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Biostimulant: The New Entrant in the Fertilizer Control Order 1985

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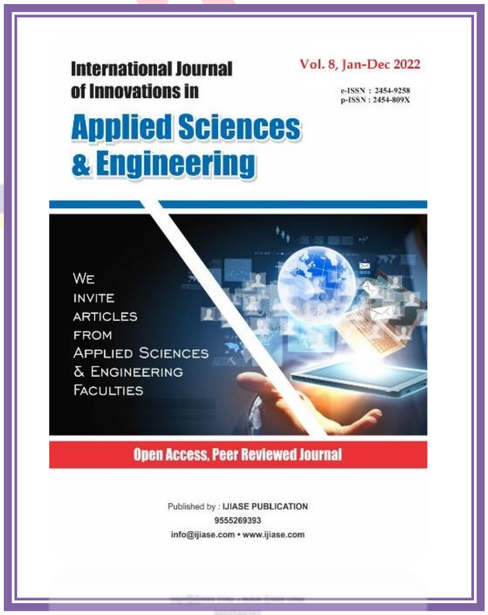

ABSTRACT

Ministry of Agriculture, Cooperation & Farmer Welfare has issued an Order Dated 23rd February 2021, regarding the addition of Biostimulants in the Fertilizer Control Order 1985. This Order may be called the Fertilizer (Inorganic, Organic or Mixed) (Control) Amendment Order, 2021.

Biostimulant means a substance or microorganism or a combination of both whose primary function when applied to plants, seeds or rhizosphere is to stimulate physiological processes in plants and to enhance its nutrient uptake, growth, yield, nutrition efficiency, crop quality and tolerance to stress, regardless of its nutrient content.

It is proposed to discuss the role of Biostimulant, as an important agrochemical for the sustainable growth, the various categories allowed, its functions, the market business potential and the market dynamics, related to its growth and business. The details of the testing requirement, and the application process to meet the requirements of the said Order will also be discussed.

Keywords: *Biostimulant, Agriculture, Organic fertilizers*

INTRODUCTION

Over the last three decades, several technical breakthroughs have been proposed to increase agricultural production systems' output by dramatically reducing synthetic agro-chemicals such as fertilizers and harmful pesticides. There are a number of biostimulants seedlings and PBs that may be used that have the ability to promote flowering and fruit setting as well as crop production and development, as well as NUE, that can work together in a synergistic manner. A plant's defenses against pests can't rely just on these levers. "Substances that enhance plant development in minute amounts," say Zhang and Schmidt from Virginia Polytechnic Institute and State University's Department of Crop and Soil Environmental Sciences. Plant biostimulant research was funded by the EU in 2012, and it was subsequently published as: "A Bibliographic Analysis of the Science of Plant Biostimulants". Biostimulants in Horticulture was published three years later, in which a novel concept of biostimulants was proposed, detailing the nature, method of action, and its impact on cereal and horticultural crops.

Depending on the content and desired outcomes, biostimulants might be sprayed on the soil or the leaves. Natural products like fulvic and humic acids have been more popular over the last two decades as plant-based chemicals. Commercially available biostimulants include the active component. Table 1 lists the biochemical and physiological alterations that these materials cause in the signaling, synthesis, and hormone regulatory pathways and cells of the host plant once they reach the tissues and cells of the plant. Fruit or vegetable waste, pulses, forages, etc. are hydrolyzed to form PBs. Amino acids and other non-protein compounds are found in the PHs. Proteins, enzymes, carbohydrates, antioxidants, and vitamins may all be used to make biopreparations.

