassium oxide (3.9%), alginic acid (1.5%), and mannitol (0.5%). It enhances plant resistance to abiotic stress, stimulates metabolic activity, and delays cellular senescence. In addition, it promotes nutrient absorption and availability through the formation of complexes with microelements; stimulates root system development; and supports key physiological processes such as photosynthesis, respiration, synthesis, and assimilate translocation. Its application has been associated with increased fruit set, improved yield uniformity, and enhanced production quality. Algevit may be applied either foliarly (200–300 mL/100 L water) or via soil application (3–5 L/ha) and is generally compatible with most plant protection products; however, prior compatibility testing is recommended.

Plant material

The plant material used in this study consisted of five *M. charantia* genotypes: Brâncuși, Rodeo, Line 1, Line 3, and Line 4. Among these, Brâncuși and Rodeo are registered Romanian cultivars, while Line 1, Line 3, and Line 4 are experimental breeding lines. For each genotype, 200 seeds of uniform size were selected and surface sterilised with a 5% sodium hypochlorite solution for 15 minutes, followed by rinsing with distilled water.

Subsequently, the seeds from each genotype were divided into two experimental groups: 100 untreated control seeds and 100 seeds treated with 0.5% Algevit. The seeds were placed in Petri dishes (9 cm diameter), with 25 seeds per dish, resulting in four Petri dishes per treatment variant. Filter paper within each dish was moistened with 20 mL of distilled water for the control group and with 0.5% aqueous Algevit solution for the treated group.

This concentration was selected based on the manufacturer's recommended application rate. To minimise positional effects and potential environmental bias, Petri dishes were repositioned daily. The experiment under greenhouse conditions at the Research Institute for Agriculture and Environment (ICAM), part of the 'Ion Ionescu de la Brad' University of Life Sciences, Iași, Romania. Germination was carried out at 22°C during the day and 18°C at night, with a relative humidity of 70%. Germination onset occurred approximately 4 days after sowing, reaching a peak between 12 and 14 days. It was monitored over a 14-day period by recording the number of germinated seeds daily. The results are presented as the cumulative germination percentage.

Seedling production

Plant material was obtained from the germinated seeds. Seedlings were transferred from the Petri dishes into pots containing a substrate composed of potting soil (FloraSol) and peat (Kekkila) in a 60:40 (v/v) ratio. Seedlings of uniform size and morphological appearance were selected and transplanted into pots under standardised conditions. A total of 20 seedlings per genotype were used, of which 10 served as control plants and 10 were treated with Algevit. The control seedlings originated from untreated seeds germinated in Petri dishes, whereas the treated seedlings were from seeds exposed to Algevit. Following transplantation, the seedlings were irrigated with 30 mL of the corresponding solution according to their experimental group.

Plant material was harvested at phenophase 102 BBCH, corresponding to the appearance of the first two fully developed leaves. For biochemical analyses, three independent composite samples were prepared for each genotype and treatment variant. Each composite sample was obtained by homogenising equal amounts of plant material collected from all 10 plants corresponding to one treatment variant.

This approach was used to reduce biological variability and to minimise experimental errors.

Spectrophotometric determinations were performed separately for each composite sample. The results are presented in the figures and used for the statistical analysis represent the arithmetic mean of the three independent measurements. Ultraviolet–visible (UV-Vis) spectra were recorded using a Specord 210 Plus spectrophotometer (Analytik Jena), employing cuvettes with a path length of 1 cm.

Determination of β-carotene, a vitamin A precursor

One gram of plant material, obtained as a composite sample as described above, was homogenised in 10 mL of 0.1% BTH solution in hexane–acetone mixture (5:1, v/v). The mixture was then ground and vigorously shaken for 10 minutes. Following filtration, 5 mL of 0.5 M methanolic KOH solution was added, and the mixture was shaken for another 30 minutes. The resulting extract was transferred to a separatory funnel and washed three times with 20 mL of distilled water. The organic phase was concentrated and adjusted to a final volume of 5 mL with methanol. From this solution, 0.5 mL was taken and diluted to a final volume of 4 mL. The absorbance of the final solution was measured spectrophotometrically at 450 nm (*Figure 1*).

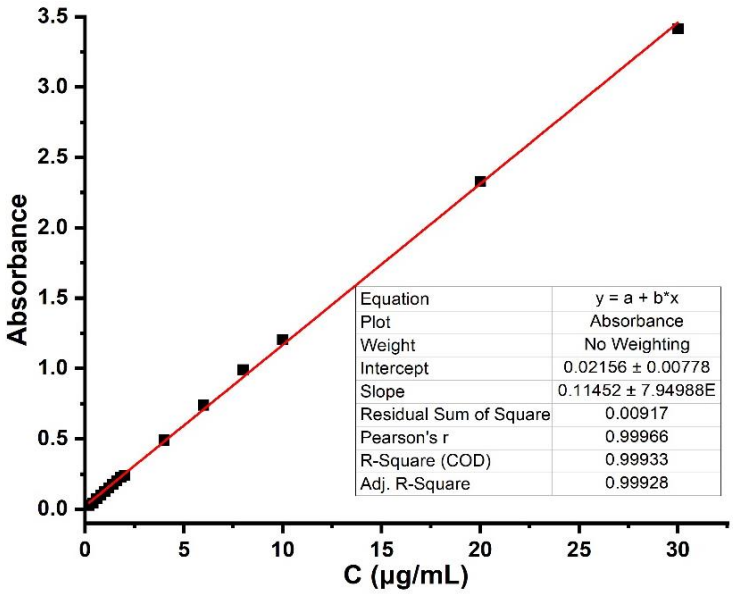

Figure 1 – Calibration curve used for the quantification of β-carotene, a precursor of vitamin A, in the range of 0.1–30 µg/mL. The limit of detection is 0.003338 and the limit of quantification is 0.011126

