

HARNESSING MICROBIAL BIOSTIMULANTS FOR ENHANCED FOOD FUNCTIONAL QUALITY: A PROMISING TOOL

Abstract

Plants exhibit a remarkable diversity of chemical compounds and the collaborative relationship between microbes and plants often triggers the synthesis of various secondary metabolites in plants. Interestingly, the interactions between microorganisms and plants can activate a series of processes, from signal reception to metabolic responses, aiding the host plant in overcoming both environmental and biological stresses. In accordance with EU Regulations 2019/1009, the utilization of non-pathogenic and non-toxigenic beneficial microbial communities, including bacteria, fungi, viruses, nematodes, and protozoa, in agriculture has been permitted. These microorganisms, known as microbial biostimulants, are gaining increasing attention as a sustainable approach to enhance plant growth and yields. The application of microbial biostimulants has been linked to elevated levels of compounds such as terpenes, phenolics, and nitrogenated compounds, as well as numerous bioactive substances. These health-promoting compounds play a significant role in human nutrition, improve sensory characteristics like aroma and color, as well as the nutritional value of food products. Surprisingly, the role of microorganisms in enhancing food quality remains relatively understudied. This review explores plant-based experiments that investigate the impact of microbial biostimulants on the production of high-quality food.

Keywords: microbial biostimulants; phenolic compounds; carotenoids; ascorbic acid; secondary metabolites; food quality.

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I. INTRODUCTION

To meet the growing demand for food in 2050, there is a pressing need to increase agricultural production by approximately 60-70% from current levels. Unfortunately, the per capita arable land globally has dwindled substantially. This means that 80% of the additional food required must be sourced from existing cultivated lands. However, a significant challenge arises as approximately 33% of the Earth's soils are already degraded, with projections indicating that over 90% could face degradation by 2050. Additionally, the impact of climate change, including extreme weather events, the spread of pests and diseases, biodiversity loss, ecosystem degradation, and water scarcity, is set to worsen with global warming. Consequently, there is a growing need to reduce the use of agrochemicals, which can have adverse effects on human health and the environment. This necessitates the exploration and adoption of new strategies within the bio-based industry.

Bio-stimulants are substances or microorganisms that, when applied to plants or soil, enhance plant growth, development, and stress tolerance. They differ from traditional fertilizers in that they don't provide primary nutrients (nitrogen, phosphorus, potassium) but instead stimulate natural processes in plants. There has been a surge of interest on different categories of biostimulants among crop growers in recent years, that enhance crop productivity, mitigate environmental impact, and promote sustainable farming practices by improving nutrient use efficiency, stress tolerance and plant health. [1]

The term biostimulant was much debated during the last decade. Roupael and Colla [2] have provided an excellent review on the Plant biostimulants discussing the state of the art and evolution of the plant biostimulants. As per the new EU regulation (2019/1009) “A plant biostimulant shall be an EU fertilising product the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: i) nutrient use efficiency, ii) tolerance to abiotic stress, iii) quality traits, or iv) availability of confined nutrients in the soil or rhizosphere” [3]. Based on this definition Bio-stimulants can be classified as microbial and non-microbial. [2] The non microbial biostimulants are categorised based on their sources: Plant-Based Bio-stimulants such as humic acids, fulvic acids, amino acids, and protein hydrolysates; Seaweed Extract -*Ascophyllum nodosum* (kelp) extracts, laminarin, and alginates; Biostimulant Enzymes - catalase, amylase and cellulase; Beneficial Compound - silicon, chitosan and salicylic acid; Bioactive Compounds - jasmonic acid, gibberellins, and auxins. Microbial Bio-stimulants: Mycorrhizal fungi, rhizobacteria, and beneficial microorganism.

Over the last decade, the utilization of plant biostimulants in agriculture has surged significantly. More recently, there has been a growing focus on experimental studies that investigate the simultaneous application of plant biostimulants. This research is driven by the overarching goal of promoting global food security and environmental sustainability while avoiding an undue increase in nutrient consumption [4,5]. Recent studies use a combination of microbial and non-microbial biostimulants that exhibited a synergistic influence on plants. This synergy is characterized by heightened nutrient absorption, increased resilience to stress, enhanced root development, and improved phenological growth [6,7,8,9].

