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The Agrochemical Effect of a Protein Hydrolyzated Biostimulant Applied in Vegetable Culture

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ABSTRACT

Currently, agriculture is facing increasing challenges related to climate change and legislative shifts regarding the inputs used. The urgent need to enhance sustainable agricultural production has driven the sector towards methods based on natural products, such as biostimulants. The application of biostimulants derived from protein hydrolysates obtained from plant biomass in horticulture brings a series of benefits that result in higher and improved yields. These fertilizing products are compliant with the relevant fertilizing product regulations. A biostimulant product containing soy-based protein hydrolysate was tested under controlled conditions on crops such as tomatoes (Precos variety), eggplants (Belona variety), bell peppers (Bianca variety), and cucumbers (Cornistar variety). The testing involved three foliar applications at a 0.5% concentration during the vegetative phase. The application of the fertilizing product with protein hydrolysate led to yield increases ranging from 28.1% (in eggplants, Belona variety) to 41.6% (in cucumbers, Cornistar variety). The three foliar treatments with the biostimulant product also enhanced the photosynthetic assimilation process by 33.6% to 50.6%. Additionally, the nutrient content (N, P, K) in the plants (leaves) showed a significant increase. Products like protein hydrolysate-based biostimulants are seen as a new, efficient generation of agricultural inputs for sustainable farming, replacing some of the traditional fertilizers used in the fertilization process and effectively correcting nutrient deficiency disorders.

Keywords: biostimulants, protein hydrolysate, foliar application, nutrients, photosynthesis.

INTRODUCTION

In alignment with the European Green Deal, which aspires to achieve climate neutrality and a sustainable framework by 2050, the imperative to establish a resilient and sustainable agricultural system is increasingly apparent. Such an initiative is vital for addressing critical challenges that impact economically viable agricultural production while adhering to ecological and sustainable principles (Tahat et al., 2020).

Addressing these challenges requires the adoption of eco-friendly and innovative agricultural practices that not only foster sustainability and circularity but also enhance the overall resilience and health of the agricultural system. Such practices improve essential physical, biological, and environmental resources, including soil nutrient mineralization, microbial diversity, and groundwater quality. Furthermore, these methods aim to reduce environmental impact, conserve biodiversity, and promote a balanced ecosystem, which are all critical for long-term agricultural productivity and ecological stability (Mäder et al., 2002; Niemiec et al., 2020; Tahat et al., 2020; Mureşan et al., 2021). In this context, a range of agricultural technologies has been proposed to enhance crop yields and improve the qualitative and functional attributes of food products. Biostimulants are among these technologies, used to support plant growth, resilience, and nutritional quality. Their application spans both conventional and organic farming systems, providing a flexible solution to the growing need for sustainable and high-quality food production (Muller et al., 2017; Regulation (EU) 848/2018; Ganugi et al., 2021; Melini et al., 2023; Kalimuthu et al., 2024).

In the context of organic agriculture in Romania, the total cultivated area has more

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than tripled between 2010 and 2021, reaching 578,718 hectares. The most significant crops include pastures and meadows (37.1%), cereals (24%), and industrial crops (20%). However, approximately 90% of the production of organic cereals and oilseeds is exported, highlighting the need for improved infrastructure for local processing. The Common Agricultural Policy (CAP) has played a crucial role in supporting this sector, although periods of stagnation, such as between 2014 and 2016, have been observed (Brumă et al., 2024).

This trend reflects not only the structural changes within Romanian agriculture but also its adaptation to the requirements of the European market. At the same time, it highlights a critical challenge: the over-application of synthetic fertilizers, which can result in nutritional imbalances and adverse ecological effects, such as water eutrophication. Addressing this issue requires a balanced approach to nutrient management, integrating sustainable agricultural practices that align both with environmental protection goals and market demands.

According to European legislation, a “plant biostimulant” refers to a product that stimulates plant nutrition processes independently of the product's nutrient content, with the sole purpose of improving one or more of the following characteristics of the plant or its rhizosphere: (a) nutrient use efficiency; (b) tolerance to abiotic stress; (c) quality traits; (d) availability of limited nutrients in the soil or rhizosphere [Regulation (EU) 2019/1009].