Determination of the total phenolic content

The total phenolic content was determined using the Folin–Ciocalteu reagent. Briefly, 1 g of plant material was homogenised with 10 mL of distilled water, and the sample was centrifuged at 8000 rpm for 10 minutes. A 0.1 mL aliquot of the supernatant was transferred into a reaction vessel, to which 0.5 mL of the Folin–Ciocalteu reagent and 2 mL of distilled water were added. The solution was homogenised by stirring and then incubated for 5 minutes to allow the initial reaction to proceed.

Subsequently, 0.75 mL of 20% Na_2CO_3 solution and 2.95 mL of distilled water were added. The solution was thoroughly mixed and incubated for another 60 minutes. Absorbance was measured at 765 nm. Quantification was performed using a calibration curve constructed in the range of 10–500 mg/L, with gallic acid as the standard reference compound (*Figure 2*)

Determination of the soluble protein content

Fresh plant material (0.5 g) was homogenised in a mortar with 2 mL of Tris buffer (pH 7.5) to obtain a uniform extract. The homogenate was transferred to centrifuge tubes and centrifuged at 8000 rpm for 15 minutes to separate the solid residue from the supernatant. A 0.6 mL aliquot of the supernatant was mixed with 2.4 mL of Bradford reagent containing Coomassie Brilliant Blue G-250. The mixture was homogenised and incubated to allow formation of the protein–dye complex. Absorbance was subsequently measured spectrophotometrically at 595 nm. The soluble protein concentration was determined using a calibration curve constructed with bovine serum albumin as the reference standard (*Figure 3*).

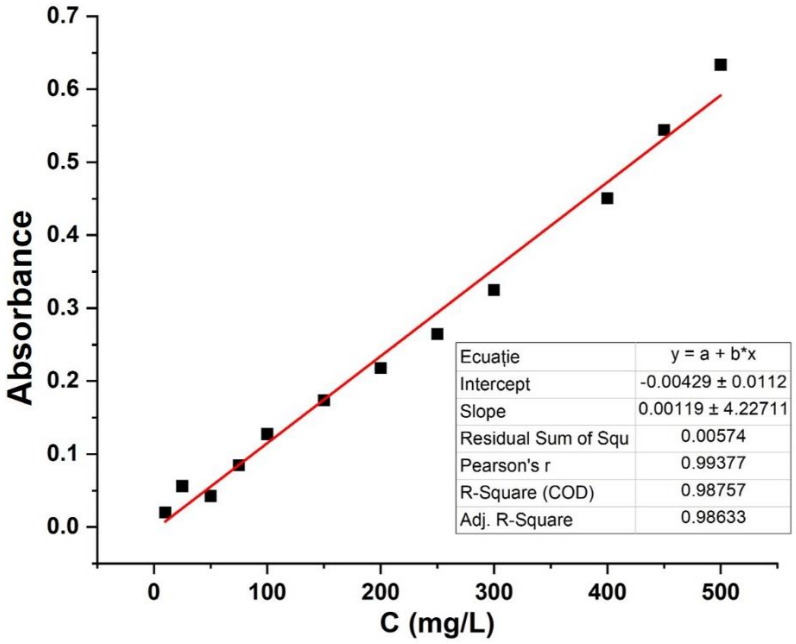

Figure 2 – Calibration curve used for the quantification of polyphenols in the range of 10–500 mg/L. The limit of determination is 0.0215 and the limit of quantification is 0.0649

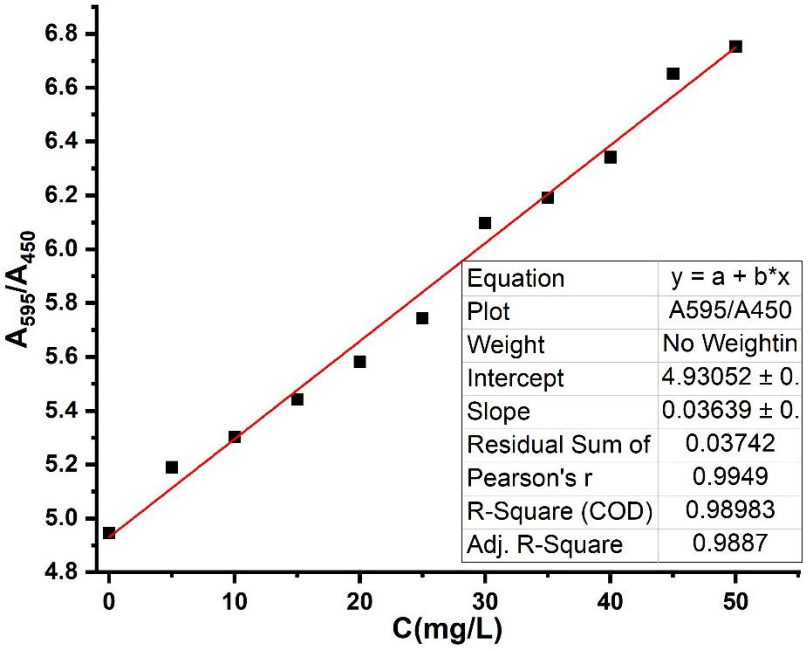

Figure 3 – Calibration curve used for the quantification of soluble proteins in the range of 5–50 mg/L. The limit of determination is 0.003338 and the limit of quantification is 0.220638

