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ORIGINAL RESEARCH PAPER

Preliminary screening of biostimulative effects of Göemar BM-86 on eggplant cultivars grown under field conditions in Poland

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Abstract

Seaweed extracts are widely used in agriculture as ecological focus substances applied to improve crop growth and quality. One of the primary benefits they bring is increased effectiveness of fruit setting as well as improved stress tolerance, essential for warm-climate crops cultivated in the nonoptimal environmental conditions of Northern and Central Europe. The aim of this study was a preliminary investigation of any genotype-dependent reaction of eggplant cultivars (*Solanum melongena*) to application of a standardized extract of the seaweed *Ascophyllum nodosum* (Göemar BM-86) under field conditions in Poland. The only statistically relevant result of this biostimulant was shown for cultivar 'Flavine' F₁, where it positively affected the early crop yield and the number of fruits per plant. Fruit quality attributes, including antioxidant activity, as well as selected mineral contents, increased as an effect of biostimulant spraying. This reaction was specific for the cultivars investigated, and it was confirmed by significant differences in the main effects between biostimulant and control treatments for almost all the properties measured. The use of this *A. nodosum* extract suggested that there could be an improvement in fruit yield and quality in selected eggplant cultivars under field conditions in the temperate climatic zone.

Keywords

Ascophyllum nodosum; abiotic stress; *Solanum melongena*; yield

Introduction

The eggplant (*Solanum melongena* L.) is a warm-climate vegetable of West Asian origin. It is grown for its fruits and has a broad spectrum of shapes, sizes and colors. A group of cultivars with dark violet and oval berries is the most popular in Europe, but white, pink, purple, or striped fruits are also offered on the market [1]. Eggplant is an economically important crop which, although of tropical and subtropical origin, is cultivated in the temperate climatic zone of Central Europe [2]. Eggplant fruits are valuable dietary components because of their low calorific value and a mineral composition beneficial for human health [3]. Recently, research carried out on subtropical crops has demonstrated the importance of investigating the effects of low temperatures on their growth, development, yield, and biological value. The direct economic application of this field of research is the introduction of subtropical species to higher geographical latitudes. Eggplant is grown only from seedlings produced in heated glasshouses or foil tunnels. Low temperatures, acting after transplantation of young seedlings to the field, can cause physiological and biochemical changes which can affect the entire plant ontogeny, flowering, and fruit setting, yield, and quality [4]. Sustainable strategies that can improve the overall crop status in unfavorable field conditions should be developed

with the particular emphasis on avoiding any adverse impact on the nutritional health value of the eggplant. Seaweed extracts (SWE), assumed to be biodegradable biostimulants, are nontoxic, nonpolluting, and nonhazardous to various organisms, and so offer a potential solution to this problem [5,6].

Biostimulants are plant extracts containing active biocompounds that affect the plant metabolome through activation of a wide range of physiological pathways. The final effect is an enhanced plant nutrition status, accelerated growth and development, as well as better tolerance to stress factors [7,8]. Some of the most effective biostimulants are SWEs, based on red, green, or brown macroalgae. These algae have been used as fertilizers by man probably since the advent of agriculture. Nowadays, this idea, supported by new technologies, has led to the development of several commercial products marketed by about 50 companies globally [9,10]. The majority of these formulations are based on the extract of the brown alga, *Ascophyllum nodosum* (L.) Le Jolis [5,11,12]. The efficiency of seaweed extracts is due to the presence of micro- and macronutrients, growth hormones, and vitamins at preferential levels [13]. Recent work has shown that extracts with addition of trace elements were able to enhance stress tolerance in crops through increased reactive oxygen species scavenging responses [14]. SWE, enriched in micronutrients, could potentially induce a significant plant reaction against environmental stresses, through an increase in antioxidant activity. Göemar BM-86 (Arysta Life Science North America, LLC) is an *A. nodosum* extract, with a standardized main micro- and macroelements content [15]. According to van Oosten et al. [8], standardization is necessary to identify and characterize how SWE affect plant metabolism. This formulation seems to be a reliable material for future investigations. In the most rigorously prepared biostimulants from leading companies, high-throughput analytical methods have been employed to ensure consistent product quality [10]. However, the mechanisms of SWE action remain largely unknown due to the heterogeneous nature of the raw materials and the complex composition of formulations [16]. According to Yakhin et al. [6], it is almost impossible to identify the components crucial for SWE biological activity and to determine the modes of action involved. All biological systems are extremely complex, and we firmly believe that the investigation of more and more aspects of biostimulant vs. plant interactions may shed new light on the effective utilization of these promising products in agriculture.

