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# Towards Sustainable Agriculture—Agronomic and Economic Effects of Biostimulant Use in Common Bean Cultivation

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**Abstract:** Today, one of the greatest challenges faced by the agriculture industry is the development of sustainable and environmentally-friendly systems to meet nutritional demands of the continuously growing global population. A number of research studies have recently been undertaken with the aim to indicate types of parameters used in plant production that would be able to improve plant growth as well as the effectiveness and quality of yield, and to help plants cope with environmental stress. The aim of this study was to verify a hypothesis that the implementation of a sustainable agricultural technology, based on the use of synthetic biostimulants, will allow not only increasing crop yield and quality but also improving the cost-effectiveness of common bean cultivation. The field experiment was conducted in three growing seasons (2016–2018). In the growing season, the plants were treated with Atonik and Tytanit biostimulants in the form of single or double spraying. We determined biometric traits, seed yield, seed number, and 1000-seed weight. Further analyses included contents of nutraceutical potential. The economic effect of using biostimulants was also calculated. The results of our experiment allowed verifying a hypothesis that the implementation of a sustainable agricultural technology based on the use of synthetic preparations was an effective method to increase plant productivity and, consequently, economic profits to farmers.

**Keywords:** yield; profitability crop; bean; growth; nutrients; nutraceutical potential; sustainability

## 1. Introduction

Today, one of the greatest challenges faced by the agriculture industry is the development of sustainable and environmentally-friendly systems to meet nutritional demands of the continuously growing global population. Considering the diminishing area of arable lands and the depleting genetic potential of crops, one of the solutions that would enable increasing crop yields and protecting produce would be the implementation of novel agricultural technologies and the development of the existing

ones [1–3]. Additional challenges posed to sustainable agricultural production include the reduction of energy consumption and more effective use of resources [4] but, simultaneously, the improvement in crop quality, especially under growth conditions that are not beneficial. This would result in higher profits farmers, which would also be translated into greater possibilities of storing plant materials after harvest and achieving higher yields of crops with quality and nutritional values that are acceptable by increasingly more aware consumers [5,6]. Considering the above, the agriculture industry has to cope with serious challenges and, undoubtedly, should be supported by many scientific disciplines [7], which would allow for its successive but modern and responsible development. Today, a compromise is needed between the increase in economic effectiveness and the accomplishment of both the social and the environmental objectives, needs, and consequences. Reaching this compromise is feasible owing to the idea of sustainable agriculture which assumes not only reaping maximum profits from farming but also simultaneous care over the natural environment and the future of both the contemporary and the next generations. The principles of sustainable development also refer to the entire agriculture industry as being strictly bound within the ecosystem that surrounds it. The use of such a system brings measurable benefits to the natural environment, thus contributing to improved quality of air and underground waters, increased soil fertility, reduced emission of greenhouse gases and consumption of energy from renewable resources, and to the increased biodiversity in agroecosystems and in the agricultural landscape [8–13].

Recent years have, however, shown that the climatic changes pose a serious challenge to crop cultivation and, by this means, also to the food production. In the upcoming decades, these two key sectors of the economy will have to deal with increasing environmental stress in many regions around the world [14]. One of the most innovative and promising solutions to these serious challenges is offered by the use of plant biostimulants (PBS), which are defined as: *“any substance or microorganism, in the form in which it is supplied to the user, applied to plants, seeds or the root environment with the intention to stimulate natural processes of plants to benefit their nutrient use efficiency and/or their tolerance to abiotic stress, regardless of its nutrients content, or any combination of such substances and/or microorganisms intended for this use”* [15,16]. Considering the above, the role of biostimulants in the agriculture industry is predicted to successively increase in the future. The market of these preparations has reached the value of 1402.15 million USD in 2014 and is estimated to increase to 2522.02 million USD by the end of 2019, assuming the annual growth rate is close to 12.5% [16]. This increase is attributable to various factors. Primarily, ample scientific research indicates that biostimulants can make crops less sensitive to stress conditions like drought, extreme temperatures, excessive moisture content in the rhizosphere as well as either excessive or insufficient exposure to light. Nevertheless, it needs to be emphasized that maximum effectiveness is accomplished only when these preparations are well adjusted to the agronomic needs of the crop, are used in a specified time and in an optimal dose [17–20]. In addition, extending the knowledge about and understanding the mechanisms of action of biostimulants may help in coping with problems stemming from their varied effectiveness under different soil or climatic conditions. According to du Jardin [21], the appropriate use of PBSs can improve soil quality and plant health, but also points the way for agricultural progress towards sustainable development and agroecology. This is mostly due to the fact that the application of biostimulants in crops cultivation leads to the diminished spread and lower intensity of diseases and pest occurrence as well as improved assimilation of nutrients, which makes a plant more resistant to external factors. Hence, the use of PBSs may lead to the reduced use or complete elimination of synthetic pesticides or fertilizers [22].

Despite the establishment of The European Biostimulant Industry Council [15], the goal of which is to develop legal regulations pertaining to the inventory of biostimulants, their registrations are still based on legal regulations set for fertilizers and plant protection agents [20,21,23,24]. A solution in this case will be the new EU Regulation on fertilizing products that will establish provisions on the introduction of fertilizers, including biostimulants, into the market. Works on this regulation are in progress, but it is already known that biostimulants will be valuable and innovative tools for farmers [22]. In field cultivations, farmers may use either synthetic or natural biostimulants to ensure

effective plant protection against biotic and abiotic factors as they contribute to the improvement of biochemical, morphological, and physiological processes in plants [25–30]. The composition of synthetic PBSs includes, most of all, plant growth regulators, polyphenolic compounds or plant stimulants such as inorganic salts or essential elements. Synthetic PBSs include, among others, preparations with commercial names Atonik and Tytanit. Atonik is also referred to as Asahi SL or Chapperone. Its composition includes three phenolic compounds: sodium para-nitrophenolate (0.3%), sodium ortho-nitrophenolate (0.2%), and sodium 5-nitroguaiacolate (0.1%), dissolved in water. However, the results obtained by many researchers showed that nitrophenolates have significant potential as protective agents, growth regulators and biostimulants, and further study is warranted. Presently, there is no evidence suggesting that nitrophenolates are toxic to pollinators, mammals, or humans and plants [31,32] and soil and water are residue-free shortly after application [33,34]. In turn, Tytanit contains 0.85% of a titanium complex, 5% of MgO, and 10% of SO<sub>3</sub>. Its formula was developed and implemented into the agricultural practice in countries of the Central and Eastern Europe as an agronomic tool for the improvement of crop productivity through stimulating the activity of certain enzymes (peroxidase, catalase), increasing chlorophyll content, stimulating photosynthesis, promoting nutrient uptake, increasing tolerance to stress, and improving crop yield quality [35].

In terms of cultivation area, common bean is ranked second in the world among the leguminous plants. India accounts for over 1/3 of its global acreage, whereas Brazil and Guatemala for ca. 20%. In Europe, it is cultivated mainly in Belarus, Romania, Portugal [36]. As a legume species, common bean provides significant amounts of nitrogen to the soil. The nitrogen is one of the key nutrients that determines the development and health of crops, whereas cost-effective practices of its management are indispensable for efficient production. For this reason, common bean is often cultivated as an inter-crop or included into crop rotation systems in biofarming [37]. Even though the yield potential of common bean is high, its seed yield is the lowest among all legumes [38]. Its lower seed yield is mainly due to its sensitivity to increasingly often occurring stress factors (drought, frosts, salinity, environment pollution with heavy metals, activity of pests and pathogens). In such cases, it seems justified to follow sustainable agrotechnical technology based on various types of biostimulants.