Colla and Rouphael [2] research has revealed a noteworthy trend in the field of biostimulants over the past decade (2009-2019). In this period, approximately 700 scientific papers have been published, encompassing both microbial and non-microbial biostimulants. A comprehensive analysis of these publications underscores a prevailing primary focus on the influence of biostimulants on crop growth, yield improvement and stress tolerance enhancement. Notably, there remains a gap in the exploration of how biostimulants contribute to enhancing the quality of food, suggesting an overlooked area of investigation in this domain. However, as consumers increasingly demand high-quality and nutritious food, there is a growing interest in studying how biostimulants can positively affect the nutritional value, flavor, aroma, and overall quality of crops. Despite the well-established connection between plant well-being and food quality, there is a notable dearth of information regarding the direct application of microbial biostimulants to enhance food quality. This deficiency in knowledge becomes apparent when examining recent bibliometric analyses, which reveal that non microbial biostimulant are frequently studied in the context of fruit quality, while microbial biostimulants remain relatively unexplored.

Research interests in this field are evolving, and the focus on specific aspects of microbial biostimulants in enhancing the quality of both plants and food products is being increasingly addressed. In their study Ganugi [10a,10b] emphasized the significance of microbial biostimulants in augmenting the production of secondary metabolites, such as phenolic compounds and carotenoids. This, in turn, has a notable impact on characteristics like aroma, color, and nutraceutical value. These compounds are known for their health benefits, including antioxidant and anti-proliferative properties. Additionally, ascorbic acid is mentioned for its role in preserving freshness and preventing spoilage due to its antioxidant activity and low pH. This potential research aims to bridge the gap between agricultural practices and the production of nutraceuticals[11,10a,10b].

The focus of this review is to provide an overview of recent studies that demonstrate improvements in food's functional traits when microbial biostimulants are applied to plants. Microbial biostimulants are often being referred as Plant probiotics. In this mini review we summarise the various functional classes and components presenting the elicitation effects of microbial biostimulants.

1. Effect of Biostimulants on Phenolic Compounds: Phenolic compounds are found in a wide range of foods, beverages, and dietary sources. These compounds belong to a group of organic substances characterized by the presence of at least one hydroxyl group directly bonded to one or more aromatic rings. The phenolic group is primarily responsible for their antioxidant properties, which are highly effective in reducing the harmful effects of reactive oxygen species and radical compounds [12].

Phenolic compounds have long piqued interest due to their significant contributions to both the color and flavor profiles of food. However, in recent times, the primary focus on these compounds has shifted towards their demonstrated bioactivity, which encompasses a range of health benefits such as antioxidative, antimicrobial, and anticarcinogenic properties. The phenolic family is incredibly diverse, encompassing a multitude of compounds, spanning from the simple monocyclic phenolic acids to the complex flavonoids. These flavonoids can be further categorized into numerous classes, including flavone, flavanone, flavonol, anthocyanin, isoflavone, and more, and they can

exist as aglycones or glycosides. Among the remarkable polyphenols, anthocyanins stand out as the largest group of water-soluble pigments found in nature. They are responsible for the vibrant and intense colors observed in various fruits and flowers [13].

Many vegetable crops experience significant advantages through their symbiotic relationship with mycorrhizal fungi (AMF), which not only enhance their nutrient uptake but also bolster their resilience against various biotic and abiotic stresses. This enhancement is thought to be achieved, at least in part, through the modulation of plant secondary metabolism [14]. Consequently, AMF may play a pivotal role in elevating the production of health-promoting phytochemicals such as polyphenols, carotenoids, flavonoids, and phytoestrogens. Additionally, this symbiotic relationship can lead to an increased activity of antioxidant enzymes [15,16]. Avio [15] investigated the impact of inoculating two varieties of lettuce, one with red leaves and the other with green leaves, using different arbuscular mycorrhizal fungi (AMF) species, *Rhizoglyphus irregularis* and *Funneliformis mosseae*. The focus was on assessing changes in the total phenolic and anthocyanin content, as well as the antioxidant activity of the leaf tissue. The findings revealed that when compared to non-inoculated plants, both cultivars exhibited a significant increase in antioxidant activity and phenolic content when inoculated with *R. irregularis*.

Additionally, in the case of red leaf lettuce, the presence of anthocyanins was more pronounced in inoculated plants compared to control plants. These results collectively suggest that *R. irregularis* exhibited a more pronounced influence on plant metabolism than *F. mosseae*. Moreover, the study suggests that mycorrhizal inoculation could serve as a valuable approach to enhance the concentration of phenolic compounds in leaf-type lettuces, provided that the appropriate AMF species is carefully selected.