Commonly used biostimulants include bacterial and fungal inoculants, biopolymers, seaweed extracts (based on algae), nitrogen derivatives, hormonal compounds, humic acids, and herbal extracts. Phytohormones, antimicrobial compounds, lipids and carbohydrates may all be found in seaweed extracts, as can proteins, amino acids, lipids, and osmolytes, which can all be used as growth supplements. Seaweed extract-based biostimulants have been shown to boost bean seed vigor and promote proline production in leaves under drought conditions in horticultural crops. It is common for biostimulants to be described as microbial inoculant or PHs, both of which contribute to stress tolerance and may improve nutrient accumulation. As an added benefit, biostimulants help to reduce dormancy in the plant and improve the root system's efficiency, as well as the photosynthetic rate and activities of other vegetative tissues. They also promote growth, increase nutrient absorption, and improve crop productivity, seed vigor, and consistency, as well as increase fruit size and ripeness. It has been summarized in Table 1 how various biostimulants have affected food and decorative crop growth, development, and production. Crop quality, growth, and production are all enhanced as a result of all of these factors. Producers, developers, politicians, scientists, and anybody else with an interest in biostimulants are all concerned about their role in sustainable agriculture. When it comes to the accumulation and biological activity of a biostimulant in a plant, there is still some dispute.

Table 1. Effect of biostimulants on the crop physiology

Plant Species	Biostimulants	Developmental Stage	Expected Outcomes
Tomato (*Solanum lycopersicum* L.)	Radifarm ®	Transplants	Enhanced roots growth
	Radifarm ® + Megafol ®	Transplants	Improved nutrients uptake and distribution
Bell peppers (*Capsicum annuum* L.)	Radifarm ® + Megafol ®	Fruit bearing	Increased macro- and micronutrient, especially Ca^{2+} ion concentration in leaves and fruits
	Benefit ®; Megafol ®; Radifarm ®; Viva ®	From transplanting to harvest	Improved fruit yield
	Benefit ®; Megafol ®; Radifarm ®; Viva ®	7th day seedlings	Increased fruit yield
Garden cress (*Lepidium sativum*) L.	Acker-Schachtelhalm Extract ®; Biplantol Universal ®; Fermentierter ® Pflanzenex trakt ®; KE-Plantasalva ®	Germination	Improved water uptake and seedling growth, germination rate
Garlic (*Allium sativum* L.)	Radifarm ®	After transplanting	Increased seedling after transplanting
Lettuce (*Lactuca sativa* L.)	Bio-algeenS-90 ®	After transplanting	Improved growth and yield characteristics; Increased ascorbic acid content and dry matter and reduced pH
Strawberry (*Fragaria x ananassa* Duch)	Megafol ® + Viva ®	Fruit bearing	Activated antioxidative defense mechanism; decreased NK fertilization; enhanced fresh and dry weight
	Kendal ® + Megafol ® + Viva ®	Flowering and fruit bearing	Increased fruit yield per plant
	Porcine blood-based biostimulant	Before flowering and onset of fruit ripening	Enhanced frost resistance, fruit weight; non-significant effects on fruit yield
Basil (*Ocimum basilicum* L.)	Radifarm ®	Transplants	Increased above ground parts and root biomass
Dog rose (*Rosa canina* L.)	Radifarm ®	Robust growth in tissue culture	Enhanced root intensification
	Radifarm ®	After transplanting	Increased seedling growth after transplanting
Wax Begonia (*Begonia semperflorens* L.)	Radifarm ®	Transplants	Increased nutrient uptake with improved growth
	Radifarm ®	After transplanting	Positive effects on morphological traits; enhanced nutrient uptake and proline level
Marigold (*Tagetes erecta* L.)	Radifarm ®	Germination	Enhanced seedling fresh weight and germination energy
Primrose (*Primula acaulis* L.)	Radifarm ®	Transplants	Increased above ground parts and root intensification
Scarlet sage (*Salvia splendens* L.)	Radifarm ®	Transplants	Improved root mass and above ground parts

