Scientific Papers. Series A. Agronomy, Vol. LXV, No. 2, 2022
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESULTS OBTAINED BY APPLYING A BIOSTIMULANT TO TOMATO AND WHEAT CROPS

Carmen SÎRBU[1], Traian Mihai CIOROIANU[1], Geanina BIREESCU[2], Daniela MIHALACHE[1], Ana Maria STĂNESCU[1]

[1]National Research and Development Institute for Soil Science, Agrochemistry and Environment Protection - RISSA Bucharest, 61 Marasti Blvd, 011464, District 1, Bucharest, Romania
[2]Institute of Biological Research, 47 Lascar Catargi Street, Iasi, Romania

Corresponding author email: licci_icpa@yahoo.com

Abstract

The paper presents a biostimulant (codified Bios) containing algae extract (Ascophyllum nodosum), hydrolysed proteins, as well as humic substances with effects on plant growth and mineral nutrition, thus reducing the negative effect of climate and technological stress. The application of the biostimulant indirectly stimulated the plants for the additional absorption of nutrients as well as the increase of the photosynthesis process.
The foliar application of the Bios product (as a 0.5% solution) led to the increase of the yield of wheat and tomato crops compared to the unfertilized control. The production increases were 36.46% for wheat and 50.12% for tomatoes. The stimulation of the photosynthesis process for both crops tested is evidenced by the increases of the concentrations of chlorophyll a and b measured in the treated leaves as compared to the control. The same trend was found for the carotene content. The nitrogen, phosphorus and potassium content of the leaves were also determined, and there were found significant increases compared to the unfertilized control.

Key words*: tomato, wheat, biostimulant, Ascophyllum nodosum, hydrolysed protein, humic substances.*

INTRODUCTION

Climate change and environmental degradation are a real threat to the world. In order to reduce the effects, the European Green Pact aims to transform the EU into a modern, competitive and resource-efficient economy, in which:

• to reach zero greenhouse gas emissions by 2050;
• dissociate economic growth from resource use;
• no person or place should be left behind (european-green-deal).

There is a current consensus that we need to make the transition from an economy based on the consumption of oil, coal and lignite to a bio-economy through the development of a sustainable circular economy (Kapoore et al., 2021).
The European model of agriculture is based on the promotion of a competitive, market-oriented sector, but taking into account the needs of current society but also future generations aiming at the integration of agriculture and civil culture with the environment.
Applying the principles of the circular economy can significantly reduce the negative impact of the extraction and use of resources on the environment and can contribute to the restoration of biodiversity and natural capital in Europe (Bruxelles, COM(2020) 98).
In this context, the identification of sustainable sources that, through minimal processing, can be sources of nutrients (especially nitrogen, phosphorus and potassium), is a global priority.
The use of organic substances with crop stimulating effects in agriculture is necessary and encouraged in the context of European sustainable development policies.
The use of biostimulators can be a viable solution for sustainable agriculture which improves the condition of crops without causing adverse side effects (du Jardin, 2015). Due to the positive effects on the growth of agricultural production, the market for these products has grown in recent years, which is why the authorities felt the need to introduce this category of products in the new Regulation (EU) 2019/1009 on fertilizers. Improving the effects of abiotic stress is the most commonly mentioned benefit of using biostimulators, as 60-70% of agricultural losses are attributed to abiotic stress (Colla et al., 2017; Yakhin et al., 2017; Ertani et al., 2013).

The application of biostimulants 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).

The role of biostimulators is well defined by the new European legislation, Regulation (EU) 2019/1009 being described as a product that stimulates plant nutritional processes regardless of the nutrient content of the product improving one or more of the following characteristics of the plant or the plant rhizosphere:

(a) nutrient use efficiency;

(b) tolerance to abiotic stress;

(c) quality traits;

(d) availability of confined nutrients in soil or rhizosphere.

In specialized studies, substances with biostimulator role are divided into 4 main categories: humic substances, protein hydrolysates, algae and microorganisms (Du Jardin, 2015).

Algae have been recognized for centuries for their beneficial effects on plants, but their market potential still seems to be underestimated. They are a renewable source with a complex composition and whose mechanisms of action on plants (Marti et al., 2004).