However, the supervision over economic outcomes of implemented technologies is necessary in every production process. The ultimate goal is to achieve some short-term or long-term economic effects not only in the production activity per se but also in scientific research. The implementation and dissemination of the cutting-edge technologies in the agricultural production must also be based on estimations of their economic effectiveness. These calculations aim to offer every farmer the possibility of choosing optimal technological variants, ensuring high profits per area unit, high yield quality, and low expenditures [39]. In common bean cultivation, a comparative analysis of the effectiveness of using various biostimulants and methods of their application should underlie the choice of an appropriate cropping system for this plant. Available literature provides sparse works into the effects of biostimulant administration methods on the profitability and effectiveness of cultivation.

This study was undertaken in order to verify a hypothesis that the implementation of a sustainable agricultural technology, based on the use of synthetic biostimulants, will allow not only increasing crop yield and its quality but also improving the cost-effectiveness of common bean cultivation. Hence, this study aimed to determine the biostimulating activity of two preparations, under real field conditions, and then to evaluate crop quality. Analyses were carried out to establish the morphological and chemical response as well as selected metabolic parameters of Mexican Black common bean plants. In this manuscript, we also evaluate economic effects resulting from biostimulant application in bean cultivation because the appropriate effectiveness and profitability coupled with reduced pesticide use will support both environmental and economic sustainability.

## 2. Materials and Methods

### 2.1. Plant Material and Growth Conditions

The experimental material originated from a field experiment conducted in the years 2016–2018 in Perespa village (50°66'N; 23°63'E, Poland) with common bean (*Phaseolus vulgaris* L.) of Mexican Black cultivar. The experiment was designed and performed in a random block system in 4 replications, on experimental plots with areas of 10 m<sup>2</sup>. Bean was cultivated on the soil belonging to the Gleyic Phaeozems, which was characterized by an alkaline pH (pH in 1M KCl: 7.4–7.5). Contents of available nutrients in the soil were at medium levels: P (12.6–14.2 mg P<sub>2</sub>O<sub>5</sub> in 100 g of soil), K (15.3–17.1 mg K<sub>2</sub>O in 100 g of soil), Mg (6.2–6.8 mg Mg in 100 g of soil), and N (8.1–9.3 mg N–NO<sub>3</sub> + N–NH<sub>4</sub> in 100 g of soil). In each experimental year, winter wheat was used as the previous crop. Bean seeds were sown on the 2 May, 2016, 2017, and 2018, with 3.5 cm gaps in rows with 45 cm spacing. Weeds were eradicated mechanically and manually. In the growing season, the plants were treated with Atonik and Tytanit biostimulants (composition provided in Table 1) according to the scheme presented in Table 2. Plants sprayed with water (being a solvent for biostimulants) served as the control sample.

**Table 1.** Composition of the tested biostimulants.

Biostimulant	Formulation
Atonik	sodium p-nitrophenolate NaC <sub>6</sub> H <sub>4</sub> NO <sub>3</sub> (3.75g/L), sodium o-nitrophenolate NaC <sub>6</sub> H <sub>4</sub> NO <sub>3</sub> (2.5 g/L), sodium 5-nitroguaiacolate NaC <sub>7</sub> H <sub>6</sub> NO <sub>4</sub> (1.25g/L); dissolved in water
Tytanit	Ti as titanium ascorbate (8.5 g/L); Mg as magnesium sulphate MgSO <sub>4</sub> (40.8 g/L); S as magnesium sulphate MgSO <sub>4</sub> (54.4 g/L)

**Table 2.** Plant developmental stages and dates of biostimulant application.

Biostimulant	Number of Sprays and Plant Developmental Stages (BBCH) in Which the Biostimulants Were Applied	Concentration	Volume of Working Solution/Working Pressure
Atonik	Single spraying: BBCH 13–15 (LSS)	0.1%	300 L·ha <sup>-1</sup> /0.30 MPa
	Single spraying: BBCH 13–15 (HSS)	0.2%	
	Double spraying: BBCH 13–15, BBCH 61 (LDS)	0.1%	
	Double spraying: BBCH 13–15, BBCH 61 (HDS)	0.2%	
Tytanit	Single spraying: BBCH 13–15 (LSS)	0.07%	300 L·ha <sup>-1</sup> /0.30 MPa
	Single spraying: BBCH 13–15 (HSS)	0.13%	
	Double spraying: BBCH 13–15, BBCH 61 (LDS)	0.07%	
	Double spraying: BBCH 13–15, BBCH 61 (HDS)	0.13%	

BBCH—Biologische Bundesanstalt, Bundessortenamt and Chemical industry; BBCH 13–15—3 leaves unfolded. BBCH 61—beginning of flowering; approximately 10% of flowers open. LSS, lower concentration single spraying; HSS, higher concentration single spraying; LDS, lower concentration double spraying; HDS, higher concentration double spraying.

Biostimulants were used when the foliar application of microelements is recommended. Their doses were established based on recommendations for other crops due to a lack of producer's information regarding the recommended terms of application and concentrations of working solutions in common bean cultivation. The terms of PBS application were established also based on the authors' experience from previous investigations [39].

Average temperatures and rainfall in three growing seasons of bean are presented in Table 3.

**Table 3.** Temperature (T) and rainfall in growing seasons 2016–2018.

Month	Year						Average from 2002 to 2015	
	2016		2017		2018		T (°C)	Rainfall (mm)
	T (°C) Average (min/max)	Rainfall (mm)	T (°C) Average (min/max)	Rainfall (mm)	T (°C) Average (min/max)	Rainfall (mm)		
IV	9.2 (−1.2/22.6)	68.4	7.7 (−1.6/23.3)	37.2	11.5 (−1.0/23.1)	29.6	8.6	41.9
V	13.8 (2.6/26.7)	61.3	13.7 (−1.4/26.9)	100.0	14.2 (1.9/25.8)	54.7	12.6	64.1
VI	18.1 (4.2/31.5)	97.1	18.3 (5.7/30.2)	38.6	18.0(5.2/30.6)	77.1	17.8	68.3
VII	19.5 (8.8/31.2)	107.6	18.5 (5.3/32.9)	61.1	19.1(7.6/32.4)	93.7	18.8	79.4
VIII	18.2 (7.1/30.7)	95.3	19.5 (4.3/34.4)	25.5	19.8 (6.3/31.9)	64.5	19.5	71.5
IX	15.2 (1.6/28.7)	41.2	13.2 (−0.3/27.3)	100.4	15.1 (1.9/26.9)	44.3	14.0	69.6
Average/Total	17.1	470.9	15.2	362.8	16.3	363.9	15.2	394.8

## 2.2. Plant Yielding and Nutritional Value Determination

### 2.2.1. Plant Yielding

Determinations were conducted for: pod number per m<sup>2</sup>, 1000-seed weight, seed number per m<sup>2</sup>, and seed weight.

### 2.2.2. Protein Content

Protein content was determined in bean seed extracts using a Bradford reagent with the method described by Redmile-Gordon et al. [40] with own modifications. A volume of 150 µL of the Bradford reagent was transferred onto a microplate, then 50 µL of either the sample or standard protein (bovine serum albumin (BSA)) was added. Afterwards, the samples were shaken at room temperature for 15 min, and the absorbance was read at a wavelength of 595 nm using an Epoch Microplate Spectrophotometer (BioTek, USA). Protein content was expressed in mg per g of fresh matter (FM).

## 2.3. Nutraceutical Potential

### 2.3.1. Extract Preparation

Ground bean seeds were subjected to the extraction process following the method presented by Świeca et al. [41]. Extraction was performed with a mixture of acetone, water, and hydrochloric acid (70:29:1, v/v/v). The prepared samples were centrifuged for 10 min (6800× g). The process of extraction was conducted in three replications for each analyzed combination. After centrifugation, the supernatant was collected and used for further laboratory analyses.