Source of Biostimulants

To put it another way, biostimulants are natural product compositions. Microbial and non-microbial biostimulants are classed according to the source of origin of their stimulant properties. In contrast, non-microbial biostimulants include plant-based products such as amino acids and non-protein substances like gelatin mixtures and herbal extracts as well as humic acid and fulvic acid, as well as microbial biostimulants like arbuscular mycorrhizal fungi (AMF), fermented products, organic wastes, and so forth. Rosemary extract, for example, encourages the development of tomatoes. There are a wide variety of biostimulants that may be produced by enzymatic hydrolysis of plant and animal sources. There is a complicated combination of PHs produced by hydrolysis (amino acids and peptides). Chemical acid or alkaline hydrolysis has been used for the processing of animal-based raw materials such as bone meal, casein, skin collagen, and fish waste (Table 3). Examples of waste products that may be hydrolyzed include fruit and vegetable waste, legumes and alfalfa hay. They are environmentally benign, cost-effective, and encourage plant development. Using a microbe-based fermentation technique enables for both the reduction of organic waste and the production of biostimulating preparations. An anaerobic digested substance may also produce them. It is possible that the dissolved organic matter has biostimulatory properties as it ferments. Animal waste, lignin biomass, and plant material are the most common substrates for dissolved organic matter. There are a variety of low-molecular-weight compounds found in marine algae that might be used to make biopreparations. These components stimulate the rhizogenesis process, which results in beneficial changes in plant anatomy and morphology (Table 2).

Table 2. Effect of biostimulants on plant metabolic and physiological responses

Source	Bioformulations	Plant Response
Consortium of beneficial fungi	*Heteroconium chaetospira*, *Glomus viscosum*, *Glomus claroideum*, *Rhymbocarpus aggregatus*, *Glomus etunicatum*, *Trichoderma* spp., *Rhizophagus intraradices*	The beneficial fungi promotes the growth and yield in tomato fruit [88] Stimulates protection against oxidative stress [89]
Marine algal biopreparations	*Gelidium pectinutum*, *Sargassum wightii*, *Enteromorpha intestinalis*, *A. nodosum*, *Ecklonia maxima*	Enhanced antioxidant capacity, chelation, extended shelf life of fruits, thermal and drought resistance [39,88]
Hydrolytic products	alfalfa hay, fruit and vegetable waste, pulses; natural and chemical (feathers, skin collagen animal tissue, casein, bone meal, fish waste	Improved yield [90], Enhanced NPK content and macro- and micronutrients in leaves [91,92] High protein content in cereals [91] Biotic and abiotic stresses tolerance [93] Improved soil fertility [94]
Anaerobic digested products	Lignin biomass, plants, and animal	Auxin-like properties [71,95] Improved nutrient availability [96]

LITERATURE REVIEW

Nimisha Mishra et al. (2013) Fertilizer application is a crucial method for maximizing crop yields in agriculture, as stated in their article Soil and water pollution have been caused by over use of chemical fertilizers. Farmers are now seeking for more environmentally friendly choices, according to their article. There are various solutions to the environmental issues that arise from the use of chemical fertilizers, such as biofertilizers. Eco-friendly fertilizers, biofertilizers work to increase soil quality and fertility. They look at how farmers see biofertilizers, how widely they're used, and what kind of marketing campaigns are already in place to promote them. Bio-fertilizer adoption in the examined region will be promoted properly using this study's recommendations, too.

Chemical fertilizers, according to Anju Rani (2014), are an essential part of contemporary technology and have had a significant influence in the expansion of agricultural productivity. There is an increasing demand-supply mismatch for fertilizers owing to India's reliance on imported supplies. Based on secondary data from 1966-67 to 2011-12, this paper analyses Haryana's increase and use of chemical fertilizers. During the time of the research, there was a significant rise in the consumption of fertilizers across the state and districts. Sirsa district has the most rise in NPK consumption of all the districts. For Sirsa and Panchkula, the CSS ranged from 7.39106 to -4.1156.

Jaga and Yogesh Patel (2012), "An Overview of Fertilizers Consumption in India: Determinants and Outlook for 2020 - A Review,". Fertilizer availability should be emphasized above higher output prices in order to guarantee that the country's agricultural output is self-sufficient, they said. The demand for fertilizer in the nation is forecast to rise to roughly 4106 million tons by 2020, and the eastern and southern regions are likely to expand faster than the north and west. A stable and accommodating regulatory framework, the availability of raw materials, financial resources, and pricing incentives will all play a crucial part in meeting the country's expanding fertilizer needs.