Numerous studies have shown the beneficial effects of algae on improving plant growth. Algae-based products improve seed germination, seedling development, increase plant tolerance to environmental factors and lead to increased production and plant quality (Zhang and Ervin, 2004; 2008; Zodape et al., 2008; Livingston et al., 2009).

Depending on the formulation, algae-based products could act as organic fertilizers or as a component of organo-mineral fertilizers, soil improvers, biostimulators and even pesticides. Due to the positive effects on the growth of agricultural production, the market for these products has grown in recent years, which is why the authorities felt the need to introduce this category of products in the new Regulation (EU) 2019/1009 on fertilizers.

Polysaccharides and oligosaccharides in green, brown and red seaweeds (marine macroalgae) corresponding to ulvans, alginates, fucans, laminarins and carrageenans have been found to stimulate the defence and protection responses of plant pathogens. Oligosaccharides obtained by depolymerization of seaweed polysaccharides induce protection against viral, fungal and bacterial infections in plants (Li et al., 2021; Drobek et al., 2019; Sharma et al., 2014).

Thus, it was observed that in tobacco plants (Xhanti NN cultivar) treated with macroalgae oligosaccharides and infected with tobacco mosaic virus (TMV), the number of necrotic lesions decreased significantly. Performing 4 weekly treatments with a solution containing polyguluronic acid (Poly-Gu), sulphated galactan (Poly-Ga) or polymannuronic acid (Poly-Ma) oligosaccharides prepared at a final concentration of 500 $\mu g\ mL^{-1}$ of each, led to a significant decrease in the number of necrotic lesions compared to the untreated control (Laporte et al., 2007).

Alginate is the predominant polysaccharide component found in cell walls and the intercellular matrix, with a degree of polymerization of 2 to 25 and accounting for 17 to 45% of the dry weight of algae in brown macroalgae (Li et al., 2021; Vera et al., 2011).

Protein hydrolysates represent another category of organic substances with the role of biostimulation of crops. They can be obtained from plant or animal sources and are an important source of nitrogen and other active biomolecules for use in agriculture.

The average nitrogen content of protein hydrolysates is in the range of 10 and 29.9% N and differs depending on the source and production method (Corte et al., 2014).

Application of protein and amino acid products have the role of providing plants with an organic source of nitrogen, thus making it possible to reduce the amount of mineral fertilizers applied (urea, ammonium nitrate).

Peptides and amino acids form complex combinations with micronutrients (Cu, Fe, Mn and Zn), thus contributing to increasing their bioavailability for the plant (Du Jardin et al., 2015).

Currently, the bulk of the market for protein hydrolysate biostimulators is represented by products resulting from the acid hydrolysis of animal proteins, the rest coming from the enzymatic hydrolysis of plant-derived proteins. Globally, most protein hydrolysates used in agriculture are produced by companies in Italy, Spain, the United States, China and India. Some of these companies were developed by the leather and meat processing industry as a way to capitalize on by-products resulting from the manufacture of biostimulators and fertilizers.

Several studies have shown that many comercial products derived from protein hydrolysates cause hormone-like activities (auxin and gibberellin), favoring the growth of roots and shoots (Colla et al., 2017; Ertani et al., 2013).

Foliar and root application have been shown to improve the absorption and efficiency of the use of macro and micronutrients (Colla et al., 2017). Improved nutrient uptake performance of protein hydrolyzed plants has been largely associated with changes in root architecture (density, length and number of lateral roots), as well as increased nutrient availability in the soil solution resulting from their complexation by peptides and amino acids and improving microbial activity (Colla et al., 2017; Du Jardin, 2015).

Protein hydrolysates can also help plants in unfavorable nutrient conditions by increasing the efficiency of their consumption. According to literature, certain amino acids (e.g., asparagine, cysteine, and glutamine) and peptides (e.g., glutathione and phytochelatin) may play an important role in plant tolerance to some toxic metals (Cu, Zn, As, Cd and Ni) by chelating and binding them (Colla et al., 2017).