Based upon the references cited, we decided to perform preliminary investigations with the application of Göemar BM-86 for eggplant cultivation under field conditions in Poland. We hypothesized that a SWE formulation will improve the flowering biology of warm-climate vegetables cultivated under field conditions of the temperate climatic zone and that this reaction will be genotype-dependent. We also expected that direct biostimulant application could increase stress tolerance of plants, especially during the generative phase of development, resulting in earlier and higher yields and modified fruit chemical composition. The study reported here assessed for the first time the potential of Göemar BM-86 to improve the yield and quality of eggplant in temperate climate field conditions, including any genotype-dependent factors.

Material and methods

Plant material and experimental design

Eggplant cultivars that were the subject of our investigation were: ‘Epic’ F₁ (Seminis Vegetable Seeds), ‘Flavine’ F₁ (Gautier Semences), ‘Gascona’ F₁ (Gautier Semences), and ‘WA 6020’ F₁ (Western Seed International BV). All were described as “early and vigorous”, so we presumed their predisposition to field cultivation in the temperate climatic zone. The biostimulant Göemar BM-86 was used as the experimental treatment. Göemar BM-86 is a standardized *A. nodosum* extract, containing: N – 5.0%, Mg – 2.4%, S – 3.2%, B – 2.07%, Mo – 0.02% [15]. It was applied as a foliar spray, three times, at a rate of 1.5 dm³ ha⁻¹; control plants were sprayed with distilled water only. The first spraying was made 2 weeks after transplanting seedlings to the field; subsequent sprayings were at 2-week intervals. A backpack-type sprayer was used in similar weather conditions conducive to the penetration of leaf tissues by the preparation [17].

The experiment was carried out at the University of Agriculture in Krakow, Poland. Eggplant seeds were sown on March 20, 2012 in seed boxes filled with a peat substrate (Klasman TS2; Klasmann-Deilmann GmbH, Germany). Seedlings were subsequently transplanted into 40-cell black multipots (VEFI, Norway) each cell having a volume of 0.23 dm³. Seedlings were grown on in a glasshouse at temperatures of 20/17 ±2°C (day/night). Fertilizer (Kristalon Green; Yara, Poland) was applied twice at a dose of 10 g dm⁻³ water as with the foliar spraying. After a 7-day gradual decrease in temperature and irrigation, transplants were finally planted out into the experimental field. The experiment was established at the Vegetable Experimental Station of University of Agriculture in Krakow, Poland (50°04' N, 19°51' E). This site is located in the warm summer humid continental climate zone (Dfb) according to the Köppen's classification. The soil is classified as a Fluvisol Cambisol (Humic) in the FAO classification with a C_{org} level of 2% and pH_{KCl} of 6.11. Seedlings were planted out on May 20, 2012 at a spacing 0.75 × 0.60 m (2.2 plants m⁻²) in a split-block design with three replicates per treatment. The experimental plots, each consisting of 12 plants, were surrounded by shelterbelts. Cultivation procedures (weeding, irrigation, plant protection against pests and diseases) were performed according to the standard recommendations for eggplant [18]. The fertilizers were applied on the basis of soil analysis to achieve a stable content of nutrients (mg dm⁻³): N – 100, P – 90, K – 220, Ca – 1,100, Mg – 70. Harvests were carried out successively from July until the end of September as maturing progressed.

