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EFFECTIVENESS OF BIOSTIMULANTS APPLIED TO WHEAT, SUNFLOWER AND SOYBEAN CROPS

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Abstract

It is known that the application of organic substances has effects to stimulate the development of plants. The paper presents the effect of two plant biostimulants (FERT - H and FERT - A) with organic matter of vegetable origin (protein hydrolyzate, respectively seaweed extract) on quality and production indicators for wheat, sunflower and soybean crops. The plant biostimulants were tested for three years by foliar application both in the experimental field in a wide range of crops, obtaining statistically significant and very significant yields compared to the unfertilized control. The obtained yields ranged between 50-60% for wheat, 8-9% for sunflower and 24-38% for soybean. Although there were differences in production between the two biostimulators, these were not significant. The plant biostimulants with natural organic matter (algae and soy hydrolysate) that were used can influence crop nutrition leading to increased product quality.

***Key words**: plant biostimulants, protein hydrolysate, seaweed extract, foliar application.*

INTRODUCTION

In the context of the Community's agricultural policy and the principles of the circular economy, we consider it is necessary to identify alternative sources (waste or by-products) which, with minimal processing, could be sources of nutrients (especially nitrogen, phosphorus potassium and trace elements) that can be used as a fertilizer in both organic and conventional agriculture.

The use of organic substances with crop stimulating effects in agriculture is necessary and encouraging in the context of European sustainable development policies. The application of biostimulants by spraying on plants, leads to the accumulation of a higher content of nutrients in their tissue and to positive metabolic changes. For these reasons, the development of new biostimulants has become a point of scientific interest (Nardi et al., 2016; He et al., 2018; Bulgari et al., 2019).

Algae extracts, humic and fulvic acids, protein hydrolysates and microorganisms have many positive effects on plants, namely: increasing production, intensifying photosynthesis, increasing the rate of absorption and utilization of nutrients, increasing resistance to biotic and abiotic stress (Van Oosten et al., 2017; Colla et al., 2015). These biostimulants can be officially used as fertilizers, which are regulated in the EU by Regulation (EU) 1009 of 2019.

Numerous studies have shown the beneficial effects of algae, useful as plant biostimulators (Hong et al., 2007; Zodape et al., 2008) and soil improvers (Jayaraj et al., 2008) on plant nutrition. Algae-based products improve seed germination, increase plant tolerance to environmental factors, and increase plant yield and quality (Zhang et al., 2008; Zodape et al., 2008).

A number of studies have shown that algae extracts have supported the development of the root system and lead to increased production in many plant species: corn (Jeannin et al., 1991), tomatoes (Crouch et al., 1992; Dobromilska et al., 2008; Kumar and Sahoo, 2011; Goñi et al., 2018; Di Stasio et al., 2018), vines (Mancuso et al., 2006), strawberries (Alam et al., 2013), and wheat (Kumar and Sahoo, 2011).

The result of the process of hydrolysis of products of plant or animal origin is divided

into two categories: protein hydrolysates (a mixture of polypeptides and amino acids of plant or animal origin) and free amino acids (proline, valine, leucine) (Van Oosten et al., 2017). Such compounds are, in fact, a mixture of soluble amino acids and peptides, resulting from enzymatic, thermal and chemical processes, obtained from proteins of animal or vegetable origin (Carillo et al., 2019; Colla et al., 2015; Shahrajabian et al., 2021).
There is considerable evidence that protein hydrolysates and certain amino acids (proline, betaine but also their precursors and derivatives) increase plant tolerance to abiotic stress (drought, high or low temperatures, salinity, oxidative conditions) (Ertani et al., 2009; Colla et al., 2014; Colla et al., 2017; Shahrajabian et al., 2021).
In the last two decades, interest in this type of product has grown steadily, as short-chain peptides in protein hydrolysates have been shown to have a higher nutritional value and can be used more efficiently than an equivalent mixture of free amino acids (McCarthy et al., 2013).
Amino acids and peptides play an important role in increasing plant tolerance to heavy metals through the sequestration process that allows them to survive in areas contaminated with metals without suffering toxic effects (Singh et al., 2016).
Phytochelatins are organic polymers related to metals and plant products and are a class of phytochemicals that can reduce the toxic effects of metals on human health (eg cadmium) (Seregin and Kozhevnikova, 2020). The paper presents the development of new fertilizers starting from natural substances and products accepted by European regulations for use in organic farming. The selected natural substances were from the class of biomaterials with biostimulatory properties, namely: protein hydrolysates from biomass and /or extracts from algae (*Ascophyllum nodosum*).
It has been suggested that organic substances in the composition of biostimulators may help plants to overcome the abiotic and biotic stresses (Battacharyya et al., 2015; Balabanova, 2021; Neshev, 2020).The new biostimulant products are characterized by a complex structure due to natural organic substances with biostimulatory effect, enriched with trace elements in chelated structures (Mg, Mn, Fe, Cu, Zn, B and S), and are intended to stimulate plant nutrition and growth, root activity and prevention and control of nutritional deficiencies.