Ertani and others (2019) There are a vast variety of biostimulants made from both animal and plant sources. Animal-derived PHs and plant-derived PHs are subcategories of PHs, which are differentiated by the hydrolysis method and the kind of protein sources used. The protein level of animal-derived materials is greater than that of vegetable-derived ones. However, the use of animal substrates for the large-scale manufacture of biostimulants is prohibitively expensive. The content of PH's amino acids varies according to the hydrolysis method and the kind of protein source. By way of example, enzymatic hydrolysis of collagen hydrolysate produces larger concentrations of glycine and proline, as well as aspartic and glutamic acids, and hydroxyl proline, than chemical hydrolysis. Animal substrates are chemically hydrolyzed to produce around 90% of the biostimulant (acid digestion). Chemical hydrolysis, on the other hand, has the potential to diminish protein hydrolysate's bioactive molecules, vitamins, and thermosensitive nutrients.

Gaurav and Jadhav, (2012), Horticultural crops have showed an increase in chlorophyll and carotenoid pigments when protein hydrolysate is applied. The maximum chlorophyll concentration was achieved by using chicken feather hydrolysate as opposed to the control. Animal-derived PHs (ISAGRO) boosted the chlorophyll content of cow pea plants, according to a study published in Subbarao et al., 2015. Commercial biostimulant AVTIWAVE also boosted the carotenoid and chlorophyll content in comparison to the control group's levels of these two nutrients (Vernieri et al., 2006). Dose-dependent chlorophyll uptake by plants was also facilitated by the Trainer (commercial biostimulant) (Colla et al., 2014a). Amino acids and peptides in the PH's may serve as a nitrogen supply, hence promoting chlorophyll and carotenoid content production.

Effect of Biostimulants on the Growth and Size of Plants

Biostimulants have a variety of ways in which they function. Fruits and vegetables benefit from the use of biostimulants, according to research. There are some studies that show that biostimulants have little influence on fruit size. Biostimulant effects were not seen because the biostimulant used was inappropriate for the cultivar under study.

For fruit farmers, biopreparations that allow for the biggest and healthiest-looking fruits that attract customers' attention are of importance. Increases in cucumber size were achieved with the use of nitrogen-amino-auxin mixtures and humic acids. In the first and second seasons, humic acids at a concentration of 3g/L enhanced the fruit's average length by 9.9 cm and 12.2 cm, respectively. Cucumbers grown in the first and second seasons with the same concentration of humic acids grew an average of 1.23 cm and 1.55 cm in diameter, respectively. Vegetables grew longer and had a greater diameter when three biostimulators were tested. As a result of one biostimulant, cucumbers grew to a length of 3.85 cm in the first growing season and 3.49 cm in the second. When using this biostimulant, the fruit's diameter rose by 1.12 and 1.56 centimeters in the first and second growth seasons. Cucumbers may be lengthened and thicker with the use of biostimulants rich in auxins and humic acids. Carboxic acids and humic acids were used to increase the size of the apricot fruit by a factor of 10. Carboxylic acids had the biggest impact on fruit size, causing an average increase in fruit width of 2.6 millimeters during the second growing season.

Consortia of microorganisms have been shown to be the best biostimulants for fruit and veg. Tomato weight was raised by using arbuscular mycorrhizal fungus and plant growth-promoting bacteria. Plant growth-promoting bacteria and the arbuscular mycorrhizal fungus Rhizophagus spp. and Rhizophagus aggregatus, Septoglycus viscosum, Claroideoglomus etunicatum, and Claridoglumus claroideum all worked together to have a beneficial impact. Arbuscular mycorrhizal fungi and P. fluorescens C7 were the most effective biostimulants for increasing tomato fruit mass, but biostimulants with arbuscular mycorrhizal fungi and P. fluorescens C7 had the best results overall, with the greatest increase in tomato fruit mass being seen. Tomatoes also grew longer as a result of the microbes. As a result of using biostimulants, the length of fruit grew from 5.49 0.03 cm in the controls to 5.88 0.03 cm to 6.05 0.02 cm. The diameter of the fruit grew from 4.24 0.02 cm to 4.62 0.03 cm to 4.64 0.03 cm to 4.78 0.02 cm, a little but noticeable rise.