Plant biostimulants are increasingly recognized as a sustainable and promising innovation in agriculture, applicable across various sectors such as horticulture, arboriculture, viticulture, cereal, and industrial crop production. These products offer substantial potential to enhance plant resilience against a range of abiotic stresses, including drought, salinity, and temperature extremes. Moreover, biostimulants contribute to improved nutrient use efficiency, allowing plants to optimize the uptake and utilization of available resources. By enhancing these physiological responses, plant biostimulants support the development of more resilient and resource-efficient agricultural ecosystems, thereby contributing to sustainable agricultural practices and long-term food security (Nephali et al., 2020; Shukla et al., 2021; Johnson et al., 2023). These developments underscore the importance of adopting modern and efficient agricultural practices that support the sustainable growth of the sector. Such practices not only aim to enhance productivity and resource efficiency but also to minimize environmental impact and promote long-term ecological balance. Furthermore, they align with European Union policies and global sustainability goals, emphasizing the need for integrated approaches that combine technological innovation, responsible resource management, and farmer education to ensure resilience and adaptability in the face of contemporary agricultural challenges.

Protein hydrolysates (PHs), defined as “mixtures of polypeptides, oligopeptides, and amino acids obtained through the partial hydrolysis of proteins”, are particularly valuable not only for their effectiveness as biostimulants but also due to their sustainable production from organic waste, fitting seamlessly into the concept of an eco-friendly circular economy. Their application in agriculture has gained significant attention due to their ability to enhance nutrient uptake, improve stress tolerance, and stimulate plant growth and development through bioactive compounds. Moreover, their origin from renewable organic waste sources reduces environmental pollution and promotes resource recycling, aligning with global sustainability goals. As part of a circular economy model, protein hydrolysates exemplify how agricultural by-products can be transformed into high-value inputs, creating a closed-loop system where waste is minimized, and productivity is maximized. This dual functionality - acting as both an effective biostimulant and a sustainable agricultural input - positions protein hydrolysates as a key innovation in modern sustainable farming practices (Schaafsma, 2009; Colla et al., 2015; Xu and Geelen, 2018).

Efficient nutrient management, particularly of nitrogen (N), phosphorus (P), and potassium (K), represents a central pillar for optimizing agricultural production while minimizing negative environmental impacts. This approach aligns with the principles of the circular economy, fostering biodiversity conservation and contributing to the reduction of the carbon footprint. By promoting a balanced and sustainable utilization of these essential nutrients, agricultural systems can achieve greater productivity, resource efficiency, and long-term environmental resilience (Rodino et al., 2024).

The exogenous application of amino acids to various crops has been shown to significantly increase chlorophyll content and photosynthetic rate, leading to enhanced yield performance, particularly under water stress conditions. This effect highlights the crucial role of amino acids in improving plant physiological responses to adverse environmental factors. By facilitating chlorophyll synthesis and optimizing photosynthetic efficiency, amino acids contribute not only to crop resilience but also to sustainable agricultural productivity, especially in regions prone to water scarcity (Terán et al., 2024).

The production of protein hydrolysates (PHs) involves chemical (acidic and alkaline), thermal, or enzymatic hydrolysis of various animal wastes and plant biomass from agro-food industries, with plant-derived PHs originating from residual proteins found in alfalfa, soybean, pea, corn, wheat condensates, distiller solubles, glycoproteins, and algal proteins (Ertani et al., 2012; Calvo et al., 2014; Colla et al., 2014, 2015; du Jardin, 2015; Halpern et al., 2015).

MATERIAL AND METHODS

A variant of a biostimulant fertilizing product with soy protein hydrolysate (HPF) was obtained, characterized physico-chemically, and tested agrochemically. The soy protein hydrolysate was obtained through enzymatic hydrolysis. The experimental variant of the biostimulant fertilizing product with soy protein hydrolysate (HPF) contains: total nitrogen 1.92-2.31%, potassium 0.76-0.92%, phosphorus 0.10-0.26%, organic matter 16.38-18.64% from the soy protein hydrolysate, iron (Fe) 0.03-0.05%, copper (Cu) 0.02-0.03%, zinc (Zn) 0.13-0.22%, manganese (Mn) 0.01-0.02%, boron (B) 0.04-0.09%, sulfur (S) 0.43-0.58%, molybdenum 0.004-0.01%, total amino acids 11.32-12.96%, free amino acids 2.97-3.41%, 0.5% solution pH 5.82-6.14, 0.5% solution conductivity 688-744 mS/cm. The raw materials used to produce these fertilizing products are permitted for use in organic farming in accordance with Regulation (EU) 848/2018. These products also comply with the requirements for fertilizing products set out in Regulation (EU) 2019/1009.