Moreover, small peptides are thought to play an important role in the biological activity of protein hydrolysates. However, only a limited number of bioactive peptides have been characterized. Therefore, more studies are needed to identify the peptides responsible for the biostimulatory activity of protein hydrolysates. These findings may also help to streamline the production process of protein hydrolysates and, implicitly, bioactive peptides (Colla et al., 2017).

Another class of substances that have a role in stimulating nutrition and which are increasingly used, are humic substances.

Humic substances can be used to improve the quality and structure of the soil, having special physical and chemical properties that act effectively to combat soil erosion, improve plant development and remove soil and environmental pollutants. These characteristics indicate that humic substances play an important role in phytoremediation technologies in degraded or polluted areas, in wastewater treatment, and use as organic fertilizers for agricultural soils (Elbehiry et al., 2020; Steverson, 1994).

Conventionally substances extracted in an alkaline solution are called humic substances (HS). In this way HS can be extracted from a wide variety of sources, including sub-bituminous coal, lignite (brown coal), peat, soil, compost and some crude organic waste (Rose et al., 2014; Steverson, 1994).

The use of HS as a biostimulant for plant growth is a beneficial approach for both farmers and the environment and is part of the circular economy concept focused on conversion to a new resource (Jindo et al., 2020).

The beneficial effects on HSs crop productivity have been elicited by the content of a variety of oxygen-containing functional groups that lead to changes in soil acidity (Nardi et al., 2002; Steverson, 1994).

There are studies that indicate that one of the major impacts of HS on plant growth is a better nutrient uptake and elongation of lateral root growth, often recognized as "auxin-like effect" (Jindo et al., 2020; Muscolo et al., 2007).

The application of HS is believed to allow plants to resist abiotic stress, such as excessive heat and salts from growing media more effectively, leading to increased yields in many crops (Suddarth et al., 2019; Abdellatif et al., 2017).

In order to evaluate the effects of plant biostimulants, it is advisable to have results obtained under controlled conditions (laboratory, greenhouse, growth chamber, phenotyping, etc.) and/or in the field (field studies) (Ricci et al., 2019).

The plant biostimulants are applied to a wide range of cultures, having no practical restrictions in this respect.

Wheat (*Triticum aestivum* L.) is the most important and cultivated crop in the world alongside maize, rice, sunflower and soybean (FAOSTAT, 2019). The EU-27's harvested production of fresh vegetables (including melons) was 60.9 million tons in 2019, about 1.1 million tons more than in 2018 and within the group of fresh vegetables, the harvested production of tomatoes was 16.5 million tons in 2019 (EUROSTAT, 2020).

The evolution of wheat areas and average production (kg/ha) in Romania (Figure 1) indicates

a steady increase in the period 2010-2019 (www.madr.ro/culturi-de-camp/cereale/grau).
The evolution of average production in the period 2010-2019 shows that average of wheat production has increased through the application of efficient management, which includes the use of fertilizers and plant protection products (Figure 1).

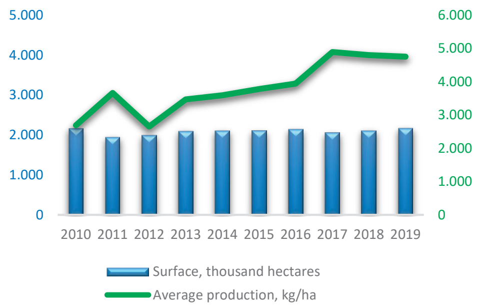

Figure 1. Trends in wheat areas and production in the period 2010-2019, in Romania

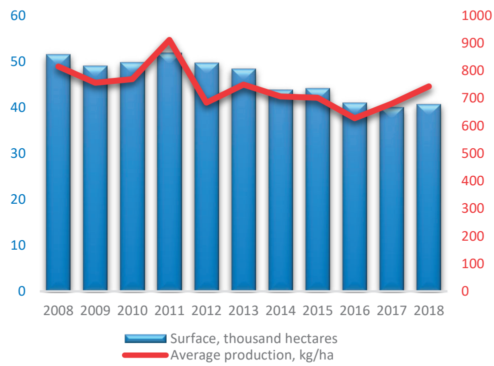

Figure 2. Trends in areas and production of tomatoes in the period 2008-2018, in Romania