A similar study by Abdelhalim [17] the influence of Arbuscular Mycorrhizal Fungi (AMF) on the phytochemical compounds and antioxidant capabilities of four sorghum varieties—Hakika, P954063, Tabat, and Tetron—was examined. Across all cultivars, there was a notable and statistically significant increase ($p < 0.05$) in the levels of total phenolic, flavonoid, carotenoids, and tannins in sorghum grains treated with AMF. Furthermore, AMF-inoculated sorghum grains exhibited enhanced antioxidant properties in terms of DPPH, TRP, and FRAP activities, again showing significant increases in comparison to non-inoculated grains ($p < 0.05$).

- 2. Effect of Biostimulants on Carotenoids :** Carotenoids, which are terpene pigments ranging in color from yellow to red, are synthesized by photoautotrophic organisms. While there is limited research on the impact of microbial biostimulants on terpenoids, carotenoids, within this category, have garnered significant attention due to their crucial role in human health. The impact of two commercial probiotics on the productivity and quality of carrots was studied by Gaveliené [20]. The commercial probiotic preparation ProbioHumus, is a composition of microorganisms: *Bacillus subtilis*, yeast *Saccharomyces cerevisiae*, lactic acid bacteria *Bifidobacterium animalis*, *B. bifidum*, *B. longum*, *Lactobacillus diacetylactis*, *L. casei*, *L. delbrueckii*, *L. plantarum*, *Lactococcus lactis*, *Streptococcus thermophilus*, phototropic bacteria *Rhodospseudomonas palustris*, and *R. sphaeroides*. Foliar application of the commercial formulation promoted the accumulation of essential compounds like monosaccharides, ascorbic acid, carotenoids,

and phenols while enhancing antioxidant activity. Notably, when compared to nonorganic farms, organic farming methods led to approximately a twofold reduction in nitrate content in carrots. In the assessment of carrot quality, a critical factor to consider is the carotenoid content. Research has indicated that the use of organic fertilizers can have a positive impact on the levels of important nutrients like β -carotenes. Additionally, when exploring the influence of microbial plant biostimulants on enhancing product quality, Chandrasekaran and coauthors [19] reported in their study that the introduction of a specific PGPR strain, *Bacillus subtilis* CBR05, led to a noteworthy improvement in tomato quality, particularly in terms of carotenoid. Inoculation of *B. subtilis* CBR05, a beneficial soil bacterium, resulted in a substantial increase in the total phenol and flavonoid contents of tomato fruits, surpassing levels observed in both control plants. Additionally, this inoculation led to the promotion of antioxidant activities and elevated the levels of carotenoids (specifically β -carotene and lycopene) in the plants. Notably, the use of *B. subtilis* CBR05 yielded the highest lycopene content, reaching 21.08 $\mu\text{g/g}$ FW, in tomato fruits. These findings underscore the versatility of the PGPR strain *B. subtilis* CBR05 in enhancing tomato production by augmenting antioxidant activities and increasing carotenoid levels in the fruits [19].

- 3. Effect of Biostimulants on Ascorbic Acid:** In the context of enhancing the ascorbic acid content in Solanaceae plants, particularly tomato and pepper (*Capsicum annum L.*) fruits, the use of microbial biostimulants has shown promise as an environmentally friendly approach. One successful approach involves the inoculation of Arbuscular Mycorrhizal Fungi (AMF) in combination with *Pseudomonas sp.* 19Fv1T. This combination of microbial biostimulants, AMF and *Pseudomonas sp.* 19Fv1T, has demonstrated positive results in increasing the ascorbic acid content of tomato fruits. It's worth noting that AMF are beneficial soil fungi that form symbiotic relationships with many plants, aiding in nutrient uptake and overall plant health. This approach likely involves a cooperative interaction between these beneficial microbes and the plants, leading to improved ascorbic acid production in tomato and pepper fruits. The exact mechanisms and pathways by which these microbes enhance ascorbic acid content may involve improved nutrient availability, protection against stressors, or other physiological changes in the plants. [10b,18]
- 4. Effect of Microbial Biostimulants on Manipulation of Fatty Acids:** The impact of two commercial bacterial strains -*Azospirillum* and *Azotobacter* on the fatty acid composition of bajra (*Pennisetum glaucum*) seeds was investigated [20]. The objectives of the study were twofold: (i) to assess how biopriming influences the manipulation of fatty acid composition, which is indicative of the quality of seedlings or sprouts, and (ii) to determine whether the seed priming process improves or inhibits the rate and uniformity of germination. The findings revealed that the stimulants had a significant impact on the fatty acid composition, leading to an increase in unsaturated fatty acids (UFA) and a reduction in saturated fatty acids (SFA). Specifically, the study showed that palmitic acid decreased by 17% in the hybrid variety and 25.1% in the local variety following priming. In contrast, linoleic acid increased by 20% in the hybrid variety and 43.2% in the local variety, while linolenic acid increased by 95.6% in the hybrid variety and 39.2% in the local variety. Additionally, the ratio of unsaturated fatty acids (TU) to saturated fatty acids (TS) increased by 40.7% in the hybrid variety and 54.1% in the local variety. The results of this study also highlighted that linoleic acid, a fatty acid from the omega-6