Microclimatic conditions in the field

Meteorological parameters were measured hourly by automatic sensors HOBO Pro RH/Temp. and HOBO Weather Station (Onset Comp. Corp., Bourne, USA) located next to the experimental plots. Data expressed as monthly means are presented in Tab. 1. The growing season in 2012 rather favoured eggplant field cultivation because of the warm July and August that encouraged generative development of plants. A cool September with lower PAR and total rainfall caused continuous decline of vegetative growth.

Tab. 1 Mean monthly temperature, photosynthetically active radiation (PAR), and the sum of rainfall in the 2012 growth season.

Month	Temperature (°C)	PAR (μmol m ⁻² s ⁻¹)	Sum of rainfall (mm)
May	15.6	692	21.4
June	17.8	621	106.0
July	20.3	437	42.8
August	18.7	363	46.4
September	14.1	241	30.6

Determination of yield parameters

Fruits at the stage of harvest maturity (typical color, shape, and weight for a cultivar), were successfully harvested and the marketable yield assessed according to the UNECE standard for eggplant [19]. For the early yield (covering the first four harvests), the number of fruits harvested per plant and average weight of the fruit were assessed.

Determination of fruit chemical composition

During the full fruiting period, 20 randomly selected fruits per treatment were harvested at the stage of harvest maturity and assigned for chemical analyses. These were performed on three replicates using the methods described below. Mineral element contents were determined for whole fruits; antioxidant activity was determined separately using fruit peel and flesh.

Plant samples were washed with distilled water and dried at 65°C for 24 h. The dried samples were ground in a blender and homogenized. Five g of dried sample were then placed in a porcelain crucible and ashed at 500°C in a muffle furnace until a grey ash residue was obtained. The residue was dissolved in 1 cm³ of nitric acid (1:2 v/v) and made up into a volume of 25 cm³ with distilled water. Fe, Zn, Ca, and Cu concentrations were determined in the digests by atomic absorption spectrophotometry (Varian Spectr AA20) with an air-acetylene flame under standard operating conditions. A colorimetric method was used to measure the phosphorus concentrations in the fruits [20].

The total antioxidant activities (TAA) of eggplant peel and flesh were determined separately using 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]). 2.5 g of fruit pulp were mixed with 80% methanol and centrifuged for 10 min, at 3,492 g and 4°C. The mixture of decanted supernatant (0.1 cm³) and 0.1 mM DPPH[•] dissolved with 4.9 cm³ of 80% methanol was incubated for 15 min in the dark at 20–22°C, and then the absorbance measured at 517 nm using an UV-VIS Helios Beta spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA). DPPH[•] radical scavenging activity was calculated using the formula: $AA (\%) = [(A_0 - A_1)/A_0] \times 100$, where *AA* is the antioxidant activity, *A*₀ – the absorbance of the reference solution, and *A*₁ the absorbance of the test solution [21].

Statistical analyses

All statistical analyses were performed using the use of Statistica 12.0 software package (StatSoft Inc., Tulsa, USA). A two-way analysis of variance followed by Tukey's HSD test was used to determine the main effects of biostimulant and genotype as well as interactions between main effects, at the $p \leq 0.05$ significance level. Tabulated data are presented as averages of three replicates.

Results

The study demonstrated that the biostimulant tested had a highly specific action in modifying the yield and chemical composition of eggplant fruits. The eggplant hybrids investigated differed significantly in their early and total marketable yield (Fig. 1). Early yield, including the first four harvests, ranged from between 0.10 kg m⁻² ('Flavine' F₁, control treatment) to 0.74 kg m⁻² ('Epic' F₁, biostimulant treatment). Marketable yield ranged between 1.61 kg m⁻² ('Gascona' F₁, control treatment) and 3.23 kg m⁻² ('Epic' F₁, biostimulant treatment). Spraying of plants with Göemar BM-86 significantly increased the early yield of 'Flavine' F₁, and a similar tendency was observed for all the cultivars investigated and statistically confirmed a main effect both for early and total marketable yields. Comparable results were found for the number of harvested fruits, with significant differences between biostimulant-treated and control plant confirmed for 'Epic' F₁, 'Flavine' F₁, and 'Gascona' F₁ (Fig. 2). Mean fruit weight increased significantly with biostimulant application for 'WA 6020' F₁ but not for 'Flavine' F₁ and 'Gascona' F₁ (Fig. 3). Analysis of the genotype-dependent differences in eggplant yield and number of fruits, regardless of biostimulant application, suggested that 'Epic' F₁, 'Flavine' F₁, and 'WA 6020' F₁ are hybrids of great potential in respect of these features.