Analysing the data presented in Figure 2 it can be observed a tendency of decreasing of the areas cultivated with tomatoes. In the last 3 years of the mentioned period there is a growing trend of the average production per hectare, which can be explained by the higher consumer demand for these vegetables, which has led to the use of fertilizers and thus to increased yields. (www.madr.ro/horticultura).
The EU is largely dependent on imports for most of the mineral fertilizers (Fertilizers in the EU, 2019) and for this reason there are attempts to supplement them with products from the plant biostimulants category. This category of products is increasingly used because they lead to an increase in the yield of agricultural crops between 5-10% according to the evaluation by The European Biostimulants Industry Council (EBIC).

MATERIALS AND METHODS

Plant materials and treatments

This study focuses on the effect of Bios biostimulant plant on two crops: tomatoes ("Precos" hybrid) in a protected area (solarium, drip irrigation) and wheat ("Izvor" cultivar) in non-irrigated field.
The experimental researches were carried out in the period 2018-2019 on unfertilized soil and were located as follows:
- in the field, on an unirrigated Chernozem for the autumn wheat crop;
- in the solarium, on an Anthrosol drip irrigated for tomato crop;
-during the vegetation period, the temperatures were higher than average with +0.6^{0}C (10.2-9.6^{0}C), with a positive deviation (+0.5^{0}C) compared to the average per years (17.9-17.4^{0}C);
- the vegetation period of 2019 year was poorer in precipitation than the average per years (337.5-282.1 mm) by 55.4 mm.
In terms of the soil enzymatic activity, catalase was determined by titrimetric method, while the dehydrogenase, invertase, and urease were determined by spectro-photo-colorimetric methods after the incubation of the soil samples at 37^{0}C during 24 hours.
The foliar fertilization with the plant biostimulant was developed as follows: application dose - 2.5 L ha^{-1}; concentration of the applied solution - 0.5%; number of treatments - 3. All three treatments were developed by the fine spraying on the whole foliage surface, during the vegetation period.
The applications with the “Bios” for both crops were chosen as follows:
- for wheat, the first treatment - in the bellows phase, the second treatment - in sprouting and the last treatment - in flowering in conditions of non-irrigation in the field;
- for tomatoes, the first treatment - 10 days after planting; the second treatment - at flowering and the last treatment - in the phase of formation and

growth of the fruit in conditions of drip irrigation in the solarium.

To perform the analysis, plant material (leaves) was harvested 7 days after the last treatment.

Agrochemical experiments for non-irrigated wheat cultivation in the field were performed on a soil with the following characteristics: total nitrogen (Nt) - 0.133%; mobile phosphorus (PAL) - 32 mg/kg; mobile potassium (KAL) - 228 mg/kg; humus (%) -2.61; pH - 6.2;

The tomato crop was placed in the protected area on a drip irrigated soil with the following characteristics: total nitrogen (Nt) - 0.221%; mobile phosphorus (PAL) - 51 mg/kg; mobile potassium (KAL) - 208 mg/kg; humus (%) - 3.0%; pH - 6.1.

The soil enzymatic activities correlated with the morphological, physical and chemical soil properties and the different management highlighted the following: high values of catalase activity in Chernozem (0.50-0.66 mL 0.02 mol/L $KMnO_4$/g soil/h) and Anthrosol (0.85-1.04 mL 0.02 mol/L $KMnO_4$/g soil/h); low values of dehydrogenase activity in Chernozem (0.5078-0.4116 µg TPF/g soil/h) correlated with the reduction of bioaccumulation processes and the destruction of active microbial cells under anaerobic conditions; higher values of dehydrogenase activity in Anthrosol (0.9444 µg TPF/g soil/h); high values of invertase activity in Chernozem (0.0669-0.0615 µg glucose/g soil/h) and Anthrosol (0.0618 µg glucose/g soil/h) correlated with the presence of loessoid deposits as parent material and a higher capacity of bacteria to use the glucose in soils formed on such parent materials; low values of urease activity (0.0249-0.0231 µg NH_4^+-N/g soil/h) in Chernozem because of the deficient aerohydric regime that led to a slower mineralization of organic matter in the unirrigated chernozem from the Ezăreni farm; higher values of urease activity (0.4634 µg NH_4^+-N/g soil/h) in Anthrosol.