### 2.3.2. Phenolics Content and Antioxidant Capacity Determination

The content of total phenolic compounds (TPC) was determined according to the method of Singleton and Rossi [42] using Folin–Ciocalteu reagent. In brief, 0.5 mL of water and then 2 mL of the Folin–Ciocalteu reagent (1:5 H<sub>2</sub>O) were added to 0.5 mL of the extract. After 3 min, 10 mL of 10% sodium carbonate was added to the mixture. After 30 min, the absorbance of the samples was measured at a wavelength of 724 nm using a UV–VIS spectrophotometer. Total phenolic compounds content was expressed in mg of gallic acid equivalents (GAE) per g of dry matter (DM).

The total flavonoid content (TFC) was determined acc. to the method proposed by Lamaison and Carnet [43]. Seed extract was mixed with a 2% methanolic solution of AlCl<sub>3</sub> × 6H<sub>2</sub>O (1:1, v/v).

After 10 min of incubation at room temperature, the absorbance of the solutions was measured at a wavelength of 430 nm using a UV–VIS spectrophotometer. The content of flavonoids was expressed in mg of quercetin equivalents (QE) per g of dry matter (DM).

The content of anthocyanins (TAC) in bean seeds was determined acc. to the method described by Fuleki and Francis [44]. Determinations were carried out using solutions of potassium chloride and sodium acetate at two pH values, i.e., 1.0 and 4.5. The solutions were mixed with bean seed extracted in a ratio of 20:1 (v/v). After 15 min, the absorbance of the samples was measured at wavelengths of 520 nm and 700 nm. After absorbance value correction at various pH values, the content of anthocyanins was computed and expressed in mg of cyanidin 3-glucoside equivalents (Cy3-GE) per g of dry matter (DM).

The reducing power was determined using the method of Pulido et al. [45]. Bean seed extracts were mixed with a phosphate buffer (200 mM, pH 6.6) and a 1% aqueous solution of  $K_3 [Fe (CN)_6]$ , in a ratio of 1:1:1 (v/v/v). After sample incubation at a temperature of 50 °C for 20 min, 0.5 mL of trichloroacetic acid (TCA) was added. Afterwards, the samples were centrifuged (6800× g, 10 min), and the resultant supernatant was mixed with distilled water and a 1% aqueous solution of iron (III) chloride (III) (2.5:2.5:0.5, v/v/v). The absorbance of the samples was measured at a wavelength of 700 nm using a UV–VIS spectrophotometer. The reducing power was expressed in mg of Trolox equivalents per g of dry matter (DM).

Ferric ion reducing ability (FRAP) was determined following the method provided by Jimenez-Alvarez et al. [46] with some modifications. The FRAP mixture was obtained by mixing an acetate buffer (3.6 pH), 2,4,6-tripyridyl-s-triazine (TPTZ—10 mM dissolved in 40 mM hydrochloric acid), and  $FeCl_3 \times 3H_2O$  (10:1:1, v/v/v). A volume of 250  $\mu$ L of the FRAP reagent and 25  $\mu$ L of the sample were transferred to a 96-well microplate, and the mixture was incubated at room temperature for 8 min. Sample absorbance was measured at a wavelength of 593 nm. The calibration curve was plotted based on a Trolox solution with concentrations from 90 to 540  $\mu$ M/mL, prepared in the extraction mixture.

The antioxidant activity was analyzed with the TEAC (Trolox equivalent antioxidant capacity) method following the procedure described by Sancho et al. [47] with own modifications. An earlier prepared  $ABTS^+$  solution (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) was adjusted to that concentration, ensuring the absorbance of  $0.7 \pm 0.02$  at 734 nm. Then, 20  $\mu$ L of the sample, acetone mixture (blank sample) or Trolox (calibration solutions) was transferred to a 96-well microplate and mixed with 280  $\mu$ L of  $ABTS^+$ . The absorbance was measured after 6 min, at a wavelength of 734 nm, at room temperature.

Proline content was determined acc. to the method of Carillo and Gibon [48]. Bean seed extract was mixed with a reaction mixture (1% solution of ninhydrin in 60% acetic acid and 20% ethyl alcohol) and incubated at 95 °C for 20 min. Absorbance was measured at a wavelength of 520, and total proline content was expressed in  $\mu$ M per mL.

Ground bean seeds were determined for content of neutral-detergent fiber (NDF), acid-detergent fiber (ADF), and lignin (ADL), in three replications acc. to the method described by Van-Soest et al. [49]. Filtration bags and Ankom apparatus (Ankom 220, USA) were used. NDF fraction content was assayed using the solution of a neutral detergent (sodium-lauryl sulfate, ethylenediaminetetraacetic salt, sodium borate, disodium phosphate, triethylene glycol), alpha-amylase (17,400 liquid units/mL, FAA Ankom Technology), and sodium sulfite (FSS Ankom Technology). ADF fraction content was determined using the solution of an acid detergent (trimethylammonium bromide, normalized sulfuric (VI) acid). Lignin content in bean seed samples was determined using the solution of sulfuric (VI) acid (VI) (Ankom Technology, FSA 72). The content of hemicellulose was computed from the difference between contents of the NDF and ADF fractions. In turn, cellulose content in been seed samples was computed from the difference between contents of ADF fraction and lignin (ADL) [49].

#### 2.4. Economic Analysis

The economic effect of biostimulant application was computed based on the value of yield increase resulting from the use of biostimulants and costs of their application [50].

Income growth resulting from the use of biostimulants ( $O_{sb}$ ) was calculated from the following formula:

$$O_{sb} = W_{pp} - K_{sb}, \text{ (EUR}\cdot\text{ha}^{-1}\text{)} \quad (1)$$

where:

$W_{pp}$ —value of yield increase, (EUR·ha<sup>-1</sup>),

$K_{sb}$ —costs of biostimulant use, (EUR·ha<sup>-1</sup>),

The value of yield increase ( $W_{pp}$ ) was computed acc. to the following formula:

$$W_{pp} = (P_{nb} - P_{nk}) \cdot C_n, \text{ (EUR}\cdot\text{ha}^{-1}\text{)} \quad (2)$$

where:

$P_{nb}$ —seed yield from the combination with biostimulant application, (t·ha<sup>-1</sup>),

$P_{nk}$ —seed yield from the control combination, (t·ha<sup>-1</sup>),

$C_n$ —average price of seeds in a given study year, (EUR·t<sup>-1</sup>).

Costs of the use of biostimulants ( $K_{sb}$ ) were computed acc. to the following formula:

$$K_{sb} = k_b + k_w + k_z, \text{ (EUR}\cdot\text{ha}^{-1}\text{)} \quad (3)$$

where:

$k_b$ —cost of biostimulant purchase, (EUR·ha<sup>-1</sup>),

$k_w$ —cost of water used for the treatment, (EUR·ha<sup>-1</sup>),

$k_z$ —cost of performing the treatment, (EUR·ha<sup>-1</sup>).

The average purchase price was established based on information from wholesale markets (Table 4). The cost of purchasing biostimulants was calculated as a mean price from 3 wholesale companies supplying farms. Water use cost was calculated based on the price of 1 m<sup>3</sup> of tap water in a village community in the Lubelskie Province. The cost of treatment performance was calculated as a mean price of the spraying service with a 1000-L tractor-mounted sprayer.

**Table 4.** Data used to compute income growth resulting from the application of biostimulants.

Specification	Unit	Value
Price of bean seeds	EUR·t <sup>-1</sup>	934.28
Price biostimulant Atonik	EUR·l <sup>-1</sup>	29.87
Price biostimulant Tytanit	EUR·l <sup>-1</sup>	12.85
Price water	EUR·m <sup>-3</sup>	1.87
Price of the application	EUR·ha <sup>-1</sup>	14.02

#### 2.5. Statistical Analysis

The materials for analyses originated from three growing seasons (2016–2018). All analyses were performed in three replications. The Shapiro-Wilk test was used to evaluate the normal distribution of data. The results were analyzed using the one-way analysis of variance. The significance of differences between mean values was estimated based on Tukey confidence intervals (significance level of  $p < 0.05$ ). The statistical analysis was performed using Statistica 13.3 software (StatSoft, Inc.).