Figure 1 illustrates the profile of total amino acids from the protein hydrolysate obtained through enzymatic hydrolysis of plant biomass (soy). The amino acids were determined using the High-Performance Liquid Chromatography (HPLC) method, providing a detailed quantification and characterization of their composition. It is worth noting that approximately 25% of the total amino acids are present in free form.

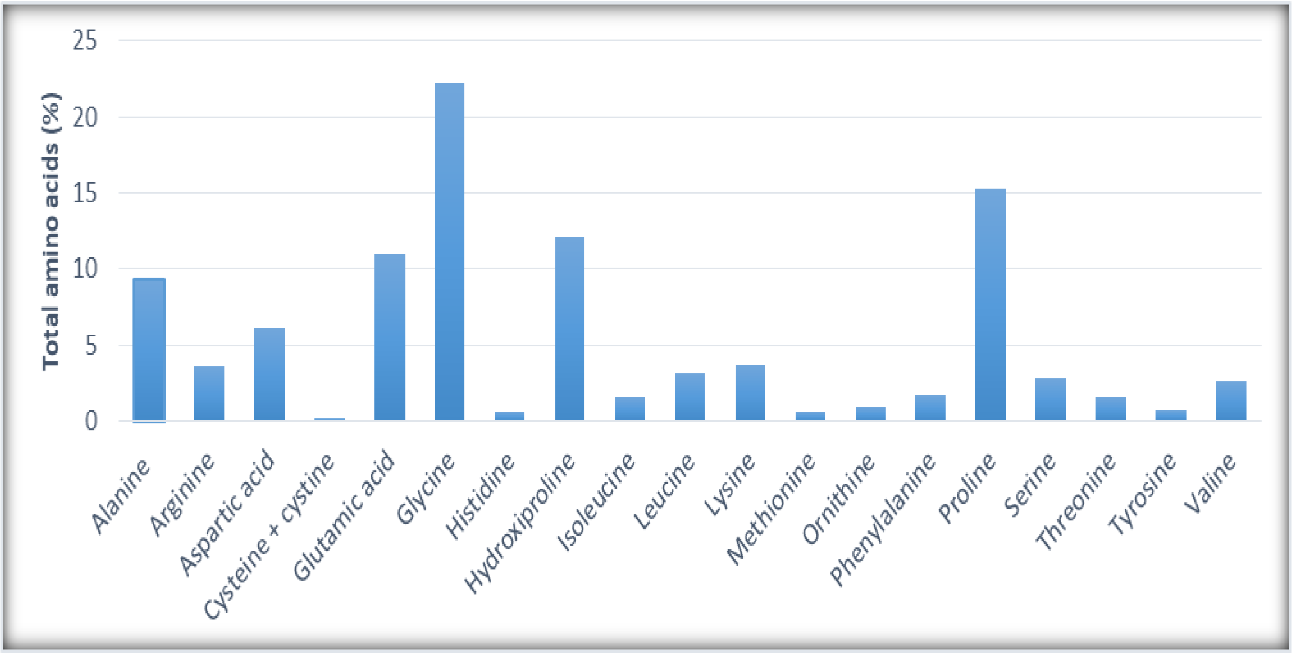

Figure 1. The profile of total amino acids from the soy protein hydrolyzate used to obtain the HPF biostimulant product

The agrochemical efficiency of the biostimulant fertilizing product was determined by applying it (0.5% solution) to four intensively cultivated vegetable crops in Romania, in protected spaces and under drip irrigation conditions. The main physical, chemical, and biological properties of the cherno-cambic hortic anthrosol were as follows: fine clay soil texture (38% and 44% clay); pH - slightly acidic (6.28 pH units) at the surface and neutral (6.85-7.11 pH units) below 60 cm depth; organic matter content 2.94-3.11%; total nitrogen 0.19%-0.16%; mobile phosphorus 48-46 ppm at the surface and 43-41 ppm below 40 cm depth; mobile potassium 220-216 ppm at the surface and 146-135 ppm below 40 cm depth, and base saturation of 83% at the surface and 90% below 40 cm depth.

Statistical analysis

The effects of the treatments were analyzed using analysis of variance (Fischer method) and Fisher's Least Significant Difference (LSD) test. All data are presented as relative values compared to the control (treated only with water), which is considered equal to 100%. Statistical significance was declared at $P \leq 0.01$.