series, was the most prominent component in both hybrid and local bajra seed varieties. Overall, microbial stimulation appeared to modify the n-3 fatty acid profile in bajra seeds and promote the speed and rate of germination. The increased levels of linoleic and linolenic fatty acids resulting from seed priming are indicative of improved seed quality, as these fatty acids are qualitative indicators of food quality. Consequently, seed priming can be considered an effective option for producing high-quality seeds, ultimately contributing to improved agricultural yields and potentially enhanced human health through the consumption of these nutrient-rich seeds.

II. CONCLUSIONS, CHALLENGES AND LIMITATIONS OF BIO-STIMULANT USE

The application of microbial biostimulants to plants has enabled the production of high-quality foods, leading to an augmentation in the concentration of essential functional compounds known for their significant role in promoting human health and active aging. Recent studies exploring the relationship between this agricultural input and food quality consistently underscore an increased presence of bioactive compounds in the treated samples.

Bio-stimulants often lack standardized definitions and regulations, leading to variability in product quality and efficacy. There's still a limited understanding of the complex mechanisms behind bio-stimulant action on plants and their interaction with the environment. Bio-stimulant effects can vary depending on plant species, environmental conditions, and application methods, making consistent results challenging. Bio-stimulants may have shorter shelf lives compared to traditional chemical fertilizers, impacting their storage and usability. Regulatory frameworks for bio-stimulants vary globally, making it difficult to ensure compliance and market access. The environmental impact of bio-stimulant production and application needs further assessment to ensure sustainability.

III. POTENTIAL AREAS FOR FUTURE RESEARCH AND DEVELOPMENT

Research should focus on a deeper understanding of the molecular and physiological mechanisms of bio-stimulants in plants, which can enhance their targeted application. Customized bio-stimulants tailored to specific crops and environmental conditions can maximize their effectiveness. Global standards and regulations for bio-stimulants ensure product quality, safety, and consistency. Explore Sustainable sourcing methods need to be for bio-stimulant ingredients to minimize environmental impact. A collaboration between researchers, industry stakeholders, and regulatory bodies advances the field of bio-stimulant development and ensure its sustainable future.

REFERENCES

- [1] G. Colla and Y. Rouphael, "Biostimulants in horticulture," *Sci. Hortic*, 196, 1–2, 2015. doi: 10.1016/j.scienta.2015.10.044.
- [2] Y. Rouphael and G. Colla, "Editorial: Biostimulants in Agriculture", *Front. Plant Sci.*, Volume 11, 2020. <https://doi.org/10.3389/fpls.2020.00040>.
- [3] EU. "Regulation of the European Parliament and of the Council 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", 2019. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2019:170:TOC>.