Statistical analysis

Prior to statistical analysis, the distribution of the experimental data was verified using the Kolmogorov–Smirnov test using an online tool (https://www.socscistatistics.com/tests/kolmogorov/calculator/) to evaluate data normality and to determine the appropriateness of parametric statistical methods. The results confirmed that the data followed a normal distribution, thereby supporting parametric analyses.

Two-way analysis of variance (ANOVA) was performed using Microsoft Excel Office 2016 to assess the statistical significance of the differences between the means of the treatment variants. The null hypothesis assumes that there are no statistically significant differences among the group means, indicating that the experimental factors do not exert a significant effect on the analysed parameters. Conversely, the alternative hypothesis assumes that there are statistically significant differences between at least two of the treatment variants. ANOVA was applied to evaluate the effects of both genotype and treatment on the measured parameters (Bevans, 2022). Post hoc Tukey tests were performed using the OriginPro 2021 software to identify specific pairs of means exhibiting statistically significant differences.

Relationships between the measured parameters were evaluated using the Pearson's correlation matrix to determine the strength and direction of associations between variables variables. The null hypothesis assumes no statistically significant correlation between variables (correlation coefficient equal to zero), while the alternative hypothesis assumes the existence of a statistically significant relationship between the parameters (Keating *et al.*, 1993; Stewart, 2023).

RESULTS

Germination

Although *M. charantia* is a highly valuable crop, it presents important challenges in both scientific research and practical applications due to its irregular germination patterns and its marked sensitivity to environmental fluctuations (Byregowda *et al.*, 2025). As shown in *Figure 4*, the progression of seed germination, expressed as cumulative percentage, showed a continuous increase over the 14-day experimental period across all studied variants. However, treatment with Algevit accelerated germination and resulted in a higher final percentage compared with the control groups.

For the control groups, germination began on day 4 for Line 3 and Line 1 (*Figure 4B* and *4E*) and on day 5 for Brâncuși, Line 4, and Rodeo (*Figure 4A*, *4C*, and *4D*). For the Algevit groups, germination began on day 3 for Line 3 and Line 1 (*Figure 4B* and *4E*). Notably, the Line 3 genotype exhibited earlier germination and higher values relative to its control, suggesting greater responsiveness to the biostimulant treatment.

During the intermediate phase of germination (days 6–10), the Algevit groups maintained higher germination rates, indicating improved speed and uniformity of germination. This effect was particularly pronounced in Line 1 and Line 3. By the end of the experimental period (day 14), all treated groups exhibited higher germination percentages than their respective

controls. The highest final germination rates were recorded in Line 3 (98%) and Line 1 (96%), whereas the control groups of Brâncuși (86%) and Line 4 (87%) had the lowest rates.