Analysis of interactions showed that biostimulant application significantly increased the TAA of 'Epic' F₁ peel and flesh, as well as 'Flavine' F₁ and 'Gascona' F₁ flesh (Fig. 4). Among the cultivars investigated, the highest TAA was for 'Flavine' F₁ peel and 'WA 6020' F₁ flesh. Regardless of the cultivar, the mean TAA of fruit peel and flesh of eggplant berries was similar: 8.75% and 8.94% DPPH[•], respectively.

The mineral element composition of eggplant fruits was significantly affected by both experimental factors (Tab. 2). Phosphorus and Zn concentrations in 'Flavine' F₁ and 'WA 6020' F₁ in fruits harvested from biostimulant-treated plants were higher than those of the control. A similar situation was found for 'Epic' F₁ and 'Gascona' F₁ for Fe as well as for 'Gascona' F₁ for Ca. Göemar BM-86 increased Zn concentrations in 'Flavine' F₁ and 'WA 6020' F₁. Regardless of any biostimulant treatment, 'Epic' F₁ fruits can be considered as the richest source of Fe, Zn, and Cu, 'WA 6020' F₁ for P and 'Gascona' F₁ for Ca.

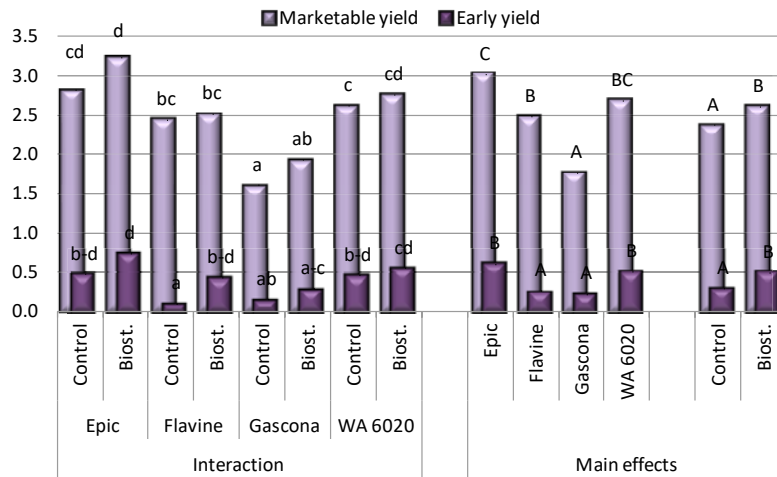


Fig. 1 The effect of cultivar and biostimulant treatment on early and marketable yield of eggplant (kg m⁻²).

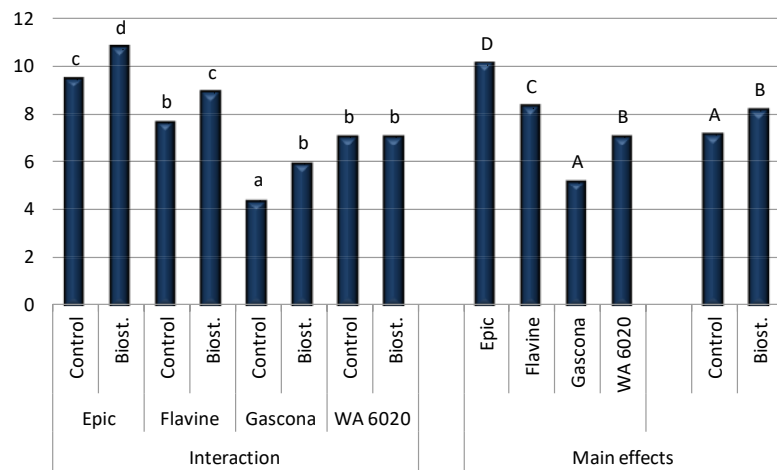


Fig. 2 The effect of cultivar and biostimulant treatment on number of eggplant fruits per m².