- [4] M.J.Van Oosten, O.Pepe, S.De Pascale, S.Silletti, and A.Maggio, “ The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants,” *Chem. Biol. Technol. Agric.*, 4, 5, 2017.
- [5] F.Sestili, Y.Rouphael, M. Cardarelli, A.Pucci, P.Bonini, R.Canaguier, and G.Colla, “Protein hydrolysate stimulates growth in tomato coupled with N-dependent gene expression involved in N assimilation,” *Front. Plant Sci.*, 9, 1233, 2018.
- [6] A.M.Castiglione, G.Mannino, V.Contartese, C.M. Berteza, and A.Ertani, “Microbial biostimulants as response to modern agriculture needs: Composition, role and application of these innovative products,” *Plants*, 10, 1533, 2021.
- [7] Y.Rouphael, and G.Colla, “Synergistic biostimulatory action: Designing the next generation of plant biostimulants for sustainable agriculture.” *Front. Plant Sci.* 2018, 9, 1655.
- [8] L.E.B.Baldotto, M.A.Baldotto, L.P.Canellas, R.Bressan-Smith, and F.L. Olivares, “Growth promotion of pineapple ‘Vitória’ by humic acids and *Burkholderia sp.* during acclimatization,” *Rev. Bras. Cienc. Solo*, 34, 1593–1600, 2010.
- [9] M.M. Bettoni, Á.F.Mogor, V.Pauletti, and N.Goicoechea, “Growth and metabolism of onion seedlings as affected by the application of humic substances, mycorrhizal inoculation and elevated CO₂,” *Sci. Hort.*, 180, 227–235, 2014.
- [10] a). P.Ganugi, E.Martinelli, and L.Lucini, “Microbial biostimulants as a sustainable approach to improve the functional quality in plant-based foods: A review,” *Curr. Opin. Food Sci.*, 41, 217–223, 2021.
b). Ganugi, Fiorini, Vincenzo Tabaglio, Federico Capra, Gokhan Zengin, Paolo Bonini, Tito Caffi, Edoardo Puglisi, Marco Trevisan, and Luigi Lucini, “The Functional Profile and Antioxidant Capacity of Tomato Fruits Are Modulated by the Interaction between Microbial Biostimulants, Soil Properties, and Soil Nitrogen Status,” *Antioxidants*, 12(2), 520, 2023. <https://doi.org/10.3390/antiox12020520>.
- [11] Y.Rouphael, G.Colla, G.Graziani, A.Ritieni, M.Cardarelli, and S.De Pascale, “ Phenolic composition, antioxidant activity and mineral profile in two seed-propagated artichoke cultivars as affected by microbial inoculants and planting time,” *Food Chem*, 234, 10–19, 2017. doi: 10.1016/j.foodchem.2017.04.175.
- [12] M. Halpern, A. Bar-Tal, M. Ofek, D. Minz, T. Muller, and U. Yermiyahu, “The use of biostimulants for enhancing nutrient uptake,” *Adv. Agron*, 130, 141–174, 2015.
- [13] Drobek Magdalena, Magdalena Fraç and Justyna Cybulska, “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, 2019. <https://doi.org/10.3390/agronomy9060335>.
- [14] Sébastien Bruisson, Pascale Maillot, Paul Schellenbaum, Bernard Walter, Katia Gindro, and Laurence Deglène-Benbrahim, “Arbuscular mycorrhizal symbiosis stimulates key genes of the phenylpropanoid biosynthesis and stilbenoid production in grapevine leaves in response to downy mildew and grey mould infection”, *Phytochemistry*, 131,92-99, Nov 2016.
- [15] C. Avio, M. Sbrana, S.Giovannetti, and Frassinetti, “Arbuscular mycorrhizal fungi affect total phenolics content and antioxidant activity in leaves of oak leaf lettuce varieties,” *Scientia Horticulturae*, Volume 224, , Pages 265-271,October 2017. <https://doi.org/10.1016/j.scienta.2017.06.022>
- [16] Rabea Schweiger and Caroline Müller, “Leaf metabolome in arbuscular mycorrhizal symbiosis,” *Current Opinion in Plant Biology*, Volume 26, Pages 120-126, August 2015. <https://doi.org/10.1016/j.pbi.2015.06.009>.
- [17] Abdelhalim Tilal Sayed, A.J. Nouralhuda, A.Tia Khitma, Sir Elkhatim , Mazahir Hamid Othman , Rainer Georg Joergensen , Salah A. Almaman , and Amro B. Hassan, “Exploring the potential of arbuscular mycorrhizal fungi (AMF) for improving health-promoting phytochemicals in sorghum”, *Rhizosphere*, 24, 100596,2022.
- [18] Gavelienė, V. Šocik, B.Jankovska-Bortkevič, E, and Sigita Jurkonienė, “ Plant Microbial Biostimulants as a Promising Tool to Enhance the Productivity and Quality of Carrot Root Crops ,” 9(9): 1850, 2021.
- [19] Murugesan Chandrasekaran, Se Chul Chun, Jae Wook Oh, Manivannan Paramasivan, Ramesh Kumar Saini and Jesudoss Joseph Sahayarayan, “South Korea as a Novel Plant Probiotic Bacterium (PPB): Implications from Total Phenolics, Flavonoids, and Carotenoids Content for Fruit Quality,” *Agronomy*, 9(12), 838,2019. <https://doi.org/10.3390/agronomy9120838>.
- [20] P. Sunitha, K. Aruna Lakshmi, and K. V. Narayana Saibaba, “Application of Response Surface Methodology for the Optimization of Growth of Pearl Millet,” *British Microbiology Research Journal*, 6(3): 154-166, 2015.