Obtaining and characterizing of the plant biostimulant "Bios"

A plant biostimulant formula has been developed for experimental testing. The fertilizer was obtained in the laboratory using raw materials that can be used in organic farming and was coded Bios for presentation in this paper. This product contains secondary nutrients and micronutrients (B, Cu, Fe, Mg, Mn, S, Zn) in a matrix of organic matter from algae extract (*Ascophyllum nodosum*), hydrolysed protein and humic substances. The analysed parameters for the test cultures were: yield, and nitrogen, phosphorus, potassium, chlorophyll pigments and carotene contents in the leaves.

Statistical analysis

Different lowercase letters mean significantly difference from other treatments at the level of $p<0.05$ according to the least significant difference (LSD) tests.

RESULTS AND DISCUSSIONS

Tested on a chernozem in a non-irrigated field, with a weak acid reaction, low nitrogen content, medium phosphorus and high potassium contents, the "Bios" biostimulator led to an increase in wheat production of 52.12% compared to the unfertilized control (Figure 3). The foliar application of the Bios product to the wheat crop (*Triticum aestivum* L.) Romanian cultivar "Izvor" led to a significant increase in production of 36.46% compared to the production obtained by untreated control (Figure 3).

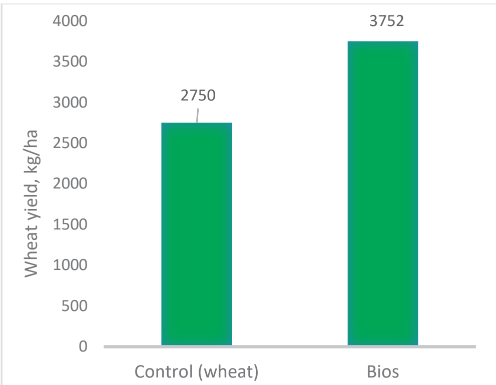

Figure 3. Wheat production (kg/ha) obtained by applying the Bios product compared to the control

The tomato crop was tested on a drip-irrigated Anthrosol which showed a weakly acidic reaction of the pH value, medium nitrogen content, low phosphorus, and medium potassium contents. Under these conditions, the application of the Bios biostimulator led to statistically positive results for tomatoes yields (Figure 4).

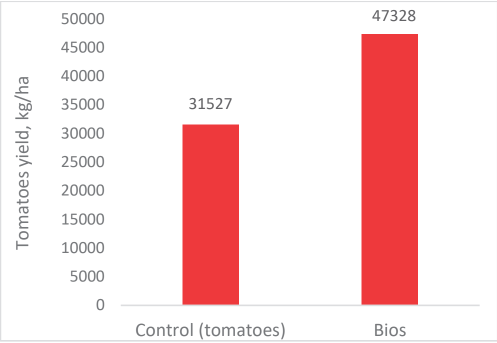

Figure 4. Tomatoes production (kg/ha) obtained by applying the Bios product compared to the control

In addition to the increase in wheat and tomato yields obtained per unit area, there was also an increase in the content of nitrogen, phosphorus and potassium, but also in the content of chlorophyll pigments in the leaves.
The accumulation of these elements in the leaves is in line with the increased production yields.
Applying plant biostimulants to different wheat cultivars can lead to increased production but also to improved crop quality (Macra and Sala, 2021).
For both cultures, the NPK content in the leaves increased significantly (Figures 5, 6), an increase that was also reflected in the chlorophyll content.