Biostimulants have a wide range of effects on plants, including fruit size, plant height, and root length. When humic acids and other biostimulants were added to cucumber plants, the plants grew taller, had more leaves, and had fewer stems. The control plants grew to a height of 78.13 cm in their first growing season. Humic acids at a concentration of 3 g/L increased plant height by an average of 14.25 cm. Humic acid-treated plants grew 13.25 cm taller in the second growing season. Biostimulators may have similar effects on the body. As compared to the controls, this biostimulant was most effective in the first and second growth seasons, increasing plant height by 14.5 cm (first growing season) and 19.75 cm (second growing season). It included nitrogen, amino acids, and auxins. The least effective biostimulant included naphthyl acetic acid among others and increased plant growth by 4.38 cm and 7.92 cm in the first and second growing seasons, respectively. The number of leaves and the number of new stems were shown to have similar correlations.

Effect of Biostimulants on Chemical Composition

A variety of chemical attributes of fruits and vegetables may be influenced by biostimulants, including dry mass, acidity, or vitamin content. The fruit's flavor is influenced directly by its chemical makeup. Fruits having an SSC concentration of more over 12 Brix are said to have an outstanding flavor. When biostimulants with polysaccharides and humic and fulvic acids as well as carboxylic acids were added to apricots in the first year of use, the average SSC value was 10.7Brix. Biostimulants increased fruit flavor greatly in the second year of use, as shown by the SSC level rising to an average of 14.1Brix. Dissolved solids content in the fruits of the three raspberry types was lowered by biostimulants comprising phenol compounds or chitosan (Pokusa, Polka, and Poranna Rosa). As a consequence, the application of biostimulants based on titanium compounds led to a higher concentration of water-soluble substances in the fruit. The ratio of dissolved solids to titratable acidity might also show the quality of the fruit. When the ratio of dissolved solids to titratable acidity is between 10 and 15, we say that the fruit is of high grade. By increasing the dissolved solids-to-titratable acidity ratio in comparison to the control (14.0), biostimulants that comprise biopolymers of polysaccharides (16.7) and humic and fulvic acids (16.1) had an adverse effect on fruit sensory quality.

However, it is impossible to establish if variations in acidity at the level of several percent have a major influence on the fruit flavor since it is dependent on the consumer's subjective judgment. It is crucial to cultivate fruit with an adequate acidity level. Biostimulant research on fruit acidity are documented in the literature, however no conclusions can be drawn from the data. In terms of fruit acidity, it's not obvious if the biostimulants utilized have a beneficial or detrimental impact. As a biostimulant, phenolic compounds and titanium compounds increased fruit acidity to 2.26 and 2.18 percent, respectively, in raspberries (control, 2.08 percent). Biostimulants comprising polysaccharides, humic and fulvic acids, and carboxylic acids all reduced the acidity of apricots. From 3.45 (control pH) to 3.7–3.8 in the second year of employing these biostimulants, fruit acidity decreased (pH after using biostimulants).

Plants benefit from biostimulants by increasing the amount of chlorophyll in their leaves, which boosts photosynthetic efficiency. Leek corn was shown to have more chlorophyll when salicylic acid-chitosan nanoparticles were applied as a biostimulant. When compared to the control's 10.72 mg/g of chlorophyll, the biostimulant-treated maize leaves (concentration 0.01 percent –0.16 percent) had an average chlorophyll content ranging from 16.43 to 25.88 mg/g. Chlorophyll concentration decreased to an average of 9.24 mg/g and 9.79

mg/g in plants exclusively treated with chitosan and salicylic acid. The chlorophyll content of Cucurbita pepo L. leaves increased by 34.6% after foliar treatment of Moringa oleifera leaf extract compared to the control (plants sprayed simply with water). Biostimulants generated during waste hydrolysis boosted both the chlorophyll content and the photosynthetic intensity in Hibiscus plants grown with the biostimulants. Compared to the control, a 15% increase in chlorophyll content resulted in a 24% increase in photosynthesis.