MATERIALS AND METHODS

Two biostimulators were selected to evaluate the effects of their application on 3 test crops: wheat, sunflower and soybeans. The compositional characteristics of the biostimulators were: FERT-H product including a hydrolysed soy protein, matrix containing secondary elements and microelements and FERT-A including an algae extract (*Ascophyllum nodosum*), matrix containing secondary elements and microelements.
For each crop, 3 foliar treatments were applied during the vegetation period with a concentration of 0.5% and a dose of 2.5 l/ha. The application times for the three crops are shown in Table 1.

Table 1. Experiment characterization and phenological observations

	Winter wheat	Sunflower	Soybean
Variety/hybrid	Trivale	Hybrid PG 4	Raluca TD
Sowing date	22.10.2020	05.05.2021	28.04.2021
Raised date	11.11. 2020	24.05.2021	14.05.2021
Maturity	03.07.2021	14.09.2021	25.09.2021
First treatment application (phenophase)	07.04.2021 (end of twinning)	05.05.2021 (1st pair of leaves)	22.05.2021 (2rd trifoliate leaf)
Second treatment application (phenophase)	28.04.2021 (elongation)	23.05.2021 (4-5 leaves)	29.05.2020 (elongation)
Third treatment application (phenophase)	16.05.2021 (heading)	04.06.2021 (7 leaves)	07.06.2021 (4th trifoliate leaf)
Basal fertilization*	100 kg/ha with complex NPK 20:20:0		

*Basal fertilization was done with complex fertilizer NPK 20:20:0 and 100 kg/ha brought 20 kg/ha N and 20 kg/ha P_2O_5

To determine the effectiveness of biostimulants, the following crops were chosen for testing: winter wheat, sunflower and soybeans. The details of the experiment are presented in Table 1. The determinations of the parameters total biomass, spikes biomass, seeds biomass and 1000 grain weight were performed by weighing and the infrared analysis (Perkin Elmer Inframatic 9500 analyzer) was used to determine protein, starch and wet gluten.

The experiment was developed on albic luvisol with clay texture, having the characteristics presented in the Table 2.

Table 2. Physico-chemical characteristics of the soil

Crops	**Humus**	**Nitrogen**	**Mobile phosphorous (P_{AL})**	**Mobile potassium (K_{AL})**	**C_{org}**	**Mobile forms of cations in solution of ammonium acetate + EDTA at pH = 7**				**pH**
						Zn	Cu	Fe	Mn	
	(%)	(%)	(mg/kg)	(mg/kg)	(%)	(mg/kg)				
Wheat	4.29	0.157	64	208	2.49	1.1	2.0	93.8	30.2	5.35
Soybean	4.53	0.163	83	189	2.63	1.7	2.4	120.4	52.3	5.64
Sunflower	4.71	0.163	58	218	2.73	1.4	2.2	115.0	46.7	5.01

Statistical analysis

Signs following the values in the tables mean significant differences compared to other treatments at levels of $p < 0.05$* $p < 0.01$** according to the tests of the least significant differences (LSD).

RESULTS AND DISCUSSIONS

Wheat (*Triticum aestivum* L.) is one of the largest growing cereal crops worldwide and its fertilization leads to yields that ensure food security.

The application of plant biostimulators to wheat crop has led to an increase in biomass and, implicitly, in the production. Significant increases compared to the unfertilized control were obtained for both the mass of the grains and the spikes (Table 3). Regarding the composition of wheat grain in protein and gluten, Fert-A led to higher values of these parameters than the unfertilized control.

Our results were in line with previous studies by Szczepanek et al. (2018) on the effect of applying seaweed products for wheat. It was also observed that the results obtained are influenced by a number of factors such as the number and timing of applications, soil characteristics, climatic conditions.

The data obtained by Stamatiadis et al. (2021) for wheat crop led to the conclusion that increased soil N uptake and/or remobilization to the reproductive organs was a key process of *A. nodosum* mode of action.