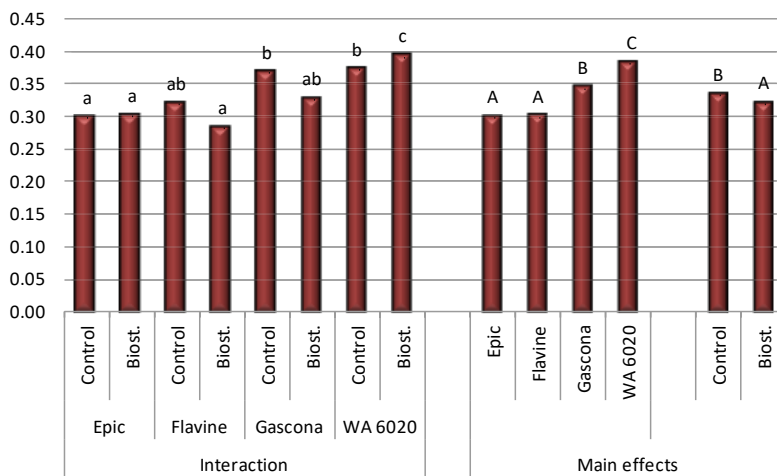


Fig. 3 The effect of cultivar and biostimulant treatment on mean weight of eggplant fruits (kg).

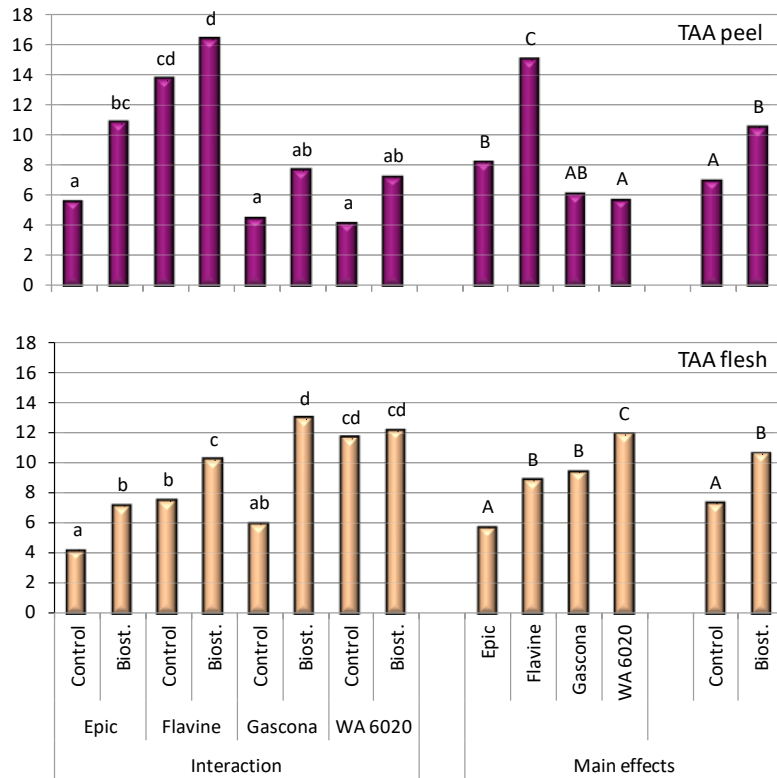


Fig. 4 The effect of cultivar and biostimulant treatment on total antioxidant activity (% DPPH) of eggplant fruit peel and flesh.

Tab. 2 The effect of cultivar and biostimulant treatment on mineral composition of eggplant fruits.