RESULTS AND DISCUSSION

The agrochemical efficiency of the biostimulant fertilizing product (0.5% solution) was determined by it applying to four intensively cultivated vegetable crops in Romania, namely tomatoes (*Solanum lycopersicum*), eggplants (*Solanum melongena*), bell peppers (*Capsicum annuum*), and cucumbers (*Cucumis sativus*). The experiment was organized under protected and drip irrigation conditions. The experiment consisted of three foliar treatments with a fine mist application over the entire leaf surface, as follows: the first treatment was applied after the flowering phase, while the second and third treatments were applied at 10-day intervals.

The agrochemical trials assessed the evolution of production yields, macronutrient content (N, P, K) in the leaves after fertilization, and photosynthetic assimilation processes.

Foliar fertilization with the HPF biostimulat during the periods of maximum nutrient demand for vegetables cultivated in protected spaces and under drip irrigation conditions had a significant influence not only on the photosynthetic activity and mineral nutrition of the plants but also on productivity indicators (Table 1-4).

Table 1. The influence of foliar fertilization with the HPF biostimulant on yield

No. Crt.	Vegetable culture	Production, Control (kg/ha)	Production, HPF biostimulant (kg/ha)	Production increase (kg/ha)	Yield increases (%)
1	Tomatoes (*Precos*)	36369	49549	13180	136.2***
2	Bell peppers (*Bianca*)	22313	29957	7644	133.7***
3	Eggplants (*Belona*)	28511	36531	8020	128.1***
4	Cucumbers (*Cornistar*)	33127	46914	13787	141.6***

Note: *** - indicates statistical significance at 0.1%; strong significance as compared to the control.

Table 2. The influence of foliar fertilization with the HPF biostimulant on mineral nutrition

No. Crt.	Vegetable culture	Nutrient	Control	HPF biostimulant	Increases (%)
1	Tomatoes (*Precos*)	Nitrogen (Nt %)	0.4736	0.6759	142.71***
		Phosphorus (P_2O_5 %)	0.2871	0.4033	140.48***
		Potasium (K_2O %)	0.2715	0.3906	143.88***
2	Bell peppers (*Bianca*)	Nitrogen (Nt %)	0.4827	0.6726	139.35***
		Phosphorus (P_2O_5 %)	0.2714	0.3754	138.32***
		Potasium (K_2O %)	0.3351	0.4701	140.29***
3	Eggplants (*Belona*)	Nitrogen (Nt %)	0.5842	0.7992	136.81***
		Phosphorus (P_2O_5 %)	0.3751	0.5092	135.76***
		Potasium (K_2O %)	0.4567	0.6273	137.36***
4	Cucumbers (*Cornistar*)	Nitrogen (Nt %)	0.4555	0.6830	149.96***
		Phosphorus (P_2O_5 %)	0.3016	0.4458	147.83***
		Potasium (K_2O %)	0.3356	0.4925	146.77***

Note: *** - indicates statistical significance at 0.1%; strong significance as compared to the control.

It has been observed that organic components, particularly protein hydrolysates, play a crucial role as plant biostimulants by activating physiological and molecular processes that stimulate growth and boost productivity. These components work by enhancing nutrient uptake, improving water-use efficiency, and promoting hormonal balance within the plant. As a result, they increase resilience to environmental stresses such as drought and salinity, ultimately leading to healthier plants with higher yield and quality (Colla et al., 2017; Rouphael et al., 2017; Rodrigues et al., 2020).

For all crops used in the agrochemical experiments, the biostimulant application of the three foliar treatments had a positive influence on the macronutrient content in the plant leaves, positively impacting foliar metabolism and thus the production increases achieved. The nutrient increases in the plants were statistically significant, exhibiting notable differences compared to the control. Specifically, phosphorus increased by 135.76% to 147.83%, nitrogen by 136.81% to 149.96%, and potassium by 137.36% to 146.77% (Tables 1 and 2). The experimental results demonstrated statistically significant increases compared to the control for nitrogen, phosphorus, and potassium content, in agreement with findings reported by other authors (Rouphael et al., 2017; Rodrigues et al., 2020).