Using chicken feathers as a biostimulant and a fertilizer resulted in a greater quality of maize production than using the fertilizer alone. Spraying the grain required the application of three different combinations of the chemical. Only fertilizer (300 kg N/ha + 120 kg K/ha) was applied in the first variation. Second, a biostimulant (3.6 L/ha) was added to the 300 kg N/ha and 120 kg K/ha fertilizer. Using 300 kg N/ha + 120 kg K/ha fertilizer and 7.2 L/ha of a biostimulant was the third option. During the course of two seasons, the therapy was administered. Biostimulant including fertilizer and the greatest dosage of biostimulant (7.2 L/ha) resulted in the highest nitrogen levels. Fertilizer with a biostimulant of 3.6 L/ha raised the nitrogen content of corn leaves by 14.4 percent and 39.1 percent in the first vegetative season, compared to maize leaves treated just with fertilizer. Fertilizer with a biostimulant concentration of 3.6 L/ha raised nitrogen content by 15%; fertilizer with a biostimulant concentration of 7.2 L/ha increased nitrogen content by 33.3% in the second growing season. The phosphorus content of maize leaves increased when a biostimulant was added to the fertilizer. A biostimulant-enriched fertilizer boosted P concentrations by 32.8 and 52.2 percent, respectively, in the first season and by 43.5 and 51.1 percent, respectively, in the second season compared to the control [33]. [34] The treatment was shown to be more effective than a control. "Kinnow" Mandarin trees responded well to the biostimulant extract of the Moringa oleifera plant. Nitrogen content increased by 1.35 and 1.42 times, respectively, when 3% Moringa oleifera extract foliar spray was used in conjunction with 0.6 percent $ZnSO_4$ and 0.25 percent K_2SO_4. Compared to control plants, trees sprayed with just 3 percent Moringa oleifera extract had phosphorus contents that were 1.09 times higher in the first season and 1.07 times higher in the second season.

Effect of the Biostimulant on Soil Enzyme Activity

Enzyme activity was significantly affected by the biostimulant (Figure 1A–C). The biostimulant boosted catalase activity in sandy and sandy loam soils by around 15% compared to the control, according to results on catalase activity. Also, in sandy soil, dehydrogenase activity rose in response to biostimulant and was 35% higher in the stimulated sample than in the control sample (3.18 + 0.05). Nonetheless, in sandy loam soil, dehydrogenase activity rose by roughly 8%. (Figure 1B). Furthermore, the biostimulant had a considerable impact on phosphatase activity in both sandy and sandy loam soils (Figure 1C), with an increase relative to control of roughly 90% and 77%, respectively (Figure 1C).

We looked at the biostimulant influence on catalase, dehydrogenase, and phosphatase activity since many soil processes and metabolisms are linked to enzyme activity. Dehydrogenase and phosphatase activity were boosted by biostimulant administration. Other research have shown similar outcomes. The use of organic fertilizers, as opposed to just inorganic ones, encourages soil microbial development and boosts enzymatic activity in this way. A soil amendment with biostimulants has been shown to result in an increase in enzyme activity because organic substrates are introduced, which encourage microbial growth and enzyme production. Another research found that soil microorganisms degraded the biostimulant's organic acids, leading to an increase in enzymatic activity. Because soil is the richest carbon source, this led to an increase in the induction of microbial metabolism. Most biostimulants, on the other hand, have a large proportion of proteins in them, which means that they significantly boost soil enzyme activity, alter the soil microbial population, and have a significant influence on soil biological characteristics. Both soil microbial activity and organic matter increased in our research, as did dehydrogenase and catalase activities in both soils with biostimulant. As a result of this, soil dehydrogenase activity fluctuations are an excellent indication of soil microbial activity. Different chemical and biological cycles, such as carbon, nitrogen, and phosphorus, are represented by the activities of dehydrogenases and catalases in soil. It has also been utilized as an indicator of soil fertility and dehydrogenase activity in the study of catalase. Both soils showed increased phosphatase activity when biostimulant was applied (sandy loam and sandy soil). Hydrolytic enzymes are driven by signals from these enzymes under nutrient-limited development circumstances because of this enzyme activity. Biostimulants have a good influence on soil enzyme metabolism and processes, which in turn improve soil characteristics and plant development.