### 3. Results

#### 3.1. Effect of Biostimulants on Biometric Traits

The use of Atonik and Tytanit biostimulants in the cultivation of the common bean of Mexican Black cultivar modified the biometric traits and yield of seed in particular years of the study. In 2016, the double application of Atonik preparation in the lower concentration increased 1000-seed weight compared to the control and to other variants of application. In 2017 and 2018, the single application of Atonik in the lower concentration decreased 1000-seed weight (Table 5). In the other combinations, no significant changes were noted in the value of this trait compared to the control sample. In turn, the application of Tytanit preparation in particular study years caused significant differences in 1000-seed weight by either increasing or decreasing it (compared to the control) in particular variants of biostimulant application. The application of both Atonik and Tytanit contributed to a significant increase in bean seed yield compared to the control. In the case of Atonik, the most beneficial effect was achieved upon double treatment of plants with this biostimulant in its higher concentration. Whereas in the case of Tytanit, the most desirable effect was achieved after its single application in the higher concentration. The increase in bean seed yield was due to the positive response of plants to treatments with both biostimulants that was manifested by an increased pod number and seed number compared to the control combination.

**Table 5.** Effect of biostimulant treatment on the biometric traits of bean.

Parameters	Biostimulant Treatment	Biostimulant							
		Atonik				Tytanit			
		Season			Average	Season			Average
		2016	2017	2018		2016	2017	2018	
1000 seed weight (g 1000 <sup>-1</sup> )	C	177.1a	152.8bc	151.7b	160.5a	177.1c	152.8b	151.7b	160.5a
	LSS	174.7a	132.4a	132.8a	146.6a	171.4b	160.3cd	161.1bc	164.3a
	HSS	174.2a	156.8c	155.1b	162.0a	155.1a	164.7d	164.0c	161.3a
	LDS	193.9b	155.6c	156.4b	168.6a	183.3d	138.8a	137.4a	153.2a
	HDS	176.4a	148.6b	148.7b	157.9a	169.5b	157.8bc	155.7bc	161.0a
Seed yield (g m <sup>-2</sup> )	C	241.8a	278.4a	286.8a	269.0a	241.8a	278.4a	286.8a	269.0a
	LSS	260.6b	360.0b	372.1b	330.9ab	304.2e	404.5bc	399.4b	369.4a
	HSS	299.6c	364.6b	378.8b	347.7ab	250.8b	436.3c	437.9c	375.0a
	LDS	358.5d	358.9b	378.9b	365.4ab	283.7d	376.4b	378.2b	346.1a
	HDS	354.7d	393.1b	399.2b	382.3b	272.1c	362.9b	385.8b	340.3a
Number of pods (per m <sup>-2</sup> )	C	350a	421a	428a	400a	350a	421a	428a	400a
	LSS	598b	605c	588d	597b	471b	606c	598d	558bc
	HSS	613bc	530b	538c	560b	527c	497b	512b	512ab
	LDS	627cd	473a	486b	529b	535c	584c	575c	565bc
	HDS	644d	627c	623e	631b	670d	617c	618d	635c
Number of seeds (per m <sup>-2</sup> )	C	1366a	1822a	1890a	1693a	1366a	1822a	1890a	1693a
	LSS	1492b	2719c	2850d	2354a	1775d	2522c	2480b	2259a
	HSS	1720c	2325b	2400b	2148a	1617c	2648c	2670c	2312a
	LDS	1849d	2305b	2421b	2192a	1548b	2711c	2753d	2337a
	HDS	2012e	2644c	2685c	2447a	1605c	2299b	2478b	2127a

Abbreviations: C, control; LSS, lower concentration single spraying; LDS, lower concentration double spraying; HSS higher concentration single spraying; HDS, higher concentration double spraying. Means in the columns, concerning the selected traits, followed by different small letters are significantly different at  $p < 0.05$ .

#### 3.2. Effect of Biostimulant Treatment on Nutraceutical Quality

The use of biostimulants in bean cultivation caused changes also in the protein content of bean seeds. Tytanit preparation increased protein content compared to the control variant, irrespective of the number of treatments and biostimulant concentration (Table 6). Plant treatment with this biostimulant in its higher concentration resulted in a higher protein content compared to its lower concentration. An increase in protein content of the seeds was also observed in plants after foliar application of Atonik

preparation. The greatest increase in protein content, compared to the control, was determined after double application of this preparation in its higher concentration. Also, the growing season had a significant effect on protein content of bean seeds, which was the lowest in 2017.

**Table 6.** Effect of biostimulant treatment on nutraceutical quality.

Parameters	Biostimulant Treatment	Biostimulant							
		Atonik				Tytanit			
		Season			Average	Season			Average
2016	2017	2018	2016	2017		2018			
Protein (mg g <sup>-1</sup> FM)	C	4970.7a	5181.7ab	5050.5a	5067.6a	4970.7a	5181.7a	4968.2a	5040.2a
	LSS	5723.0c	4782.1a	5759.5c	5421.5a	5860.0b	4701.1a	5824.2c	5461.8a
	HSS	5216.3ab	5588.7b	5299.1ab	5368.0a	5872.8b	4839.2a	5795.5c	5502.5a
	LDS	5540.2bc	4600.6a	5573.2bc	5238.0a	5506.1b	4797.4a	5489.2b	5264.2a
	HDS	5711.3bc	5186.5ab	5664.7c	5520.8a	5964.5b	4869.7a	5919.7c	5584.6a
FRAP (μM trolox/mL)	C	945.7a	5181.7a	959.2a	2362.2a	945.7ab	1148.7b	918.6ab	1004.3ab
	LSS	1020.7a	4782.1a	1042.9ab	2281.9a	1084.0bc	983.4a	1071.4cd	1046.3ab
	HSS	1076.6a	5588.7a	1079.9ab	2581.7a	1019.5bc	1110.0ab	989.9bc	1039.8ab
	LDS	1003.8a	4600.6a	1038.7ab	2214.4a	792.6a	985.9a	793.5a	857.3a
	HDS	1131.0a	5186.5a	1135.4b	2484.3a	1204.6c	1074.7ab	1164.7d	1148.0b
ABTS (μM trolox/mL)	C	946.1a	1003.1ab	984.3ab	977.8a	946.1a	1003.1a	955.4a	968.2a
	LSS	873.3a	1543.4ab	923.9a	1113.5a	1184.9ab	1391.1ab	1191.6bc	1255.9ab
	HSS	1129.8ab	1206.8ab	1121.2bc	1152.6a	1230.5ab	1567.5b	1170.4b	1322.8b
	LDS	1147.9ab	1659.0b	1138.7bc	1315.2a	1130.4ab	1262.9ab	1118.9ab	1170.7ab
	HDS	1283.5b	959.5a	1251.9c	1165.0a	1366.8b	1310.2ab	1345.7c	1340.9b
Prolina (μM mL <sup>-1</sup> )	C	5.912a	4.982ab	5.651a	5.515a	5.912a	4.982a	5.465a	5.453a
	LSS	4.672a	5.418b	4.580a	4.890a	4.758a	4.140a	4.737a	4.545a
	HSS	5.026a	4.604ab	4.594a	4.741a	4.969a	5.286a	4.765a	5.007a
	LDS	5.616a	4.255a	5.179a	5.017a	4.978a	5.543a	4.758a	5.093a
	HDS	5.318a	4.708ab	5.190a	5.072a	5.187a	5.552a	4.618a	5.119a

Abbreviations: C, control; LSS, lower concentration single spraying; LDS, lower concentration double spraying; HSS higher concentration single spraying; HDS, higher concentration double spraying. Means in the columns, concerning the selected traits, followed by different small letters are significantly different at  $p < 0.05$ .