Table 3. The influence of foliar fertilization with the HPF biostimulant on the assimilatory pigment content in leaves

Vegetable culture	Chlorophyll a		Chlorophyll b		Carotenoid		Total pigments	
	(mg/g fresh substance)							
	Control	HPF	Control	HPF	Control	HPF	Control	HPF
Tomatoes (*Precos*)	0.7212	1.0299	0.6134	0.8620	0.5043	0.6990	1.8389	2.5909
Bell peppers (*Bianca*)	0.7124	0.9927	0.4632	0.6402	0.4175	0.5744	1.5931	2.2073
Eggplants (*Belona*)	0.6917	0.9235	0.4823	0.6493	0.4251	0.5644	1.5991	2.1372
Cucumbers (*Cornistar*)	0.5177	0.7826	0.4281	0.6452	0.3915	0.5866	1.3373	2.0144

Table 4. The influence of foliar fertilization with the HPF biostimulant on the total assimilatory pigment content in leaves

No. crt.	Vegetable culture	Increases in total assimilatory pigment (mg/g fresh substance)	Increases in total assimilatory pigment (%)
1	Tomatoes (*Precos*)	0.7520	140.89***
2	Bell peppers (*Bianca*)	0.6142	138.55***
3	Eggplants (*Belona*)	0.5381	133.65***
4	Cucumbers (*Cornistar*)	0.6771	150.63***

Note: *** - indicates statistical significance at 0.1%; strong significance as compared to the control.

These results underscore the positive impact of biostimulant application on nutrient absorption and plant health, confirming that well-timed foliar treatments can significantly boost both crop productivity and nutrient use efficiency.

The ANOVA analysis revealed significant differences between the treated and untreated plots ($p<0.01$), confirming the effectiveness of biostimulant application on photosynthetic parameters and yield performance.

The extraradicular application of the HPF fertilizer at a 0.5% concentration stimulated the photosynthetic assimilation process, resulting in highly significant increases, both for each individual assimilatory pigment and for the total content of assimilatory pigments. The application of HPF increased the chlorophyll and carotenoid content in the plant leaves (Tables 3 and 4).

Our results are consistent with the studies conducted by Colla et al. (2017), which demonstrated a significant increase in photosynthesis and yield following the foliar application of protein hydrolysate. As well, Petcu et al. (2021) shown that some innovative fertilizers (hydroxyapatite and iron oxide nanoparticles) have a positive effect on photosintesis and yield of corn and winter wheat plants.

The data obtained indicate a positive correlation between nutrient content (N, P, K) and the concentration of chlorophyll pigments in plant leaves. An increase in nitrogen content promoted chlorophyll synthesis, while phosphorus contributed to enhanced photosynthetic activity through its role in energy metabolism. Potassium, in turn, optimized water and stomatal balance, thereby facilitating the synthesis of photosynthetic pigments. These findings align with the existing literature, which emphasizes the direct influence of these macronutrients on photosynthetic efficiency and the accumulation of chlorophyll pigments (Taiz et al., 2015; Jiang et al., 2017; Chen et al., 2021).

The application of the soy protein hydrolysate-based biostimulant had the greatest impact during the post-flowering period in tomatoes and cucumbers. It is recommended to apply at least three foliar treatments at 10-day intervals to maximize nutrient absorption and photosynthetic efficiency.

The results also showed that the photosynthetic efficiency promoted the increase in production and the absorption of substances by the plant cell and are in in agreement with findings reported by other authors (Madeira and Varennes, 2005; Padilla et al., 2018; ALKahtani et al., 2020; Pereira et al., 2021).

CONCLUSIONS

Foliar fertilization with the HPF biostimulant had a significant impact on production increases for all tested crops. The highest production gains were observed in cucumbers, followed by tomatoes, bell peppers, and eggplants. Foliar application of the biostimulant product containing soy protein hydrolysate led to production increases ranging from 28.1% (in eggplants, Belona variety) to 41.6% (in cucumbers, Cornistar variety) compared to the control.

The three foliar treatments applied resulted in statistically significant increases in carotenoid content, chlorophyll pigments, and total assimilatory pigment content, with values ranging between 33.6% and 50.6%.

Moreover, by stimulating the biosynthesis of assimilatory pigments, it shortens the duration of the organogenesis stages. The content of essential nutrients (N, P, K) in plant leaves showed a significant increase.

Biofertilizers are a modern and indispensable component of environmentally friendly agricultural technologies, thus contributing to ensuring food safety and security. These products play an essential role in the development of a sustainable agricultural system, capable of addressing current challenges such as climate change and increasing food demand.

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