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Bio-stimulants and its influence on growth and yield of millets: A review

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Abstract
Bio-stimulants are a diverse group of substances and microorganisms which when applied to plants or soil stimulate natural processes to enhance nutrient uptake, stress tolerance and overall plant growth and development. They are classified mainly as acids which include humic substance and amino acid substances, microorganisms which include beneficial fungi and bacteria, seaweed extracts. Bio-stimulants plays an important role in enhancing soil health, mitigating abiotic stress and improving plant resilience to environmental challenges such as drought, salinity and disease. In recent years, there has been a growing interest in bio-stimulants as sustainable alternatives to traditional agricultural practices driven by their ability to improve crop productivity while reducing reliance on synthetic inputs. Bio-stimulants enhance millets growth and yield by stimulating root development, improving nutrient uptake efficiency and promoting physiological processes like photosynthesis and stress tolerance. Current challenges and opportunities in bio-stimulant research emphasizing the need for standardized testing protocols and knowledge dissemination to support their widespread adoption in sustainable agriculture. This review provides an overview of the various types of bio-stimulants including humic substances, seaweed extracts and beneficial microorganisms and the influence of different bio-stimulants on the growth and yield of millets.

Keywords: Bio-stimulants, millets, humic substances, seaweed extract, beneficial microorganisms

Introduction
Millets play a crucial role in agriculture and food security due to their superior nutrients and climate resilience properties. They are rich in minerals, B-vitamins and antioxidants, making them nutritious and climate change-compliant crops. Millets can thrive in extreme conditions like drought and flooded areas and they have a low glycemic index, gluten-free protein and high efficiency of water use making them environmentally friendly (Antony Ceasar and Maharajan, 2022) [1]. The world is facing agrarian and nutritional challenges and millets have the potential to become new staple crops especially in hunger hotspots (Priya *et al.*, 2023) [48]. They might support equitable and sustainable agricultural growth, contributing to global food and nutritional security. However, there are challenges in millet cultivation and sustainable solutions are needed to further increase grain production, reduce climatic uncertainties and mitigate climate change (Kumar *et al.*, 2018) [29]. Therefore, promoting millets is critical for reducing over-reliance on commonly grown crops, boosting diverse diets, and enhancing food security.

Plant bio-stimulants in agriculture refers to substances of biological origin or microorganisms which when applied to plants either through soil application, root drench, foliar spray or in combinations is intended to stimulate natural processes in the plant that is responsible for efficient plant nutrient use efficiency and growth processes and/or an increase in the tolerance to abiotic and biotic stress, irrespective of the plant-beneficial nutrient content of the substances (Yakhin *et al.*, 2017) [67]. These bio-stimulants play a significant role in improving the general health, vitality and growth of plants as well as protecting them against infections (Drobek *et al.*, 2019) [15].

Bio-stimulants are linked to millets through their potential to enhance stress tolerance, improve nutritional quality and promote sustainable agriculture. Research has shown that bio-stimulants can help millet plants to tolerate stress factors like salinity, contributing to sustainable cultivation in the face of climate challenges. Additionally, bio-stimulants can enhance the nutritional content of millets, making them more attractive for both farmers and consumers. Furthermore, the use of bio-stimulants supports sustainable agricultural practices by reducing the need for synthetic fertilizers. Overall, bio-stimulants offer promising benefits for the cultivation of millets, addressing the need for resilient and nutritious crops in the context of global food security.

Classification of Bio-Stimulants

I. Acids

(i) Humic substances

Humic substances are diverse organic molecules generated in soil through microbial breakdown of deceased organic material (Nardi *et al.*, 2007) [37-38]. They represent one of the most prevalent organic compounds globally (Sutton and Sposito, 2005) [62], constituting approximately 60 per cent of soil organic matter worldwide (Muscolo *et al.*, 2007) [37]. Formerly perceived as large interconnected polymers, it's now widely accepted that humic substances comprise numerous small organic compounds held together by hydrophobic interactions and hydrogen bonds (Piccolo., 2002; Simpson *et al.*, 2002; Sutton and Sposito., 2005) [42, 60, 62].

Humic substances can be harvested from various sources, including soil (Nardi *et al.*, 2000; Varanini *et al.*, 1993; Zandonadi *et al.*, 2007) [39, 65, 69], municipal waste (Ayuso *et al.*, 1996) [3], vermicompost and earthworm casts (Canellas *et al.*, 2002; Russell *et al.*, 2006) [9, 53], different coal deposits (Kulikova and Perminova., 2002) [28] and peat (Ayuso *et al.*, 1996; Schmidt *et al.*, 2007) [3, 57].