The evaluation of the effects of the number of doses and concentrations of the biostimulants tested on the antioxidant activity of bean seeds was based on determinations of their capability to scavenge an ABTS<sup>+</sup> cation-radical and their capability to reduce a TPTZ-Fe (III) complex to a TPTZ-Fe (II) complex.

When considering the antioxidant activity of bean seeds in particular combinations in terms of their ABTS<sup>+</sup> radical scavenging capability, the use of Tytanit and Atonik preparations in the growing season contributed to the enhancement of this capability, irrespective of the number of treatments and solution concentration (Table 6). A statistically significant increase in the radical scavenging capability was determined in the combination in which plants were double sprayed with the higher concentration of Tytanit.

A significant increase in the antioxidant activity of bean seeds was also obtained after plant treatment with Atonik biostimulant in the case of its double application in the lower concentration. Considering growing seasons, the highest antioxidant activity of bean seeds was determined in 2017, compared to the other study years.

The ferric ion reducing capability of bean seeds was found to depend on the type of biostimulant, and on the number of treatments and solution concentration. Statistically significant differences were observed in the case of the higher and the lower concentration of Tytanit preparation after its double application. Double plant spraying with its solution in the concentration of 0.07% decreased the FRAP value by ca. 14.64% compared to the control. In turn, double plant treatment with Tytanit in its higher concentration contributed to FRAP value increase by 14.31% on average, compared to the control.

The use of Atonik biostimulant in higher concentrations contributed to FRAP values decrease compared to the control variant. The greatest decrease in FRAP value was noted after double plant

spraying with this biostimulant. However, the higher concentrations of Atonik solution increased FRAP values compared to the control.

### 3.3. Effect of Biostimulant Treatment on the Antioxidant Potential

A complex evaluation of the effects the tested biostimulants had on the antioxidant activity of bean seeds assayed with ABTS and FRAP tests shows that higher values of the activity were achieved after plant treatment with Atonik than with Tytanit.

No statistically significant differences were determined in proline content, irrespective of the number of doses and concentrations of the analyzed biostimulants. A significant difference was, however, noted in particular study years, which suggests that proline content of bean seeds is determined by weather conditions in a given growing season.

Despite a lack of significant differences in proline content of bean seeds, its decrease was noted compared to the control in all combinations studied. The greatest decrease in the content of this amino acid occurred after single plant spraying with Tytanit in its lower concentration (0.07%).

The content of anthocyanins in bean seeds after plant treatment with Atonik biostimulant was the same as in the control samples, except for the combination with double plant spraying with the lower concentration of its solution, in which anthocyanins content decreased compared to the control. In the case of Tytanit, decrease was observed in anthocyanins content in bean seeds after double plant treatment with its solution in the higher concentration (Table 7). In the other combinations, the content of these compounds increased compared to the control samples, but the differences were statistically insignificant.

**Table 7.** Effect of biostimulant treatment on the antioxidant potential in common bean seeds.

Parameters	Biostimulant Treatment	Biostimulant							
		Atonik				Tytanit			
		Season		Average	Season		Average		
2016	2017	2018	2016		2017	2018			
Anthocyanins (mg g <sup>-1</sup> DM)	C	0.010b	0.007b	0.009a	0.009a	0.010b	0.007b	0.011b	0.009ab
	LSS	0.009ab	0.009c	0.009a	0.009a	0.010b	0.009c	0.010b	0.010b
	HSS	0.010b	0.007b	0.009a	0.009a	0.013c	0.006b	0.014c	0.011b
	LDS	0.008a	0.004a	0.007a	0.006a	0.014c	0.015d	0.014c	0.014b
	HDS	0.009ab	0.009c	0.009a	0.009a	0.004a	0.003a	0.003a	0.003a
Total flavonoids (mg g <sup>-1</sup> DM)	C	1166.3b	1094.7b	1134.9b	1132.0a	1166.3c	1094.7a	1210.0c	1157.0a
	LSS	1007.1a	1161.4c	970.8a	1046.4a	787.6b	1569.5e	822.7b	1059.9a
	HSS	1305.1d	898.0a	1258.2c	1153.8a	1476.1e	1449.6d	1503.5e	1476.4a
	LDS	1190.9c	1191.1d	1138.8b	1173.6a	682.3a	1316.8c	651.2a	883.4a
	HDS	1778.9e	1110.5b	1724.8d	1538.1a	1302.5d	1253.7b	1329.1d	1295.1a
Reducing power (mg TE g <sup>-1</sup> DM)	C	2.459b	2.967b	2.302b	2.576a	2.459b	2.967c	2.638b	2.688a
	LSS	0.043a	2.794a	0.166a	1.001a	3.182c	2.694c	3.407c	3.094a
	HSS	2.643c	4.357d	2.466b	3.155a	3.223c	0.139a	3.450c	2.271a
	LDS	2.684c	2.776a	2.421b	2.627a	2.178a	2.723d	1.930a	2.277a
	HDS	3.172d	3.852c	2.933c	3.319a	2.162a	2.474b	1.999a	2.212a
Total phenols (mg g <sup>-1</sup> DM)	C	23.555b	26.252b	22.082b	23.963a	23.555a	26.252a	25.083a	24.963a
	LSS	22.460a	29.000d	20.983a	24.148a	28.846b	31.199b	30.096c	30.047a
	HSS	28.011d	28.631c	26.129d	27.590ab	28.898b	43.981e	30.185c	34.355a
	LDS	24.391c	23.103a	22.930c	23.475a	28.871b	42.812d	27.578b	33.087a
	HDS	38.258e	29.154d	36.847e	34.753b	28.809b	36.912c	27.310b	31.010a

Abbreviations: C, control; LSS, lower concentration single spraying; LDS, lower concentration double spraying; HSS higher concentration single spraying; HDS, higher concentration double spraying. Means in the columns, concerning the selected traits, followed by different small letters are significantly different at  $p < 0.05$ .

Atmospheric conditions occurring in the growing season of 2017 resulted in a lower content of anthocyanins in bean seeds compared to the other study years. The complex analysis of biostimulant

use in common bean cultivation in terms of anthocyanins content shows that Tytanit preparation had a more beneficial effect on their content in bean seeds.

The content of flavonoids in bean seeds depended on both the number of treatments and concentration of the biostimulants. The double application of Tytanit in its lower concentration resulted in the lowest flavonoid content in bean seeds. However, single plant spraying with this biostimulant in the same concentration caused the highest content of these compounds in bean seeds. The use of Atonik biostimulant in the form of single plant spraying with its lower concentration caused an insignificant decrease in flavonoids content of the seeds (Table 7). In contrast, a significant increase in their content compared to the control was determined after double plant spraying with Atonik in the higher concentration (0.2%). Flavonoids content of bean seeds was also significantly determined by the growing season of the field experiment.

The content of polyphenolic compounds in bean seeds differed in particular study years. Plant treatment with Tytanit increased polyphenol content compared to the control, regardless of the number of treatments and solution concentration. In the case of Atonik biostimulant, only double plant treatment with its solution in the lower concentration caused an insignificant decrease in the content of these compounds compared to the control material. However, the same number of treatments but with the solution in the higher concentration allowed achieving the highest content of polyphenols in bean seeds compared to the control, and the differences observed were statistically significant.

The complex analysis of biostimulants effect on polyphenol content demonstrated Tytanit preparation to be more effective in increasing polyphenol content in bean seeds.

The antioxidant potential of bean seeds was also analyzed based on the reducing power (RP). Its values were determined not only by the type, number of treatments, and concentration of the biostimulant, but also by meteorological conditions in a given growing season.