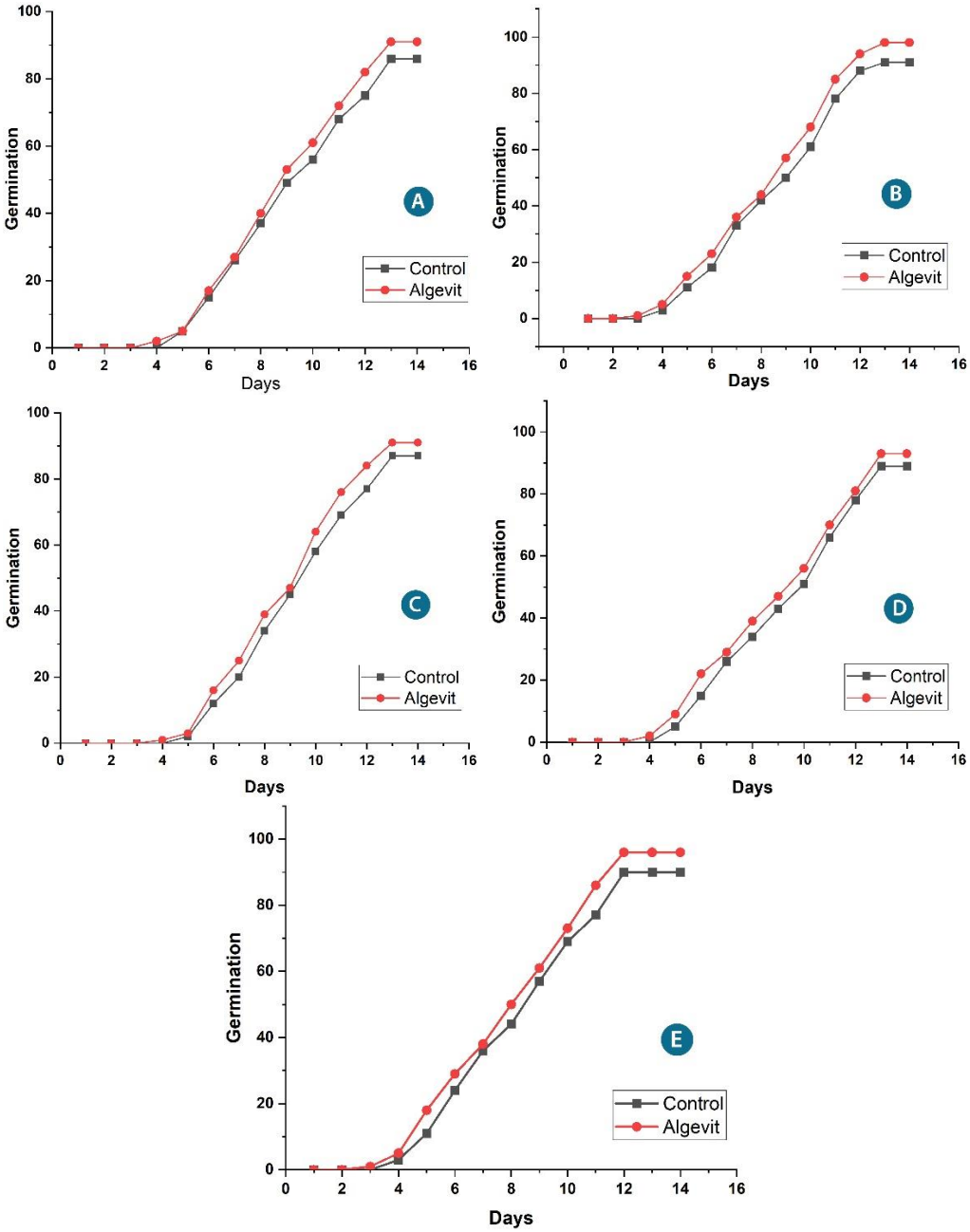

Figure 4 – The influence of Algevit treatment on the germination of *Momordica charantia* (A) Brâncuși, (B) Line 3, (C) Line 4, (D) Rodeo, and (E) Line 1 seeds over 14 days

Vitamin A (β-carotene) content

The control groups exhibited relatively small variation in the provitamin A content (measured indirectly based on the β-carotene content), ranging from 3.14 mg/L (Line 4 and Rodeo) to 3.95 mg/L (Line 1). There was a significant difference between Line 3 and Line 1 (*Figure 5*).

For each genotype, the Algevit group showed a significant increase in the β-carotene content compared with the control group, with the highest value for Line 1 (8.19 μg/mL), and the lowest for Rodeo (4.25 μg/mL) (*Figure 5*).

The most pronounced increase occurred for Line 1 (107%), followed by Line 4 (93%) and Line 3 (81%). These results suggest that Algevit stimulated the synthesis of vitamin A precursor compounds and enhanced the enzymatic activity involved in these processes. Differences between the genotypes may indicate genetic variability in the capacity to benefit from Algevit treatment, with Line 1 showing the greatest benefit (*Table 1*).

Total phenolic content

Compared with the vitamin A (β-carotene) content, the total phenolic content in the control groups showed a wider range of values (*Figure 6*), highlighting more pronounced variability between the genotypes. The total phenolic content in the control groups ranging from 263 mg/L (Brâncuşi) to 173 mg/L (Line 1). The application of Algevit increased the total phenolic content in all five genotypes, but this effect was not as strong as for the vitamin A (β-carotene) content. The values ranged from 276 mg/L (Line 3) to 203.33 mg/L (Line 1). Among the genotypes, Line 1 showed the greatest different between the control and Algevit groups (17%), and Line 3 genotype also showed a significant increase (7.8%). On the other hand, Brâncuşi, Line 4, and Rodeo showed lower increases. These results suggest that Algevit exerts a positive effect on the metabolism of phenolic compounds, but the effect is weaker than that for carotenoids. Variability between the genotypes indicates that there is genetic influence on polyphenol accumulation, with Line 1 standing out for its greatest response to Algevit (*Table 1*).

Soluble protein content

Similarly to the vitamin A (β-carotene) and total phenolic contents, the soluble protein content also increased after Algevit treatment across all analysed genotypes. In the control groups, the soluble protein content exhibited genotype-dependent variability, highlighting underlying genetic differences, as confirmed by two-way ANOVA (*Table 1*) and post hoc Tukey tests (*Figure 7*). It ranged from 36.59 mg/L (Line 1) to 19.86 mg/L (Brâncuşi). Following Algevit treatment, the soluble protein content increased for all genotypes, ranging from 45.16 mg/L (Line 1) to 33.30 mg/L (Rodeo). Line 1 showed the greatest increase in the Algevit group compared with the control group (23%), and Line 3 and Brâncuşi also showed significant increases. The changes for Rodeo and Line 4 were not as pronounced. These results suggest that Algevit enhances protein metabolism.