(ii) Amino acid substances

Commercially available amino acid bio-stimulants are mostly mixtures of different amino acid and short peptides, rather than pure substances (Du Jardin, 2012) [16]. These mixtures called protein hydrolysates. These are derived from the hydrolysis of proteins from plant (Schiavon *et al.*, 2008) [56], animal (Maini, 2006) [34] and microbial sources (Du Jardin, 2012) [16], often from industrial and agricultural waste products such as crop residues (Du Jardin, 2012) [16], animal skin (Vasileva-Tonkova *et al.*, 2007) [66] and feathers (Grazziotin *et al.*, 2007) [21].

II. Micro-Organisms

Microorganisms in the soil play a pivotal role in plant growth by influencing rhizosphere-associated processes. Plant growth receives significant stimulation from microorganisms such as mycorrhizal fungi and bacteria. The introduction of microbial inoculants into the soil contributes positively to the soil biome found to be environmentally friendly. The majority of bio-stimulant microorganisms originate from beneficial fungal groups, notably arbuscular mycorrhizal fungi, and free-living bacteria. Among these, plant growth-promoting bacteria (PGPB) and plant growth-promoting rhizobacteria (PGPR) isolated from plant rhizospheres, exhibit the capability to function as bio-fertilizers, thereby fostering plant growth (Kumari *et al.*, 2023) [30].

(i) Beneficial fungi

Fungal-based products applied to plants for enhancing nutrition efficiency, stress tolerance, crop yield and product quality are deemed to be categorized as bio-stimulants (Dalpé and Monreal, 2004) [14]. However, their widespread application faces significant challenges, primarily due to the technical complexities associated with the large-scale propagation of arbuscular mycorrhizal fungi (AMF) owing to their biotrophic nature and the fundamental gaps in understanding the factors governing host specificities and population dynamics of mycorrhizal communities in agroecosystems. Nevertheless, certain fungal endophytes, such as Trichoderma spp. (Ascomycota) and Sebacinales (Basidiomycota) distinct from mycorrhizal species, exhibit the capability to colonize roots and facilitate nutrient transfer to their hosts through poorly elucidated mechanisms (Behie and Bidochka, 2014) [5]. These endophytes, increasingly recognized as plant inoculants easier to propagate *in vitro* and as model organisms for exploring nutrient transfer mechanisms between fungal endosymbionts and plants are gaining attention. Among them, Trichoderma spp., extensively studied for their bio pesticidal (mycoparasitic) and biocontrol (inducer of disease resistance) properties have also been harnessed by biotechnological industries as enzyme sources (Mukherjee *et al.*, 2013; Nicolás *et al.*, 2014) [36, 40]. Moreover, substantial evidence suggests that these fungal endophytes induce various plant responses including enhanced tolerance to abiotic stress, improved nutrient utilization efficiency and promotion of organ growth and morphogenesis (Colla *et al*., 2015; Shoresh *et al*., 2010) [12, 58]. Consequently, based on these effects these fungal endophytes could be regarded as bio-stimulants, although their agricultural applications currently rely on assertions primarily as biopesticides.

(ii) Beneficial bacteria

Plant Growth-Promoting Rhizobacteria (PGPRs) exhibit multifunctionality, exerting influence across various facets of plant life including nutrition, growth, morphogenesis, development and responses to both biotic and abiotic stress, as well as interactions within agroecosystems (Du Jardin *et al*., 2015) [17]. While many of these functions are typically carried out by the same bacterial strains some are specific to particular strains, while others rely on synergies within bacterial consortia. The agricultural utilization of PGPRs faces constraints due to this intricate complexity along with the variable responses exhibited by different plant cultivars and environmental conditions. Furthermore, technical challenges associated with the formulation of PGPR inoculants contribute to inconsistent practical outcomes (Arora *et al*., 2011; Brahmaprakash and Sahu, 2012) [2, 8]. Despite these obstacles, the global market for bacterial bio-stimulants is expanding with PGPR inoculants now being recognized as a kind to "plant probiotics," demonstrating efficacy in enhancing both plant nutrition and immunity (Berendsen *et al*., 2012) [6].

III. Seaweed Extracts

Seaweed has been utilized as a fertilizer in coastal areas for centuries (Craigie, 2011) [13]. The first patented method for converting seaweed into a liquid form for agricultural purposes dates back to 1912 (Booth, 1969) [7]. Typically derived from *Ascophyllum nodosum,* a brown seaweed prevalent in the North Atlantic, liquified seaweed is the primary product used, although other species such as *Durvillaea antarctica, Durvillaea potatorum, Ecklonia maxima and Macrocystis pyrifera* are also employed (Khan *et al.*, 2009) [26].