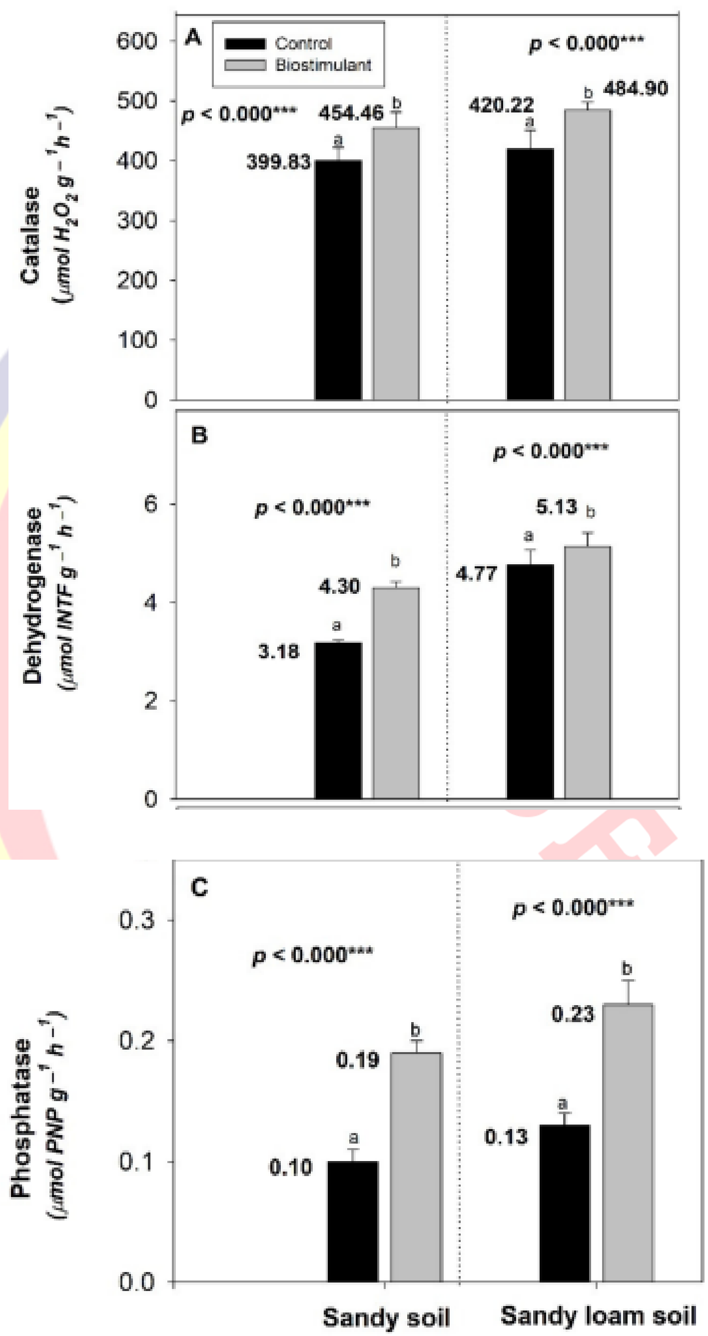

Figure 1. Mean values of catalase (A), dehydrogenase (B), and phosphatase (C) enzymatic activities of soil samples treated by biostimulant and control from the two types of soils.