Two-way ANOVA was employed to evaluate the effects of genotype, treatment, and their interaction on the analysed biochemical parameters. The results revealed highly significant

differences ($p < 0.001$) among genotypes and between the control and treatment groups for all investigated parameters.

Moreover, the interaction between genotype and treatment was also highly significant ($p < 0.001$), indicating that the response to biostimulant application was dependent on the *M. charantia* genotype. Overall, these findings demonstrate the positive effect of Algevit on the biochemical metabolism of bitter melon genotypes and highlight genotype-specific differences in physiological and metabolic responsiveness to treatment.

Pearson correlation coefficients were calculated to assess relationships between germination rate, the vitamin A (β-carotene) content, the total polyphenol content, and the soluble protein content (*Figure 8*). The correlation matrix uses the following notations: B, L3, L4, R, and L1 correspond to the five *M. charantia* genotypes (Brâncuși, Line 3, Line 4, Rodeo, and Line 1, respectively). The letters C and A denote the control and Algevit treatments.

The abbreviations G, vA, PT, and SP represent germination rate, the vitamin A (β-carotene) content, the total phenolic content, and the soluble protein content, respectively. Colour gradients range from deep blue, indicating strong negative correlations ($r = -1$), through green, indicating no correlation ($r = 0$), to deep yellow, indicating strong positive correlations ($r = 1$).

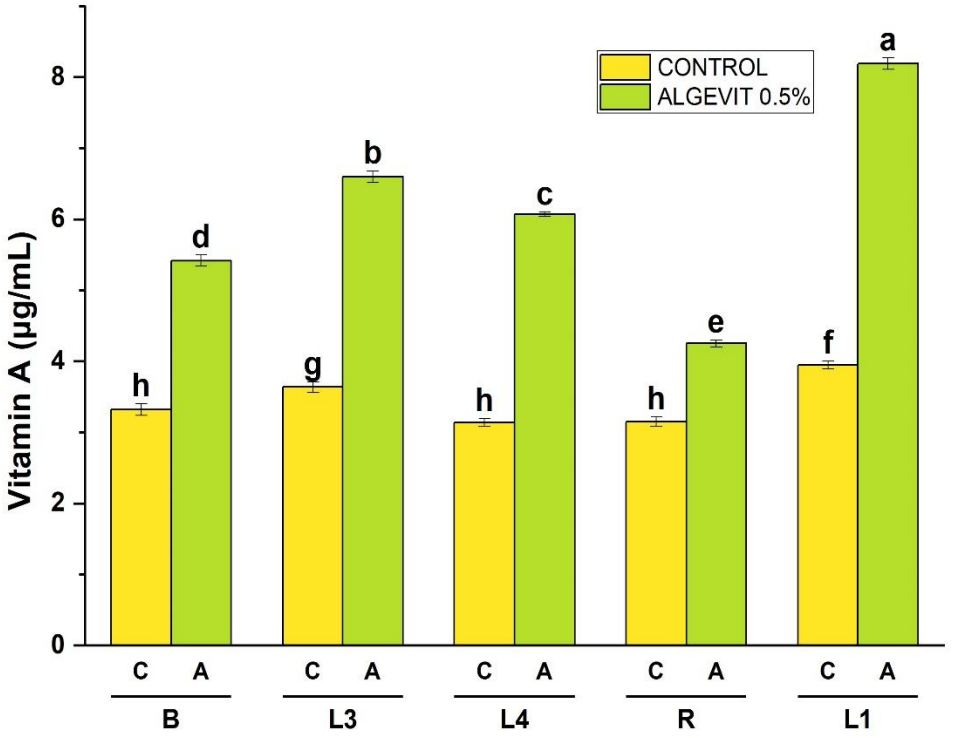

Figure 5 – The effect of Algevit treatment on the vitamin A (β-carotene) content in the *Momordica charantia* genotypes. Error bars represent the standard deviation, and the letters indicate the results of post hoc Tukey tests ($p < 0.05$, $n = 3$)