Seaweed extracts contains various plant nutrients and its application to plants cultivated in deficient soils can enhance growth and nutrient absorption due to the increased availability of these nutrients. Positive impact on potassium uptake (Beckett and Van Staden., 1989) [4] was noted when seaweed extract was subjected to ash at high heating at 450 °C for 1 hour.

Table 1: Seaweed application methods to increase tolerance towards drought.

Seaweed Description	Application Methods	Effects on Crop Performances	References
A. nodosum (alkaline extraction)	Foliar or drench	Foliar applications were effective in fostering photosynthetic rate after recovery.	Frioni *et al*., 2021 [19]
A. nodosum (alkaline extraction)	Foliar	Increased water conductivity, lower leaf temperature during stress, faster recovery of photosynthetic capacity	Tombesi *et al*., 2021 [63]
Seaweed (undefined origin) containing Alginic acid 16%	Foliar	Increased ABA, proline, total phenol and soluble carbohydrates	Irani *et al*., 2021 [23]
A. nodosum (alkaline extraction)	Foliar or drench spray, once per week	Increased vegetative growth and water use efficiency.	Spann *et al*., 2010 [61]
Seaweed origin not defined	Foliar	Improved fruit quality.	Kapur *et al*., 2018 [25]

IV. Other Bio-Stimulants

Inorganic salts containing beneficial elements such as aluminium, silicon, sodium, selenium and cobalt in forms like chlorides, carbonates, silicates, and phosphates serve not only as essential nutrients but also contribute to plant growth and improve physiological conditions, thereby enhancing resistance to abiotic stress (Pilon-Smits *et al*., 2009) [44]. Various sources including industrial and agricultural waste, manures, vermicompost and sewage waste function as bio-stimulants (Yakhin *et al*., 2017) [67]. Additionally, polypeptides, oligopeptides and amino acids found in hydrolysed protein-rich waste components act as bio-stimulants.

Mechanism Action of Bio-Stimulants

Hormonal Effects

The ability of bio-stimulants to influence plant hormone levels and signalling pathways, leading to changes in plant growth and development. For example, bio-stimulants containing cytokinin is a type of plant hormone have been found to promote cell division and shoot growth, while those containing auxins have been found to promote root growth and development (Rouphael *et al.*, 2020) [51]. Additionally, bio-stimulants containing gibberellins have been found to promote stem elongation and flowering, while those containing abscisic acid have been found to enhance plant tolerance to abiotic stress.

Enhanced Plant Defence Mechanisms

Bio-stimulants can enhance plant defence mechanisms by stimulating the production of phytohormones, antioxidants and other compounds that protect plants from biotic and abiotic stressors. For example, bio-stimulants containing chitosan, a natural polymer derived from chitin have been found to enhance plant defence mechanisms against fungal and bacterial pathogens (Rouphael *et al.*, 2020) [51]. Additionally, bio-stimulants containing salicylic acid, jasmonic acid and other plant hormones have been found to enhance plant resistance to pests and diseases. Bio-stimulants can also enhance plant defence mechanisms by promoting the production of secondary metabolites such as flavonoids and phenolic compounds, which have antioxidant and antimicrobial properties.

Other Mechanisms

Mechanism

Reference

Improving soil structure and soil microbial activity Piccolo *et al.*, 1997 [43]

Increase activity of NO_3-assimilation Vaccaro *et al.*, 2009 [64]

Increase H^+ ATPase activity Zandonadi *et al.*, 2010 [70]

Changes in root morphology Malik and Azam 1985 [35]

Chelation or solubilization nutrients Chen *et al.*, 2004 [10]

Role of Bio-Stimulants

Improving Seed Germination and Early Root Growth:

Bio-stimulants are known to contribute better seed germination and induce biological activity of plants, promoting root and shoot growth as well as leaf area (Simoes *et al.*, 2022) [59]. They also improve microbial activity, organic matter and enzymatic activity in soil which can enhance root growth and establishment (Yousfi *et al.*, 2021) [68]. Studies revealed that fucoidan fractions of *Turbinaria decurrens* have been found to promote seed germination, organogenesis and adventitious root formation in finger millet and eggplant (Kaniyassery *et al.*, 2024) [24]. Few studies revealed that bio-stimulant did not significantly influence germination seed index under low-temperature thermal stress in millet (Popko *et al.*, 2018) [45].