Canonical Multivariable Analysis

A canonical analysis (Figure 2) of all parameters studied was achieved with the two types of soils and the two treatments (Control and biostimulant). The two axes 1 and 2 explained together 99% of the total variance in the two types of soils and variables. The results showed that root length (RL) was associated with microbial activity, organic matter, and catalase activity (Figure 2). Moreover, RL was also related to Ca2+, Mg2+, P, and K+, whereas pH and EC were not associated with roots growth.

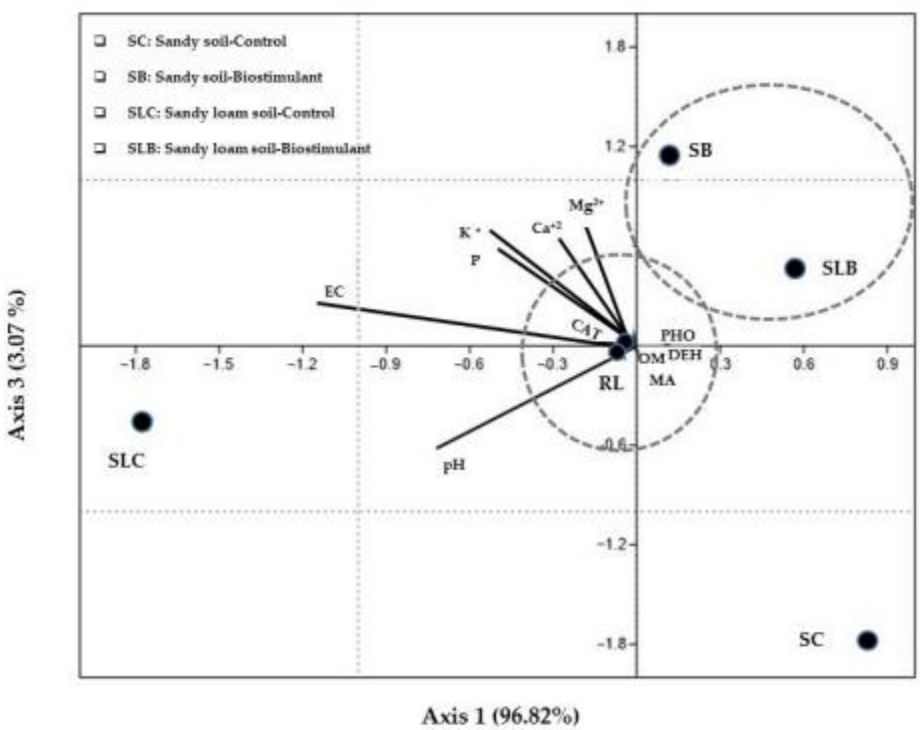

Figure 2. Canonical analysis using, as a variable, all parameters measured for the two types of soil. CAT, Catalase; PHO, Phosphatase; DEH, Dehydrogenase; MA; Microbial activity; OM, Organic matter; RL, Root length.

In turn, the two types of soil with biostimulant application corresponded closely to enzyme activity and Ca2+, Mg2+, P, and K+ . However, the control of both soils (without application of biostimulant) was separated from the two soils with biostimulant and from enzymes activities and nutrient elements.

CONCLUSION

Growing fruit and vegetables is made easier with the use of biostimulants, which are naturally occurring substances. Despite the fact that biostimulants have been thoroughly studied in recent years and shown to have a good influence, they are seldom included into conventional technical advancements. As a result of this, farmers are concerned about rising expenses and a decrease in plant quality and quantity, which eventually affects the profitability of their crops. In order to maximize yields and quality for a certain plant variety, biopreparations must be prioritized and biostimulant seedlings must be established using biostimulant PBs. Biopreparations that provide a wide range of services, are easy to use, and may be combined with other agents are in high demand in the market. In addition to improving soil quality and yield, biostimulants have no adverse effects on humans, animals or the environment. They also enhance the biodiversity of beneficial microorganisms and have a good effect on human health and the environment. A commercial usage of biostimulants would reduce the quantity of mineral fertilizers put into the environment, thereby lowering soil, water, and air pollution. Global warming is a very pressing issue; thus, this is extremely critical.

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