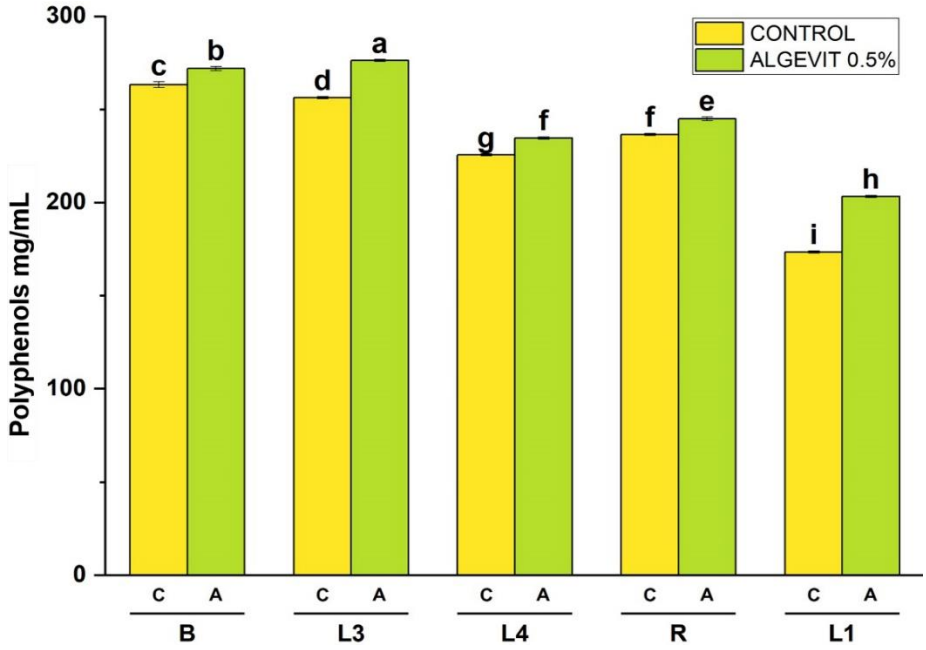

Figure 6 – The effect of Algevit treatment on the total phenolic content in the *Momordica charantia* genotypes. The error bars represent the standard deviation, and the letters indicate the results of post hoc Tukey tests ($p < 0.05$, $n = 3$)

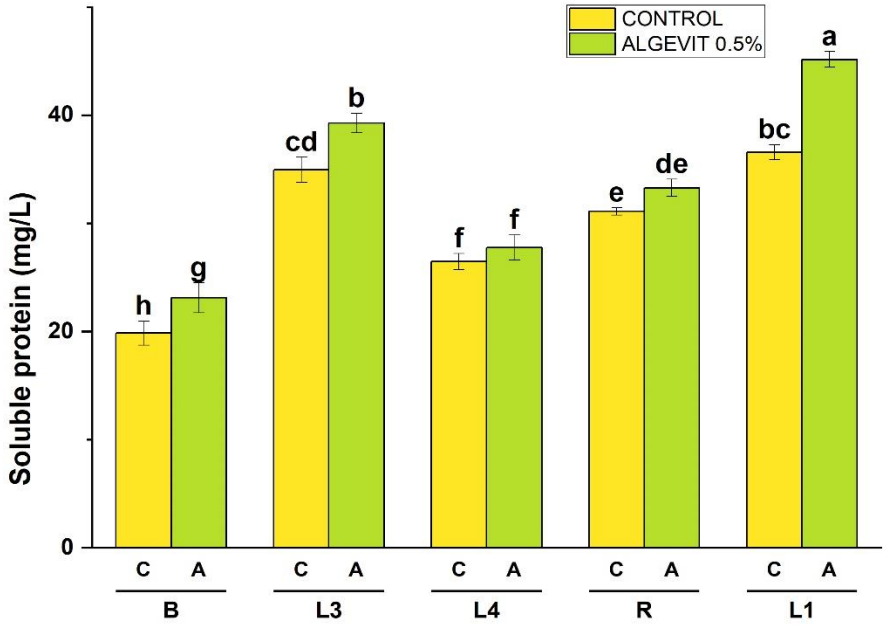

Figure 7 – The effect of Algevit treatment on the soluble protein content in *Momordica charantia* genotypes. The error bars represent the standard deviation, and the letters indicate the results of post hoc Tukey tests ($p < 0.05$, $n = 3$)

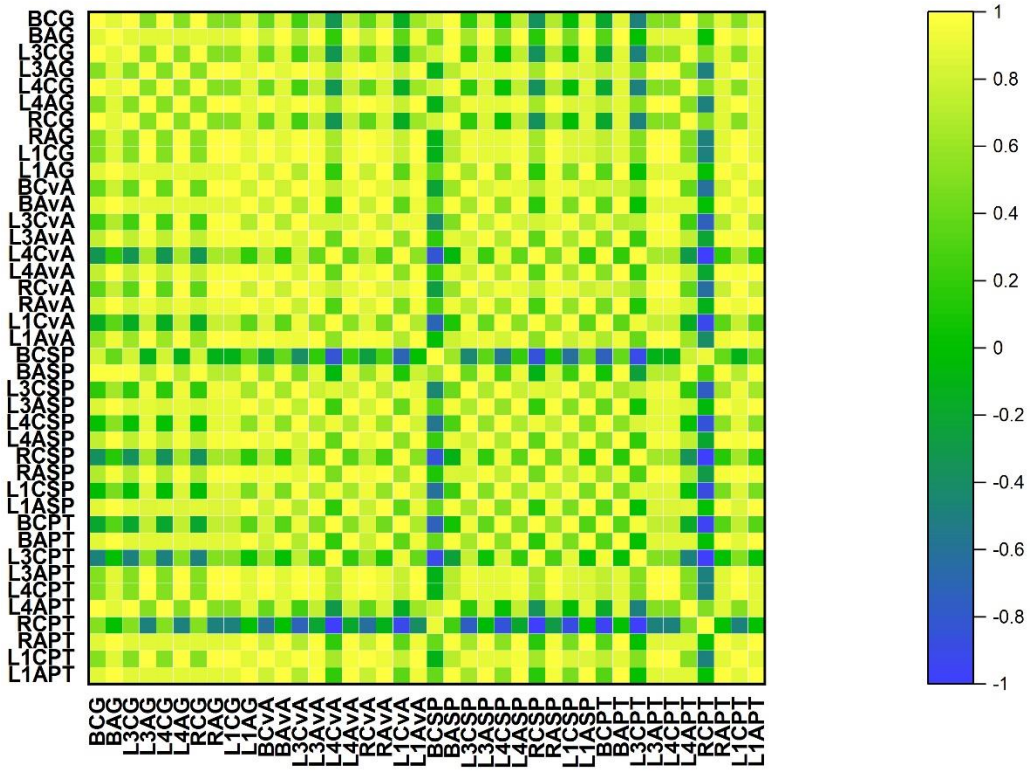

Figure 8 – Pearson correlation matrix for the germination rate, the vitamin A (β-carotene) content, the total phenolic content, and the soluble protein content for the five *Momordica charantia* genotypes and the two treatments