The use of the biostimulant, containing mainly phenolic compounds, in its higher concentration resulted in RP value increase compared to the control samples. However, single plant spraying with Atonik in the concentration of 0.1% caused a 2-fold decrease in RP value. The double application of this biostimulant in its lower concentration had a negligible effect on reducing power of the seeds, and its mean values achieved were similar to those determined in the control samples.

The foliar application of Tytanit also caused differences in the reducing power of bean seeds. An increase in RP value was observed only after single plant treatment with this biostimulant in its lower concentration. In the other combinations, RP values decreased slightly compared to the control and differences noted were statistically insignificant.

#### *3.4. Effect of Biostimulant Treatment on Fiber Content*

The use of Tytanit biostimulant in common bean cultivation caused a decrease in the content of neutral-detergent fiber (NDF) in seeds compared to the control samples (Table 8). None of the combinations tested caused NDF content to approach that determined in the untreated plants. The greatest, over 2-fold, decrease in NDF content occurred after single plant spraying with this biostimulant in its lower concentration.

Plant treatment with Atonik preparation also resulted in a decreased content of the NDF fraction in seeds compared to the control samples, except for the single spraying with the higher concentration after which NDF content increased in seeds by ca. 17.57%.

Contents of acid-detergent fiber (ADF) varied depending on the type of biostimulant, number of treatments, and solution concentration. Meteorological conditions occurring in that growing season also determined ADF content in bean seeds.

Plant treatment with Tytanit preparation increased ADF content only in one of the analyzed combinations, i.e., after double plant spraying with this biostimulant in its higher concentration. ADF contents determined in the other combinations were similar to these found in the control samples.

The greatest, nearly 1.5-fold, decrease in the content of acid-detergent fiber fraction was determined after double plant spraying with Atonik in its higher concentration. In turn, the plants responded with

the greatest increase (by 21.98% on average compared to the control) of the ADF fraction content after single application of this biostimulant in its lowest concentration.

Lignin content analysis showed that double plant spraying with Atonik in the lower concentration (0.1%) caused a nearly 2.5-fold increase in its level in bean seeds compared to the control sample (Table 8). An opposite observation was made after double plant spraying with this biostimulant in its higher concentration, which resulted in almost 2-fold decrease in ADL fraction content.

**Table 8.** Effect of biostimulant treatment on fiber content in common bean seeds.

Parameters	Biostimulant Treatment	Biostimulant							
		Atonik				Tytanit			
		Season		Average	Season		Average		
2016	2017	2018	2016		2017	2018			
NDF (% DM)	C	10.919a	26.601e	9.110a	15.543a	10.919d	24.583c	12.539c	16.014a
	LSS	12.606c	14.521b	11.337c	12.821a	8.405a	11.494a	6.811a	8.903a
	HSS	22.628e	11.341a	20.852e	18.274a	9.339b	17.147b	10.910b	12.465a
	LDS	14.328d	16.447c	12.632d	14.469a	12.694e	16.631b	10.896b	13.407a
	HDS	11.558b	17.699d	10.123b	13.127a	10.176c	9.454a	11.706bc	10.445a
ADF (% DM)	C	8.651b	8.702c	7.842b	8.398a	8.651b	8.702b	7.769b	8.374a
	LSS	9.474c	12.600e	8.659c	10.244a	10.429d	6.062a	9.566c	8.686a
	HSS	9.867c	6.027a	8.890c	8.261a	6.754a	10.048c	7.584b	8.129a
	LDS	9.921c	8.920d	9.063c	9.301a	6.840a	12.730e	5.976a	8.515a
	HDS	7.249a	6.892b	6.403a	6.848a	9.018c	11.423d	9.877c	10.106a
ADL (% DM)	C	1.676b	1.322b	1.514b	1.504a	1.676d	1.322b	1.939c	1.646a
	LSS	1.678b	3.849d	1.481b	2.336a	3.322e	0.591a	3.208d	2.374a
	HSS	2.041c	0.632a	1.885c	1.519a	0.722b	1.537c	0.895b	1.051a
	LDS	4.915d	1.468c	4.690d	3.691a	0.398a	4.279d	0.162a	1.613a
	HDS	0.643a	1.443c	0.487a	0.858a	1.002c	4.614e	0.789b	2.135a
HCEL (% DM)	C	2.267a	17.899e	1.268a	7.145a	2.267c	15.881c	4.770c	7.639a
	LSS	3.132b	1.920a	2.678b	2.577a	0.000a	5.432b	0.000a	1.811a
	HSS	12.761d	5.314b	11.962c	10.012a	2.585d	7.099b	3.326bc	4.337a
	LDS	4.407c	7.527c	3.569b	5.168a	5.855e	3.901ab	4.920c	4.892a
	HDS	4.310c	10.807d	3.720b	6.279a	1.157b	0.000a	1.829b	0.995a
CEL (% DM)	C	6.976b	7.380b	6.328bc	6.895a	6.976c	7.380c	5.829a	6.728a
	LSS	7.796c	8.751c	7.178c	7.908a	7.106c	5.471a	6.358b	6.312a
	HSS	7.825c	5.395a	7.005c	6.742a	6.033a	8.511d	6.689b	7.078a
	LDS	5.006a	7.452b	4.373a	5.610a	6.441b	8.451d	5.813a	6.902a
	HDS	6.605b	5.448a	5.916b	5.990a	8.017d	6.809b	9.088c	7.971a

Abbreviations: C, control; LSS, lower concentration single spraying; LDS, lower concentration double spraying; HSS higher concentration single spraying; HDS, higher concentration double spraying. Means in the columns, concerning the selected traits, followed by different small letters are significantly different at  $p < 0.05$ .

The single foliar application of Tytanit in the lower concentration caused an increase in lignin content compared to the untreated samples. This increase was also noted after double plant treatment with this preparation in its higher concentration. Analyses conducted in the other combinations showed a decrease in ADL fraction content. The differences observed in lignin content of bean seeds after plant treatment with Tytanit biostimulant were statistically insignificant.

The Atonik biostimulant was more effective in increasing the lignin content of bean seeds compared to the control treatment. Lignin content was additionally determined by weather conditions occurring in a given growing season.

The analysis of hemicelluloses (HCEL) in bean seeds after plant treatment with Atonik biostimulant demonstrated their content to increase by 40.13% on average only in the combination in which plants were sprayed with Atonik solution in the higher concentration. An opposite observation was made after the use of Tytanit, where the content of hemicelluloses did not increase compared to the control samples in any of the combinations tested. In all combinations, HCEL content was observed to decrease. The greatest, over 7.5-fold, decrease was determined after double plant treatment with this preparation in its higher concentration.

The foliar application Atonik biostimulant during common bean growing season caused changes in cellulose (CEL) content in its seeds. An increase in cellulose content by 15.19% on average was determined after single application of Atonik in its lower concentration. In the other combinations, CEL content was lower than in the control samples.

The greatest increase in cellulose content of bean seeds was due to the double plant treatment with Tytanit in its higher concentration and reached 18.46% on average compared to the control material. A decrease in the CEL content of bean seeds represented plant response to the single application of this preparation in the lower concentration, i.e., 0.07%.

In 2017, the mean cellulose content determined in bean seeds was higher than in the other study years.

### 3.5. Economic Analysis

A comparative analysis of the economic effects of biostimulant use and modes of application in common bean cultivation should underlie decision about their use. The conducted analyses confirmed the seed yield increase caused by the applied biostimulants to be the factor improving the profitability of bean production for dry seeds. In all combinations tested, the application of the studied biostimulants had a positive effect on bean seed yield and increased the cost-effectiveness of the cultivation. In 2016, the profitability of biostimulant use ranged from 49.51 to 1059.96 EUR·ha<sup>-1</sup> (Figures 1 and 2). The highest profitability was demonstrated in the case of the double foliar application of Atonik preparation in both concentrations tested. An increase in incomes after the use of Tytanit biostimulant was relatively high and the highest after plant treatment with working solutions in the lower concentrations.