Cultivar	Treatment	P	Fe	Zn	Ca	Cu
		(g 100 g ⁻¹ FW)				
'Epic'	Control	11.22 ^{a*}	0.395 ^b	0.334 ^{de}	0.268 ^a	0.240 ^d
	Biost.	18.19 ^a	0.565 ^c	0.339 ^{de}	0.334 ^a	0.227 ^d
'Flavine'	Control	12.61 ^a	0.357 ^{ab}	0.253 ^b	0.260 ^a	0.158 ^c
	Biost.	15.56 ^b	0.361 ^{ab}	0.368 ^e	0.344 ^a	0.177 ^c
'Gascona'	Control	12.51 ^a	0.237 ^a	0.271 ^{bc}	0.307 ^a	0.097 ^a
	Biost.	12.61 ^a	0.470 ^{bc}	0.266 ^{bc}	0.681 ^b	0.111 ^{ab}
'WA 6020'	Control	15.30 ^b	0.357 ^{ab}	0.173 ^a	0.225 ^a	0.144 ^{bc}
	Biost.	17.55 ^c	0.476 ^{bc}	0.310 ^{cd}	0.278 ^a	0.158 ^c
Mean for cultivar	Epic	14.71 ^B	0.480 ^B	0.337 ^B	0.301 ^A	0.234 ^C
	Flavine	14.09 ^B	0.359 ^A	0.311 ^B	0.301 ^A	0.167 ^B
	Gascona	12.56 ^A	0.355 ^A	0.269 ^A	0.494 ^B	0.104 ^A
	WA 6020	16.42 ^C	0.416 ^{AB}	0.241 ^A	0.251 ^A	0.151 ^B
Mean for treatment	Control	12.91 ^A	0.336 ^A	0.258 ^A	0.265 ^A	0.160 ^A
	Biost.	15.98 ^B	0.468 ^B	0.321 ^B	0.409 ^B	0.168 ^A

* Means within a column followed by different letters (capital letters for main effects and lowercase letters for interaction effects) are significantly different at $p \leq 0.05$ according to Tukey's HSD test.

Discussion

Plant hormones, vitamins, as well as the mineral contents of *A. nodosum* extracts have been shown to enhance growth and yield in some fruits and vegetables such as tomato [5], but the opposite has also been reported [16]. Lola-Luz et al. [22] were unable to show any statistically significant increase in yield of broccoli as an effect of the application of three commercial seaweed extracts. In our study, the only statistically relevant result of a biostimulant was for 'Flavine' F₁ eggplants where it positively affected the early crop yield and the number of fruits per plant. A similar tendency was observed for the other cultivars. This tendency was not confirmed by the analyses of variance for the interaction of genotype and treatment but it was significant for the main effect of the biostimulant treatment. The increase in number of fruits harvested from 'Flavine' F₁ sprayed with biostimulant could be due to more intensive fruit set resulting in a greater number of smaller fruits. The reaction of 'WA 6020' F₁ was different because the direct effect of biostimulant application was a significantly greater fruit weight but not the number of harvested fruits. According to Abd El-Gawad et al. [23], an increase in eggplant yield as an effect of SWE application could be the result of stimulation of root and shoot growth and thus an increase in nutrient accumulation (e.g., from exploitation of deeper soil horizons). Yakhin et al. [6] confirmed that SWE stimulated plant productivity by enhancing the absorption of nutrients from soil, increasing photosynthetic efficiency, as well as an upregulation of antioxidant activity.

The protective effect of biostimulants against environmental stresses is associated with a reduction of stress-induced reactive oxygen species, as well as an activation of the antioxidant defence system of plants [6]. TAA of fruit extracts collected from multicolored eggplant varieties has been found to depend mainly on the chlorogenic acid content [24]. The importance of eggplant as an antioxidant source is tied to the relatively high content of phenolic acids in the fruit flesh [25] and/or the anthocyanins in the peel [24,26]. In the present study, for the cultivar with a light, pink-violet skin ('WA 6020' F₁), TAA was probably influenced by the phenolics content of the flesh, but for the cultivar with a dark violet skin ('Flavine' F₁), it was rather the peel anthocyanins. Both the peel and flesh of eggplant fruits seemingly participate in the total antioxidant activity, which was also affected positively by biostimulant treatment.