Table 1 – Two-way analysis of variance results to assess *Momordica charantia* genotype, treatment, and genotype × treatment interaction on the vitamin A (β-carotene), total phenolic, and soluble protein contents

Source of variation	SS	df	MS	F	P-value	F crit	Significance
Vitamin A (β-carotene)							
Genotype	1433.314	4	358.3285	400.7914	8.77E-19	2.866081	***
Treatment	115.7944	1	115.7944	129.5164	3.45E-10	4.351244	***
Interaction	48.22199	4	12.0555	13.48411	1.74E-05	2.866081	***
Total polyphenols							
Genotype	18.8391133	4	4.709778	1045.069	6.56E-23	2.866081	***
Treatment	53.3333333	1	53.33333	11834.32	3.3E-29	4.351244	***
Interaction	8.13356666	4	2.033392	451.1964	2.72E-19	2.866081	***
Soluble proteins							
Genotype	25200.47	4	6300.117	9450.175	1.88E-32	2.866081	***
Treatment	1717.633	1	1717.633	2576.45	1.3E-22	4.351244	***
Interaction	540.8667	4	135.2167	202.825	6.99E-16	2.866081	***

Note: NS, p > 0.05; * p < 0.05;** p < 0.01; *** p < 0.001;**** p < 0.001

There were strong positive correlations, particularly in Line 1 and Line 3 under Algevit treatment, supporting the significant differences observed in *Figure 4*, *Figure 5*, *Figure 6*, and *Figure 7*. In Line 1, the strongest correlation was observed between the total phenolic content and the soluble protein content (L1A–SP and L1A–PT; r = 0.99), whereas the lowest correlation was found between the germination rate and the vitamin A (β-carotene) content (L1A–G and L1A–vA; r = 0.70). Similarly, in Line 3, the strongest correlation was recorded between the soluble protein content and the vitamin A (β-carotene) content (L3A–SP and L3A–vA; r = 0.98).

DISCUSSION

Germination is a critical stage in the plant life cycle and is strongly influenced by environmental factors such as temperature, humidity, and oxygen availability. Optimising this process is essential to ensure rapid, uniform germination (Affecting, 2023; Hanson *et al.*, 2018). Natural biostimulants may contribute to overcoming seed dormancy and initiating germination by stimulating enzymatic reactions involved in embryonic metabolism, thereby facilitating the transition from a quiescent to an active physiological state (Gupta *et al.*, 2022). They also modulate hormonal balance, particularly the gibberellin/abscisic acid ratio (Liang *et al.*, 2026). Mackinnon *et al.* (2008) demonstrated that *A. nodosum* extract (which Algevit contains) enhances α-amylase activity in barley seeds, promoting starch mobilisation required for germination and early seedling growth.

In the present study, application of Algevit significantly improved germination across all five studied genotypes, reducing the time to germination onset and increasing final germination percentages. From the earliest stages, the treated groups exhibited higher values than the control groups, indicating accelerated activation of metabolic processes involved in germination. Physiologically, these effects may be attributed to bioactive compounds present in the seaweed extract, including phytohormones (auxins and cytokinins), amino acids, and other organic metabolites involved in the regulation of embryonic metabolism. These compounds enhance imbibition, activate hydrolytic enzymes responsible for reserve mobilisation, and stimulate cellular respiration, thereby promoting faster and more uniform germination. The variability in response among genotypes suggests genetic differences in sensitivity to exogenous stimulation, with Line 1 and Line 3 exhibiting the strongest responses.

According to the literature, seaweed-based biostimulants may also improve the physicochemical properties of the growth substrate, thereby increasing nutrient availability (Xie *et al.*, 2022). In addition, amino acids act as osmoprotectants and metabolic precursors, stabilising cellular structures and supporting early developmental processes. Exogenous amino acid application has been shown to increase germination rate and enhance photosynthetic pigment synthesis in *A. cepa* seedlings (Abdelkader *et al.*, 2023).

Similarly, seaweed extracts have been reported to stimulate germination and early growth in onion (*Allium cepa L.*), effects associated with the presence of phytohormones and polysaccharides (Zhang *et al.*, 2024).