Table 3. Efficacy of FERT-H and FERT-A for wheat crop

Variants	**Total biomass, kg/ha**	**Spikes biomass, kg/ha**	**Seeds biomass, kg/ha**	**1000 grain weight, g**	**Protein %**	**Starch %**	**Wet gluten %**
Control	5520	2650	1730	34.2	7.9	72.7	12.8
FERT - H	7150**	4050**	2770**	35.1*	7.6	74.4	12.2
FERT - A	7730***	3860**	2600**	35.0*	8.0	72.2	14.2

**, *indicates statistical significance at 1% and 5%

It can be seen that the increase in biomass in wheat crops led to the effect of "dilution" in terms of protein content in the case of the variant to which the product FERT - H was applied (Table 3). The results concerning the sunflower crop, except those for 1000-grain weight indicated significant and distinct significant differences between treatments and control variant (Table 4). The results obtained in the case of seed production were significantly higher than the unfertilized control. The differences between the two biostimulants products were not significant.

The use of different doses of a biostimulator based on *Ascophyllum nodosum* has led to increases in root and stem masses corresponding to the concentrations used in treatments (Santos et al., 2019).

Table 4. Efficacy of FERT-H and FERT-A for sunflower crop

Variants	Total biomass, kg/ha	Calatidium biomass, kg/ha	Seeds biomass, kg/ha	1000-grain weigh, g
Control	11600	5333	2890	67.7
FERT - H	11933	6533**	3360*	66.7
FERT - A	13867*	6310*	3320*	65.9

**, *indicates statistical significance at 1% and 5%

The application of the product FERT - A to the sunflower crop led to the increase of calatidium biomass and seeds biomass but to the decrease of the parameter "1000-grain weigh" related to the control (Table 4).
The parameters determined for the application of biostimulants to soybean crop were higher and distinctly statistically significant compared to the unfertilized control (Table 5).
The differences between the two biostimulants were not statistically significant, higher productions being obtained for the FERT-H variant.
Experiments conducted by Briglia et al. (2019) have shown that the application of biostimulators based on various combinations of seaweed and plant extracts formulated with selected micronutrients, such as Mn, Zn, Mo, on the metabolism of soybean nitrogen, transport of metal ions (mainly zinc and iron), sulphate reduction and amino acid biosynthesis were positively regulated.

Table 5. Efficacy of FERT-H and FERT-A for soybean crop

Variants	Total biomass, kg/ha	Pods biomass, kg/ha	Seeds biomass, kg/ha	1000-grain weigh, g	Protein %	Oil %	Fiber %
Control	4767	2780	1480	117	33.8	24.8	5.9
FERT - H	5833**	3460**	2010**	125	35.5	24.8	5.7
FERT - A	5467*	3350**	1880**	119	35.6	25.3	5.6

**, *indicates statistical significance at 1% and 5%

The application of the two biostimulants with organic substances lead to increased production compared to the unfertilized control, that ranged between 50% and 60% for the wheat crop, 8% and 9% for the sunflower, respectively, between 24% and 38% for the soybean. The highest productions were observed in the case of the FERT-H biostimulant variant (Figure 1).

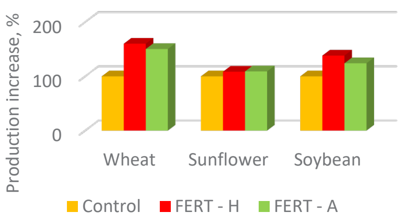

Figure 1. Production increases obtained for wheat, sunflower and soybean crops depending on the biostimulant applied

The production obtained by applying plant biostimulants based on algae (FERT - A) and soy protein hydrolyzate (FERT - H) showed increases for the three crops tested. In the case of sunflower cultivation, production increases were close, i.e. 8% for the product with organic content from algae and 9% for the one based on hydrolyzate. In contrast, in the case of wheat and soybean crops, the highest increase was obtained in the case of the FERT - H variant (60% for wheat and 38% for soybeans).

CONCLUSIONS

The production of wheat, sunflower and soybeans obtained as a result of the application of biostimulants FERT - H and FERT - A led to increases in production compared to the untreated control.
It was noticed, that biostimulators with natural organic matter content (algae/soy hydrolyzate) can influence crop nutrition leading to increased product quality. Some seed quality indices for the three crops declined as production increased, with the phenomenon of dilution occurring.

In conclusion, the application of biostimulators can be considered a sustainable choice in terms of environmental protection but also economic and is a solution in the current crisis of mineral fertilizers.

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