After the second year of bean production, the analysis of the effects of biostimulant application on the economic effectiveness of the production process (Figures 1 and 2) demonstrated increased profitability of the use of both preparations. In 2017, the total profitability from using Atonik biostimulant ranged from 721.61 (double plant spraying with the lower dose) to 1010.51 EUR·ha<sup>-1</sup> (twice as high dose). Different economic effects were observed after the application of Tytanit, as the average higher profitability was due to the single application of this preparation in both concentrations tested.

In the third year of cultivation, the highest profitability of biostimulant use was demonstrated after single plant spraying with Tytanit in both concentrations. For Atonik, the greatest economic profits were noted after double plant treatment with its higher concentration.

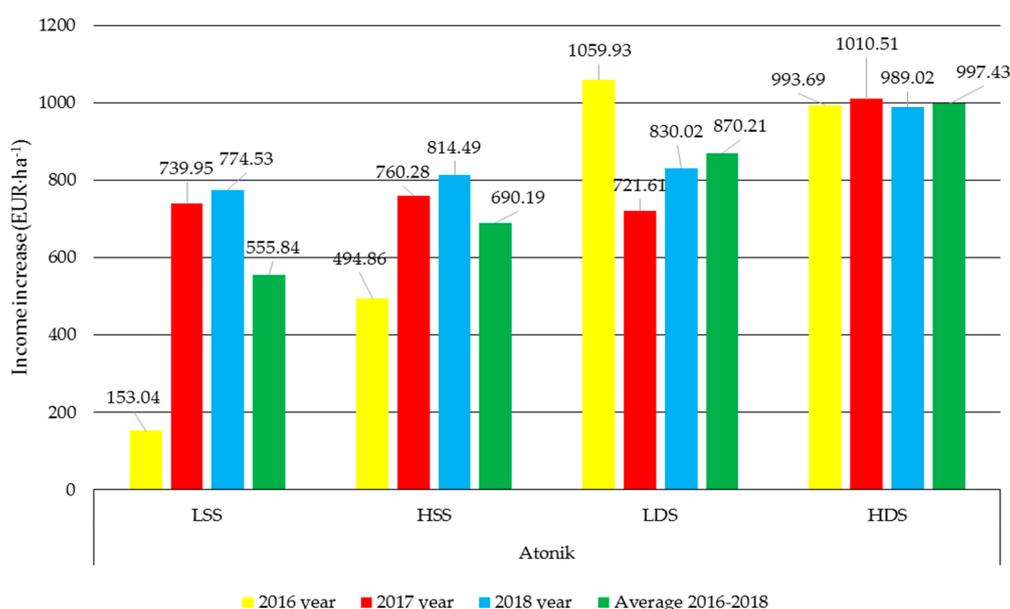
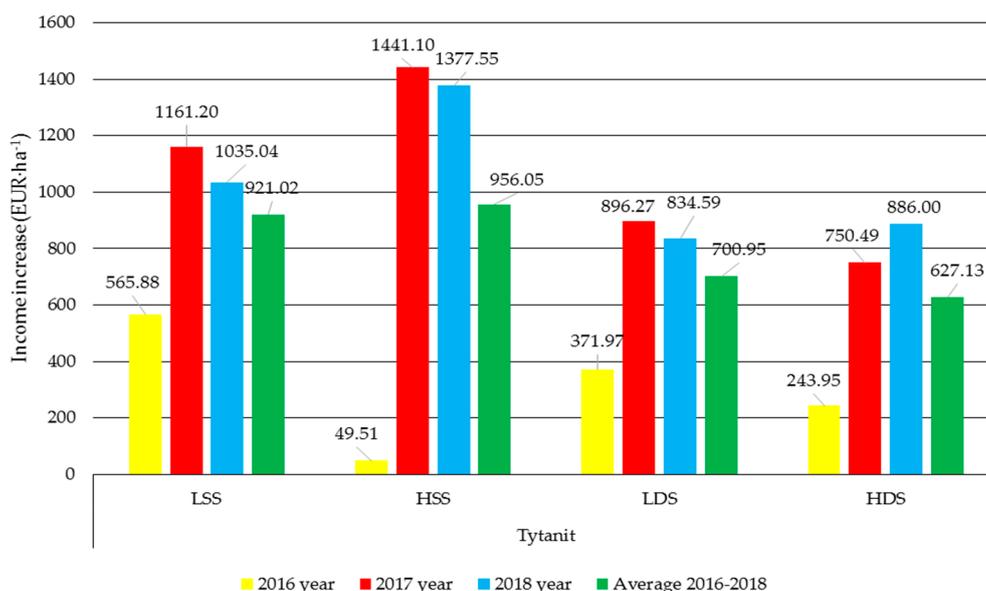


Figure 1. Economic effects of biostimulant Atonik use.



**Figure 2.** Economic effects of biostimulant Tytanit use.

#### 4. Discussion

The results of our study indicate a positive effect of biostimulants tested not only on bean seed yield but also on the yield quality traits. Both protein content and the antioxidant potential of seeds, produced with the use of biostimulants, were significantly increased compared to the control samples. The more beneficial effect on average was due to the application of the biostimulant containing titanium compounds. The use of the Atonik preparation resulted in increased antioxidant capability (FRAP) and reducing power (RP) compared to the control samples and to the second plant growth regulator tested in the study. This could stem from the effect of this biostimulant on the increased inhibition of IAA oxidase which enhances natural synthesis of endogenous auxins in plants [33,51–53]. This is due to Atonik composition, because the phosphorylated form of para-nitrophenolate, being a substrate for phosphatases, determines IAA activity enhancement [54] because it exhibits effects analogous to ATP [55]. According to Przybysz et al. [56], plant treatment with Atonik influences nitrogen metabolism in these plants, which may stimulate the activity of nitrate reductase.

The concentration of antioxidants in plants is extremely important to their protection against oxidative damage induced by reactive oxygen species (ROS). The analysis of the antioxidant capability of bean seeds demonstrated the positive effect of biostimulants tested, which may indicate that they supported the development of the plant antioxidative system, composed of both antioxidant enzymes and antioxidant organic molecules [57,58]. Noteworthy is the fact that seeds of bean treated with biostimulants had a higher content of flavonoids. A high amount of these compounds is accumulated in vacuoles. Flavonoids are commonly believed to be strong antioxidants which effectively scavenge hydrogen peroxide and other ROS [59,60]. It needs to be emphasized, however, that the antioxidant system of plants is a vast and complicated network of metabolites and biomolecules which are capable of both ROS production and elimination. In addition, this dualism of nature of antioxidant biomolecules facilitated the selection and evolution of ROS as signaling molecules. For this reason, the evolution of the antioxidant system allowed ROS to play a double role. Today, owing to the antioxidant system, which had initially been perceived as detrimental, ROS have become important signaling molecules. This, in turn, suggests that they play a meaningful role both in plant growth and development as well as in the fight with environmental stress. The latest conclusions from scientific research indicate, however, that the demonstrated signaling role of ROS will require redefining the notion of ‘oxidative stress’ considering ‘oxidative signaling’ [61].