Enhancing nutrient uptake and fertilizer efficiency

Bio-stimulants have been found to positively influence nutrient use efficiency (NUE) and photosynthetic physiological processes in plants, indicating their potential to enhance nutrient uptake and utilization (Castiglione *et al.*, 2021). Additionally, bio-stimulants promoted root and shoot growth, as well as leaf area, under unfavourable environmental stress conditions, which may contribute to improved nutrient availability for millet crops (Simoes *et al.*, 2022) [59]. Studies has revealed that the application of bio-stimulants has been associated with increased absorption and utilization of fertilizers in crop cultivation, potentially leading to improved crop yield and quality (Savarese *et al.*, 2022) [55].

Mitigating Abiotic Stress Resistance

The use of bio-stimulants in millet cultivation has been investigated as a strategy for mitigating abiotic stress particularly salinity and enhancing the tolerance of millet plants to unfavourable environmental conditions. Research has shown that bio-stimulants play an important role as alleviators of abiotic stress in sustainable agriculture, particularly in the context of salinity stress tolerance in millets (Mahadik and Kumudini, 2020) [33]. A study evaluating the influence of a bio-stimulant on growth of millets, specifically focusing on tolerance to salinity stress, the research showed that the combined application of bio-stimulant and nutsedge stimulated growth and forage production in millets (Simoes *et al.*, 2022) [59]. Application of microbial bio-stimulants have been recognized for their potential to ameliorate climate change-associated abiotic stresses on foxtail millets crops, indicating their broader applicability in mitigating various environmental stress including salinity, drought, cold, and heat stress (Fadiji *et al.*, 2022) [18].

Influence of Bio-Stimulants on Growth of Millets

Bio-stimulants enhance millets growth by stimulating root development, improving nutrient uptake efficiency and promoting physiological processes like photosynthesis and stress tolerance. They also improve soil health, fostering a conducive environment for millet growth, ultimately leading to increased yield and quality.

Foliar application of 0.1% humic acid and 3% panchagavya recorded significantly higher plant height, number of tillers per meter and leaf area in foxtail millet (Chethan *et al.*, 2022) [11]. Biogas slurry application along with 3% panchagavya spray at 30, 45 and 60 days after sowing enhanced the plant height, leaf area index and dry matter production in foxtail millet (Priya and Sathya, 2019) [47]. Application of 50% of recommended dose of nitrogen as soil application and 50% of recommended dose of nitrogen as foliar spray and application of 100% phosphorus and potassium through water-soluble fertilizer (at 20 and 40 days after transplanting) along with foliar application of humic acid 0.1% (at 20 and 40 days after transplanting) recorded significantly higher growth parameters *viz*., plant height, number of tillers plant^{-1} and dry matter production in finger millet (Gokul and Senthil, 2019) [20].

Hemalatha and Paramasivan (2020) [22] revealed that the application of 100% poultry manure in combination with foliar spray of 3% panchagavya at 30 and 45 days after transplanting was found to be superior with respect to growth characters in finger millet. Foliar application of 1% urea, 0.2% sulphate of potash and 0.1% humic acid along with recommended dose of fertilizer recorded significantly higher leaf area, leaf area index, leaf area duration, total dry matter accumulation, absolute growth rate, crop growth rate, relative growth rate of foxtail millet (Prashant *et al.*, 2022) [46].

Influence of Bio-Stimulants on Yield of Millets

The use of 75% recommended dose of nitrogen along with foliar application of panchagavya at 4% concentration and jeevamrut at the rate of 500 liters ha^{-1}, accompanied by irrigation at 30 and 45 days after sowing, resulted in a greater number of effective tillers per plant, earhead length, earhead girth, grain weight per earhead, 1000 grain weight, as well as grain yield and straw yield of summer pearl millet (Patel *et al.*, 2021) [41]. The leaf application (100%) of humic acid to common millet (*Panicum miliaceum L.*) gave a significantly higher grain yield than the control (Saruhan *et al.*, 2011) [54].

Methods of Application

Bio-stimulants can be administered at various stages of a crop's growth cycle from seedling to grain filling stages. The timing of bio-stimulant application depends exclusively on the crop type and growth stage. To optimize the use of bio-stimulants and streamline application, it is advantageous to apply them to seeds or seedlings. However, there are three common methods for bio-stimulant application (Reddy *et al.*, 2023) [50].