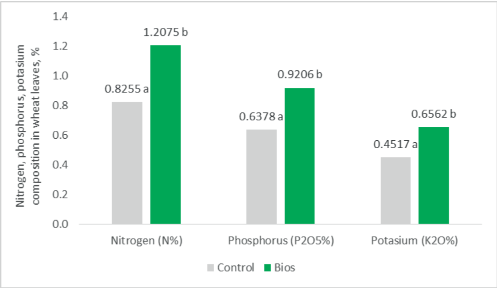

Figure 5. Nitrogen, phosphorus and potassium content (%) in wheat leaves

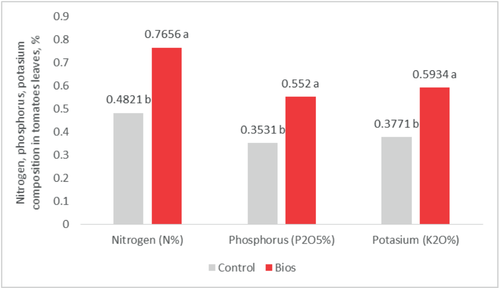

Figure 6. Nitrogen, phosphorus and potassium content (%) in tomato leaves

The foliar application of the biostimulant plant Bios, indirectly stimulated the plants for the additional supply of nutrients as well as the increase of the photosynthesis process.
Similar results were obtained by Popko et al. (2018) who tested the effect of plant growth biostimulants based on amino acids on yield and grain quality of winter wheat.
It has also been observed that the application of biostimulants to tomatoes can maintain high and economically convenient yields, even if the dose of NPK fertilization is decreased (Klokić et al., 2020; Khan et al., 2019)
The stimulation of the photosynthesis process is highlighted by the yields but also by the concentrations measured for chlorophyll a and b and carotene, both for wheat crop (Figure 7) and for tomato crop (Figure 8).

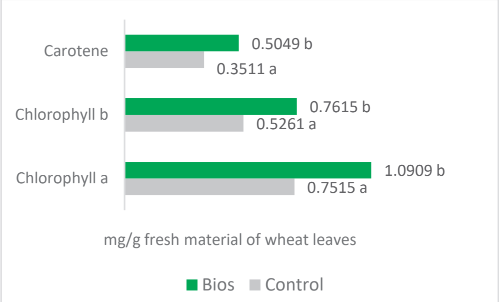

Figure 7. The content of chlorophyll pigments (mg/g fresh material) in wheat leaves

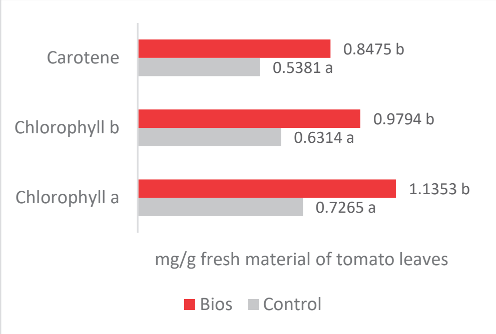

Figure 8. The content of chlorophyll pigments (mg/g fresh material) in tomato leaves

Plant biostimulants through their compounds have the ability to modify the physiological processes in plants.

There are many studies that show that the application at tomato of plant biostimulants leads to increased chlorophyll concentration and photosynthetic activity, which automatically leads to increased yield (Koleška et al., 2017), a fact also proved in this study.

Also, our results regarding the influence of the foliar fertilization on the photosynthesis showed that the percentages of increased photosynthesis are higher than the production increases because not all photosynthetic yield was directed to increase production. Part of the photosynthetic yield was maintained by the plant cell in order to accumulate reserve substances.

CONCLUSIONS

Bios product was tested by foliar application to tomatoes and wheat, obtaining statistically significant increases in production compared to the unfertilized control.

The production increase obtained compared to the unfertilized control was 36.46% for tomato cultivation and 50.12% for wheat.

By foliar application of the Bios product, the total content of chlorophyll pigments increased significantly for the tested crops, an increase also manifested in the case of NPK content in the leaves.

The soil enzymatic activities correlated with the morphological, physical and chemical soil properties, as well as with the soil management, respond to the these soils and their management in the investigated agroecosystems.

ACKNOWLEDGEMENTS

This research was conducted under the NUCLEU Program, Contract No. 19 34N/2019- Sustainable soils for high-performance agri-culture and a healthy environment - SAPS, Project PN 19 34 03 01 "Innovative products for sustainable agriculture and food security in the context of global change" and a grant of the Romanian Ministry of Research, Innovation and Digitization, project number 44 PFE /2021, Program 1 - Development of national research-development system, Subprogramme 1.2 - Institutional performance - RDI Excellence Financing Projects.