The relationship between fruit mineral composition and biostimulant application in field-grown vegetables was more complicated due to the complex composition of biostimulants and their interaction with plant constituents. The SWE surely served as a source of additional amounts of nutrients [23]. Other researchers suggest that foliar seaweed extract application improved root growth and development as well as nutrient uptake by roots [27]. Turan and Köse [28] reported that SWE improved Cu uptake of grapevines probably by increased permeability of the cell membranes. In our study, however, differences between treatments regarding Cu accumulation in eggplant fruits were not significant. Majkowska-Gadomska and Wierzbicka [29] reported that application of the synthetic biostimulant Asahi SL significantly decreased N but increased K and Cu contents in two eggplant cultivars grown in unheated tunnels in northern Poland. Di Stasio et al. [16] and Dobromilska et al. [30] also demonstrated that two *A. nodosum* preparations enhanced the accumulation of minerals in tomato fruits.

Seaweed extract can ameliorate crop physiological status and thus contribute to final yield and its characteristics. Identification of the biologically active chemicals is necessary and also the mechanisms of action involved in plant growth responses and stress mitigation in biostimulant preparations such as those of *A. nodosum*. However, the complex nature of seaweed-derived preparations (e.g., as organic fertilizers) requires characterization of their biological functions in plants for which an explicit mode of action has not yet been defined. This need is in agreement with the current definition of biostimulants proposed by Yakhin et al. [6], "a formulated product of biological origin that improves plant productivity as a consequence of the novel or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds".

Conclusions

Biostimulants are promising, environmentally-friendly, and ecologically desirable substances that can support crop productivity and quality. The present study allowed a more detailed description of the relationships between biostimulants and a crop cultivated under field conditions. *Ascophyllum nodosum* extract, as a component of the standardized formulation Göemar BM-86, was successively applied to increase yield and its characteristics in eggplant. This biostimulant significantly affected the majority of selected properties of eggplant fruits. The genotype-dependent reaction of eggplant to biostimulant application showed that Göemar BM-86 effects could be beneficial for selected cultivars.

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Wstępna analiza stymulującego działania Göemar BM-86 na wybrane odmiany oberżyny uprawiane w warunkach polowych w Polsce

Streszczenie

Ekstrakty z alg morskich są szeroko wykorzystywane w rolnictwie jako proekologiczne substancje stymulujące wielkość i jakość plonu roślin uprawnych. Korzystne efekty ich stosowania obejmują m.in. zwiększenie efektywności wiązania owoców jak również poprawę tolerancji na czynniki stresowe, kluczową dla roślin klimatu podzwrotnikowego, uprawianych w odbiegających od

optymalnych warunkach środowiskowych. Celem prezentowanych badań była wstępna analiza reakcji czterech odmian oberżyny (*Solanum melongena* L.) na zastosowanie standaryzowanego ekstraktu z *Ascophyllum nodosum* (Göemar BM-86) w uprawie polowej w Polsce. Stwierdzono, że aplikacja biostymulatora, istotnie zwiększyła plon wczesny oraz liczbę owoców u odmiany 'Flavine' F₁. Generalnie, cechy jakościowe owoców, w tym aktywność antyoksydacyjna oraz zawartość wybranych pierwiastków mineralnych wzrosły w efekcie zastosowania biostymulatora, a analiza efektów głównych wykazała istotne różnice pomiędzy obiektami w których aplikowano biostimulant a kontrolą dla większości badanych parametrów. Wykorzystanie ekstraktu z *A. nodosum* można uznać za obiecującą metodę zwiększającą plonowanie i poprawiającą jakość owoców u wybranych odmian oberżyny uprawianych w warunkach polowych w strefie klimatu umiarkowanego.