1. **Seeds:** Pre-sowing: Bio-priming or pelleting represents the most prevalent method of applying bio-stimulants of microbial origin before sowing seeds. Alternatively, protein hydrolysates or seaweed extract can also be used for seed treatment. This approach is particularly common for field-grown crops such as cereals and pulses application of bio-stimulant enhances seed germination, crop stand uniformity, plant vigour and yield. Seed priming prepares the crop plants for potential adverse growing conditions during subsequent growth stages.
2. **Seedlings:** Pre-transplanting: This method is commonly employed for raising seedlings intended for transplantation. Bio-stimulants can be applied in ways in nursery soil bed, growing media or through foliar spray, which include mycorrhizal fungi, Trichoderma, humic substances, protein hydrolysates, and plant growth-promoting bacteria (PGPB). Alternatively, water-soluble liquid-based bio-stimulants such as seaweed extracts, protein hydrolysates, and organic acids are applied through foliar application at specific intervals during the nursery stage. Both approaches ensure the production of high-quality, uniform, sturdy seedlings that perform well after transplanting in field conditions.
3. **Crops:** After sowing or transplanting: On-site application of bio-stimulants is conducted through fertigation and foliar application. Organic acids like humic and fulvic acids are particularly efficient in fertigation, stimulating root growth and mitigating transplanting shock. Inorganic compounds, protein hydrolysates, seaweed extracts, and plant extracts are applied through foliar spray, either once or multiple times, depending on the crop and growth stage. Foliar application is typically performed during active vegetative growth, flowering, and fruit set stages. In anticipation of stress conditions, bio-stimulants can be applied as a foliar spray to prepare crops to withstand adverse climatic conditions. This mode of application enhances flowering, fruit set quality and increases tolerance to abiotic stresses.

Challenges

Standardization of Bio-Stimulants

One significant challenge is the lack of standardization in bio-stimulant products. Many bio-stimulants are available in market in various forms i.e. soluble powder, granular form or liquid, and can be applied as foliar sprays or in soil near the root zone. However, the lack of standardized guidelines for their composition and application can lead to variability in their effectiveness making it difficult for farmers to select the most suitable products for their specific needs (Ma *et al.*, 2022) [32]. Need of standardization for measuring the effectiveness of bio-stimulants to distinguish the variation in their effectiveness and to ensure consistency and reproducibility in the beneficial effects they offer (Li *et al.*, 2022) [31].

The Economic Viability and Cost-Effectiveness:

The economic viability and cost-effectiveness of bio-stimulants present several challenges as evidenced by various research papers. Some of the key challenges include the need to minimize costs associated with the purchase and application of bio-stimulants as well as the importance of achieving good physiological effects with small doses to ensure higher yields and quality of crops ultimately leading to higher incomes for farmers (Kocira *et al.*, 2020) [27].

Future Perspectives and Research Directions

Potential for Bio-Stimulant Development

Exploring synergistic interactions for plant growth: The agricultural sector's pursuit of decreased environmental impact and increased efficiency has led to the exploration of synergistic interactions among plant bio-stimulants. In recent years, limited experimental studies on testing the additive and synergistic effects of various plant bio-stimulants categories have demonstrated that combinations of non-microbial is in need holistic studies. Plant bio-stimulants or microbial inoculants with humic acid, seaweed extracts or plant hormones give more reproducible benefits to plant growth and production.

Increasing the efficiency of bio-stimulant application: To maximize the benefits of bio-stimulants, it is essential to optimize their application methods, concentrations and time of application. Future research should focus on developing better-targeted products and delving deeper into the interactions of bio-stimulants with indigenous plant systems to achieve greater efficacy and wider adoption (Rouphael *et al.,* 2018) [52].

Long-Term Effects and Sustainability
Future research should focus on understanding the long-term effects of bio-stimulant applications on plant growth, soil health, and overall sustainability. Additionally, exploring the potential for bio-stimulants to reduce fertilizer requirements and protect crops from abiotic stress could lead to more sustainable and environmentally friendly agricultural practices (Rakkammal *et al.,* 2023) [49].

Conclusion
In conclusion, millets hold immense promise for enhancing global food security and promoting sustainable agriculture due to their nutrient density and resilience to adverse climatic conditions. Their ability to thrive in extreme environments and offer high nutritional value makes them ideal candidates for staple crops, especially in regions facing food scarcity. However, challenges in their cultivation necessitate innovative solutions for increased productivity and climate adaptability. Bio-stimulants emerge as a vital tool in this context, improving stress tolerance, nutrient uptake, and overall plant health, thereby supporting the sustainable growth of millets. By integrating bio-stimulants into millet cultivation, we can enhance crop resilience, reduce dependency on synthetic fertilizers, and promote agricultural sustainability. Addressing the standardization and economic viability of bio-stimulants is crucial for maximizing their benefits. Overall, the combined use of millets and bio-stimulants offers a promising pathway to secure food and nutrition for future generations, highlighting the need for continued research and development in this area.

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