REFERENCES

Abdellatif, I. M. Y., Abdel-Ati, Y. Y., Abdel-Mageed, Y. T., & Hassan, M. A. M. M. (2017). Effect of humic acid on growth and productivity of tomato plants under heat stress. *Journal of Horticultural Research*, *25*(2), 59-66.

Bruxelles, *11.3.2020 COM(2020) 98 final,* Accessed on January 10th, 2022.

Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R., & Rouphael, Y. (2017). Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. *Frontiers in plant science*, *8*, 2202.

Corte, L., Dell'Abate, M.T., Magini, A., Migliore, M., Felici, B., Roscini, L., Sardella, R., Tancini, B., Emiliani, C., Cardinali, G. and Benedetti, A. (2014). Assessment of safety and efficiency of nitrogen organic fertilizers from animal-based protein hydrolysates a laboratory multidisciplinary approach. *Journal of the Science of Food and Agriculture*, *94*(2), 235-245.

Drobek, M., Frąc, M., & Cybulska, J. (2019). Plant biostimulants: Importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress—A review. *Agronomy*, *9*(6), 335.

Du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*, *196*, 3-14.

Elbehiry, F., Elbasiouny, H., Ali, R., & Brevik, E. C. (2020). Enhanced immobilization and phytore-mediation of heavy metals in landfill contaminated soils. *Water, Air, & Soil Pollution*, *231*(5), 1-20.

Ertani, A., Schiavon, M., Muscolo, A., & Nardi, S. (2013). Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed Zea mays L. plants. *Plant and soil*, *364*(1), 145-158.

EUROSTAT, Agriculture, forestry and fishery statistics, 2020.

FAOSTAT, 2019. Crops and livestock products. http://www.fao.org/faostat/en/#data/QCL.

Fertilisers in the EU Prices, trade and use, no 15, June 2019, European Commission.

https://ec.europa.eu/info/ strategy / priorities-2019-2024 / european-green-deal_ro

https://www.madr.ro/culturi-de-camp/cereale/grau.html

https://www.madr.ro/horticultura/fructe-si-legume.html

Jindo, K., Olivares, F. L., Malcher, D. J. D. P., Sánchez-Monedero, M. A., Kempenaar, C., & Canellas, L. P. (2020). From lab to field: role of humic substances under open-field and greenhouse conditions as biostimulant and biocontrol agent. *Frontiers in plant science*, 426.

Kapoore, R. V., Wood, E. E., & Llewellyn, C. A. (2021). Algae biostimulants: A critical look at microalgal biostimulants for sustainable agricultural practices. *Biotechnology Advances*, *49*, 107754.

Khan, M.Z., Ahmed, H., Ahmed, S., Khan, A., Khan, R.U., Hussain, F., Hayat, A. and Sarwar, S. (2019). Formulation of humic substances coated fertilizer and its use to enhance K fertilizer use efficiency for tomato under greenhouse conditions. *Journal of Plant Nutrition*, *42*(6), 626-633.

Klokić, I., Koleška, I., Hasanagić, D., Murtić, S., Bosančić, B., & Todorović, V. (2020). Biostimulants' influence on tomato fruit characteristics at conventional and low-input NPK regime. *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science*, *70*(3), 233-240.

Koleška, I., Hasanagić, D., Todorović, V., Murtić, S., Klokić, I., Parađiković, N., & Kukavica, B. (2017). Biostimulant prevents yield loss and reduces oxidative damage in tomato plants grown on reduced NPK nutrition. *Journal of Plant Interactions*, *12*(1), 209-218.

Laporte, D., Vera, J., Chandía, N. P., Zúñiga, E. A., Matsuhiro, B., & Moenne, A. (2007). Structurally unrelated algal oligosaccharides differentially stimulate growth and defense against tobacco mosaic virus in tobacco plants. *Journal of Applied Phycology*, *19*(1), 79-88.

Li, Y., Zheng, Y., Zhang, Y., Yang, Y., Wang, P., Imre, B., Wang, D. (2021). Brown Algae Carbohydrates: Structures, Pharmaceutical Properties, and Research Challenges. *Marine Drugs*, *19*(11), 620.