The content of β-carotene, a precursor of vitamin A, increased significantly in all *M. charantia* genotypes after Algevit treatment. Elevated carotenoid levels are associated with higher photosynthetic capacity due to their role in protecting photosynthetic pigments and stabilising photosynthetic complexes (Sandmann, 2021; Stra *et al.*, 2023). *Ascophyllum nodosum* extracts are rich in phytohormones, including auxins, cytokinins, and gibberellins. Khan *et al.* (2011) demonstrated cytokinin-like activity of these extracts in *Arabidopsis thaliana*, evidenced by the increased expression of the *ARR5* gene, a molecular marker associated with the cytokinin response. These phytohormones may regulate genes involved in photosynthesis, leading to increased carotenoid accumulation, which is essential for thylakoid membrane structure and protection of chlorophyll under high light intensity (Nair *et al.*, 2022; Shukla *et al.*, 2019). De Saeger *et al.* (2020) further reported that *A. nodosum* extracts enhance photosynthetic efficiency by modulating genes involved in photosystem II function, Rubisco activity, CO_2 transport, and thylakoid organisation.

As shown in *Figure 5*, Line 1 exhibited the strongest protective capacity of the photosynthetic apparatus, potentially contributing to increased carbohydrate synthesis. Higher carbohydrate availability, resulting from enhanced photosynthesis, is closely associated with elevated protein and polyphenol levels, as reflected in *Figure 6* and *Figure 7* (Caretto *et al.*, 2015; Garoff *et al.*, 1982). Similar effects have been reported in *Solanum lycopersicum*, where *A. nodosum* extracts increased the photosynthetic pigment content and improved photosynthetic performance (Zhang *et al.*, 2023). Carotenoids, as precursors of abscisic acid, play a key role in regulating plant physiological responses. The significant increase observed in treated variants suggests potential abscisic acid accumulation, which may induce a priming effect, preparing plants for rapid response to stress conditions (Nair *et al.*, 2022; Shukla *et al.*, 2019; Wei *et al.*, 2021).

Polyphenols are complex secondary metabolites involved in plant adaptation to environmental stress. They function as antioxidants by scavenging reactive oxygen species and maintaining redox homeostasis. Additionally, they regulate gene expression associated with secondary metabolism, thereby influencing growth, differentiation, and tissue integrity (Wang *et al.*, 2025). The increased total phenolic content in treated plants may be attributed to bioactive compounds in *A. nodosum* extracts, which are rich in polysaccharides and phenolic substances with metabolic regulatory functions (Pereira, 2023). These extracts modulate hormonal pathways, including auxins, cytokinins, and abscisic acid. These extracts are reported in the literature as having the ability to modulate hormonal pathways (auxins, cytokinins, abscisic acid). *Ascophyllum nodosum* extracts modulate abscisic acid metabolism by regulating genes involved in its biosynthesis and degradation, thereby promoting

polyphenol biosynthesis (de Saeger *et al.*, 2020). This increase may also be associated with stimulation of the phenylpropanoid pathway, which is responsible for the biosynthesis of most phenolic compounds (Ninkuu *et al.*, 2025; Wang *et al.*, 2020). The higher values observed in Line 1 and Line 3 may reflect the metabolic link between primary metabolism, which supplies carbon skeletons via photosynthesis, and secondary metabolism, where polyphenols represent major end products (Zagoskina *et al.*, 2023). Similar responses have been reported in banana, where seaweed extracts enhanced enzymatic activity and increased the accumulation of phenols, flavonoids, and other antioxidant compounds (Chinnasamy *et al.*, 2024). Such variability is commonly reported and reflects genotypic differences in regulation of the phenylpropanoid pathway (Cavallini *et al.*, 2015; Wang *et al.*, 2020; Weisshaar and Jenkins, 1998).

A comparable pattern was observed for soluble proteins, which represent a key component of primary metabolism. Enhanced carotenoid protection of the photosynthetic apparatus likely contributes to increased photosynthetic efficiency, thereby stimulating primary metabolism and protein synthesis. This may explain the observed correlation between the vitamin A (β-carotene) and soluble protein contents in Line 1 and Line 3. Deepika *et al.* (2025) reported similar findings in spinach treated with *A. nodosum*–based biostimulants, where increased protein content was associated with enhanced photosynthetic activity, chlorophyll accumulation, and improved nutrient uptake. Increased levels of soluble proteins and polyphenols may also enhance tissue sensitivity to subsequent stress exposure, enabling a faster physiological response to adverse environmental conditions (Khan, 2025; Yong *et al.*, 2015)

Overall, the observed effects can be explained through a mechanism in which improved protection of the photosynthetic apparatus enhances carbon assimilation, leading to increased primary metabolism, higher protein synthesis, and stimulation of secondary metabolite accumulation, including polyphenols. This integrated metabolic enhancement may also contribute to improved stress tolerance and adaptive capacity in treated plants.

CONCLUSIONS

The application of the seaweed-based biostimulant Algevit resulted in significant improvements in the analysed parameters, manifested through enhanced germination, increased levels of bioactive metabolites, and optimised plant metabolic activity. These results highlight the efficacy of seaweed extracts in supporting early physiological processes.

The observed increases in the vitamin A (β-carotene), total phenolic, and soluble protein contents suggest an intensification of photosynthetic activity as a primary carbon source supporting the synthesis of these biochemical compounds. This response may be associated with the high content of amino acids and phytohormones present in the biostimulant formulation. Among the studied genotypes, Line 1 exhibited the highest compatibility with Algevit treatment, showing the greatest values for all analysed biochemical parameters and

the strongest metabolic correlations, indicating enhanced metabolic adaptability to the applied biostimulant.

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