Plants respond to environmental stress at many levels, including at biochemical, cellular, and molecular levels. This response may be triggered by the activation of stress hormones, production of osmolytes, elimination of reactive oxygen species (ROS) or accumulation of proteins protecting against stress [62,63]. According to Ashraf and Foolad [64], proline is one example of plant osmolytes; its synthesis is stimulated by several stress conditions. Its roles include, most of all, osmotic regulation, stabilization of subcellular structures, scavenging free radicals, buffering the redox potential, and gene induction. Proline content in plants is determined by multiple factors, including plant species and cultivar, plant organs and phenological stage, as well as by the type and intensity of abiotic stress [64–67]. Plants exposed to stress exhibited increased accumulation of proline [68]. Investigations conducted by Hong et al., [69] demonstrated even that the elimination of enzyme feedback inhibition from proline metabolism in the *Arabidopsis* plants led to a significant accumulation of this osmolyte, which in turn ensured increased plant tolerance to the extreme osmotic stress. It needs to be emphasized, however, that—according to many authors—proline accumulation does not always occurs in plants under conditions of abiotic stress. However, a strong correlation is often observed in higher plants between tolerance to stress factors and accumulation of this osmolyte [64,67,69]. Therefore, considering the study results and speculations, a hypothesis has been advanced that plants that superproduce proline will be more resistant to drought stress, which may result in their higher productivity compared to the control plants or in the maintenance of the assumed productivity even under the exposure to the stress factor. However, based on results of his study, Carvalho [70] concluded that proline metabolism is regulated through the elicitor mechanism. According to Djanaguiraman et al., [53,71], Atonik also affects the production of proline and polyols in plants, i.e., two extremely important and compatible metabolites involved in anti-stress mechanisms. The results of our study demonstrated a similar effect of Tytanit biostimulant, because proline content decreased in bean seeds upon its use compared to the untreated samples. Nevertheless, proline content cannot be directly perceived as a specific marker of tolerance to one specific stress condition because its accumulation reflects only the response to general abiotic stress [72]. Szabados and Savouré, [73] demonstrated that the high content of proline allows plants to maintain a low water potential through the additional uptake of water from the environment. However, a study conducted by Patané et al. [74] proved that the genotypes of tomatoes characterized by a higher sensitivity to water shortages in the soil responded to the drought-induced stress with suppressed synthesis of proline in leaves. Therefore, according to Goñi et al. [75], the high accumulation of proline cannot be treated as a general response of crops exposed to stress or to the use of biostimulants. In addition, the study conducted by this author demonstrated that the magnitude of this osmolyte accumulation may depend on biostimulant type, the method of its application or crop class. Nevertheless, scientists do agree that proline plays a key role in plants regeneration after stress [75].

Investigations addressing the effects of the use of biostimulants on contents of dietary fiber fractions are relatively new. Therefore, sparse information may be found in the available literature concerning results of such analyses. The concept of the division and analysis of the fractions of cell walls, neutral-detergent fiber (NDF) and acid-detergent fiber (ADF), has been proposed by Van Soest. He advanced a hypothesis that feedstuffs contain cell wall constituents (CWC) and cell contents (CC); and that the CWS, i.e., NDF and ADF, are factors which reduce their intake, digestibility, and energy value [76]. An unexpected outcome of our study was the finding that the NDF content decreased in almost all analyzed combinations of plant treatment with Tytanit and Atonik biostimulants, compared to the control samples. In the case of the ADF fraction, a similar tendency was observed after plant treatment with Atonik preparation. In turn, the use of the titanium-containing biostimulant resulted in the increased ADF content, especially after the double treatment. However, the differences observed were statistically insignificant. Some scarce study results show that the use of biostimulants can determine the content and technological characteristics of dietary fiber [77]. But according to Wang et al. [78], the process of dietary fiber biosynthesis is extremely complicated and determined not only by the nutritional status of plants but also by the effects of multiple abiotic factors. The stage

of its biosynthesis itself entails the enhanced synthesis of gibberellins which directly determine the micronaire, length or strength of the fiber [78]. In the opinion of Silva et al. [77], the foliar application of biostimulants may lead to the increased accumulation of gibberellins in plants, which in turn triggers changes in the production process of both dietary fiber and its fractions. Apart from ADF and NDF, the main cell wall components include cellulose, hemicelluloses, and lignin [79–81]. Our study demonstrated that their contents in bean seeds originating from the combinations with biostimulants were lower than in the control samples. Only in the case of Tytanit was the cellulose content increased, but differences observed were statistically insignificant. It needs to be emphasized, however, that cellulose is not digested by monogastric animals, but may be degraded, to some extent, in the gastrointestinal tract upon fermentation by intestinal microflora [81,82]. Worthy of notice is also the reduced content of hemicelluloses in bean seeds, because this group of compounds includes polysaccharides exhibiting anti-nutritional effects and are non-homogenous in terms of their chemical structure and physicochemical properties [83]. Some of the compounds exist in an insoluble form in the environment of the gastrointestinal tract of animals, but the majority of these compounds are soluble which is determined, to a large extent, by the presence of L-arabinose which—next to D-glucose, D-galactose and D-glucuronic acid—represents a branch of the main chain of this polysaccharide [81,84,85].

Even though biostimulants offer novel possibilities and although their use in agriculture is in many cases deemed beneficial for improved crop yielding, the exact mechanism of their action still remains unknown, which is indicated by the presented results concerning the contents of dietary fiber in bean seeds [86,87], because the effect of biostimulants on plants is not only a consequence of their direct role in metabolism regulation, but also of their multi-faceted action. The most important, however, is the fact that—unlike hormones—biostimulants improve metabolic processes in plants without modifying their natural pathways [87,88].

The analysis of the average income from cultivation during the three-year experiment demonstrated that in the case of Atonik preparation used in higher concentrations, its double application at appropriate developmental stages of plants contributed to the achievement of the greatest seed yield increase, and thus to the stability of incomes gained by farmers. For this reason, this method of biostimulant application seems to be the most beneficial from an economic perspective. The size and value of crop yields as well as costs incurred on their cultivation determine the profitability of common bean cultivation, but most of all affect incomes [89]. According to Santoso et al. [90], the application of biostimulants will allow farmers to achieve additional incomes from higher crop yields achieved owing to the use of these preparations. These additional incomes will be significantly higher than the costs of the preparations and labor and may exceed even 20 times the expenditures incurred. In the available literature, there are few papers on the impact of the biostimulant application on profitability and crop efficiency. However, the results of economic analysis are reflected in the research of Zarzecka et al. [89] and Mystkowska [91]. These authors stated that the factor determining profitability of potato production was the value of harvested crops, which depended on their quality. Zarzecka et al. [89] showed that the highest economic effect was obtained after the application of the Atonik. Gugala et al. [92] also concluded that the use of such preparations not only favorably stimulates the yield increase, but also determines the quality of seeds, which is connected with a higher selling price. Anderson et al. [93] proved that the use of biostimulants in sweetcorn cultivation led to yield increase. However, financial analysis showed that applying biostimulants to seeds is a very significant cost. Therefore, for greater profitability, these researchers recommended the use of biostimulants using appropriate methods.

## 5. Conclusions

The application of the tested biostimulants induced the beneficial responses of common bean plants that were manifested in terms of size and the quality of seed yield. The results of the present study point to the need for more exhaustive explanations of the basic mechanisms responsible for the positive effects

of these preparations on the analyzed crops, which will represent a highly important issue in future research. However, the use of the tested preparations in order to improve crop productivity seems to be a justified agronomical approach in the context of food safety, sustainable development, and the effective use of expenditures. The increased protection of plants against environmental factors after the use of biostimulants, which results in increased productivity and profitability of crop production, represents a potential form of sustainability support in agricultural farms. The results of our experiment allowed verifying a hypothesis that the implementation of a sustainable agricultural technology based on the use of synthetic preparations was an effective method to increase plant productivity and, consequently, economic profits to farmers. However, it is not only crop yields and economic aspects that should determine the use of specific biostimulants. Nowadays, ecological considerations come to the fore. Due to the fact that synthetic biostimulants, including Atonik, are mixtures of potentially toxic compounds, scientific research should indicate a specific range of concentrations for working solutions that can be used by farmers in practice. This will enable sustainable crop management with minimal impact on the natural environment.

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