Livingston, D. P., Hincha, D. K., & Heyer, A. G. (2009). Fructan and its relationship to abiotic stress tolerance in plants. *Cellular and Molecular Life Sciences*, *66*(13), 2007-2023.

Macra, G., & Sala, F. (2021). variation of some production parameters in wheat in relation to foliar biostimulator, cultivars and crops site in Romania. *Scientific Papers: Management, Economic Engineering in Agriculture & Rural Development*, *21*(4).

Manilal, A., Sujith, S., Kiran, G. S., Selvin, J., Shakir, C., Gandhimathi, R., & Lipton, A. P. (2009). Antimicrobial potential and seasonality of red algae collected from the southwest coast of India tested against shrimp, human and phytopathogens. *Annals of Microbiology*, *59*(2), 207-219.

Martí, R., Uriz, M. J., & Turon, X. (2004). Seasonal and spatial variation of species toxicity in Mediterranean seaweed communities: correlation to biotic and abiotic factors. *Marine Ecology Progress Series*, *282*, 73-85.

Muscolo, A., Sidari, M., Francioso, O., Tugnoli, V., & Nardi, S. (2007). The auxin-like activity of humic substances is related to membrane interactions in carrot cell cultures. *Journal of Chemical Ecology*, *33*(1), 115-129.

Nardi, S., Pizzeghello, D., Schiavon, M., & Ertani, A. (2016). Plant biostimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. *Scientia Agricola*, *73*, 18-23.

Nardi, S., Pizzeghello, D., Muscolo, A., & Vianello, A. (2002). Physiological effects of humic substances on higher plants. *Soil Biology and Biochemistry*, *34*(11), 1527-1536.

Popko, M., Michalak, I., Wilk, R., Gramza, M., Chojnacka, K., & Górecki, H. (2018). Effect of the new plant growth biostimulants based on amino acids on yield and grain quality of winter wheat. *Molecules*, *23*(2), 470.

Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003

Ricci, M., Tilbury, L., Daridon, B., & Sukalac, K. (2019). General principles to justify plant biostimulant claims. *Frontiers in plant science*, *10*, 494.

Rose, M. T., Patti, A. F., Little, K. R., Brown, A. L., Jackson, W. R., & Cavagnaro, T. R. (2014). A meta-analysis and review of plant-growth response to humic substances: practical implications for agriculture. In *Advances in agronomy*, Vol. 124, pp. 37-89). Academic Press.

Sharma, H. S., Fleming, C., Selby, C., Rao, J. R., & Martin, T. (2014). Plant biostimulants: a review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. *Journal of applied phycology*, *26*(1), 465-490.

Stevenson, F. J. 1994. Humus Chemistry: Genesis, Composition, Reactions. Wiley & Sons, New York,USA

Suddarth, S.R.P.; Ferreira, J.F.S.; Cavalcante, L.F.; Fraga, V.S.; Anderson, R.G.; Halvorson, J.J.; Bezerra, F.T.C.; Medeiros, S.A.S.; Costa, C.R.G.; Dias, N.S. (2019). Can humic substances improve soil fertility under salt stress and drought conditions? *Journal of Environmental Quality*, *48*(6), 1605-1613.

Vera, J., Castro, J., Gonzalez, A., & Moenne, A. (2011). Seaweed polysaccharides and derived oligosaccharides stimulate defense responses and protection against pathogens in plants. *Marine drugs*, *9*(12), 2514-2525.

Yakhin, O. I., Lubyanov, A. A., Yakhin, I. A., Brown, P. H. (2017). Biostimulants in plant science: a global perspective. *Front. Plant Sci*. 7:2049.

Zhang, X., & Ervin, E. H. (2008). Impact of seaweed extract-based cytokinins and zeatin riboside on creeping bentgrass heat tolerance. *Crop Science*, *48*(1), 364-370.

Zhang, X., & Ervin, E. H. (2004). Cytokinin - containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. *Crop science*, *44*(5), 1737-1745.

Zodape, S. T., Kawarkhe, V. J., Patolia, J. S., & Warade, A. D. (2008). Effect of liquid seaweed fertilizer on yield and quality of okra (*Abelmoschus esculentus* L.*),* J. Sci. Ind. Res., 67: 1115-1117.