Plant Archives Vol. 25, No. 1, 2025 pp. 2785-2793 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.1.403

EFFECTS OF HUMIC ACID ON WHEAT GROWTH AND YIELD: A REVIEW

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(Date of Receiving-29-12-2024; Date of Acceptance-10-03-2025)

ABSTRACT

Wheat (*Triticum aestivum* L.) is a crucial crop, providing 20% of caloricintake for many populations worldwide. Soil organic matter, an essentialcomponent of soil, directly influences soil fertility and texture. Humicsubstances, derived from biomolecules' physical, chemical, andmicrobiological transformation, are integral to soil humus. Humic acid as a part of Humus is a natural ingredient of soil and contributes toward improved biological properties of soil. Humic acid hasbecome a standard method for enhancing crop growth, yield and soil fertility. Humic acid is an organic compound that is formed when biomass residues decompose and transform over a long period of time. It is a major component of organic matter in soil, peat, coal, and sediments. Humic acid can significantly increase the length of wheat roots and shoots. For example, a study found that a small concentration of humic acid in water increased root length by 500%. It can improve seed germination and seedling growth. Pre-sowing treatment with humic acid can stimulate germination. Humic acid can be used as a sustainable method for wheat production to reduce the negative effects of climate change and chemical fertilizers. Soil organic matter isone of the most essentialparts of the soil, and it directly affects soil fertility and texture. Humic substances are part of humus in soil organic matter produced from biomolecules' physical, chemical,and microbiological transformation. Humic acids are an essentialsoil component that can improve nutrient availability and impactother important chemical, biological, and physical properties of soils. Similarly, Humic acid not only positivelyaffects the growth and yield of plantsbut also affects grain quality. Humic acid is a natural biological organic that has a high effect on plant growth and quality.

Key words: Humic acid, wheat, organic, foliar application, bio-molecules

Introduction

Humic sustances (HSs) are biogenic, naturally occurring, heterogeneous organic compounds with a high molecular weight, resistance, and a yellow to black color. More prevalent organic components on Earth are called HSs, and they can be found in peat, brown coal, soil, water, lake sediments, and shales. Approximately 25% of the planet's organic carbon comes from HSs. These compounds are a class of naturally occurring complex molecular structures that cannot be classified as proteins, polysaccharides, or polynucleotides since they are the result of the breakdown of plant and animal wastes (Nardi *et al*. 2002). One of the most important abiotic factors influencing crop production worldwide is salinity. It has a number of detrimental impacts on the development and growth of several plants, including wheat(Saddiq *et al*., 2021). Natural soil salinity or the result of subpar human activity, such as farming practices, the growing use of saline water, and insufficient agronomic skills, can both occur(Ramlow *et al*., 2019). A high level of soluble saltsespecially NaCl cause salinity stress in soil and water (Hopmans *et al*., 2021, Parihar *et al*., 2015). It seriously harms agricultural productivity, especially in arid and semi-arid regions of the world (Liu *et al*., 2020). Saline soil reduces the development and productivity of many field crops such as rice, wheat, maize, cotton, sugarcane, and sorghum (Hussain *et al*., 2019, Chandio *et al*., 2017). The reduced productivity of salty soils can be due to a variety of factors, including salt toxicity and lack of organic matter and minerals, notably N, P, and K (Saddiq *et al*., 2019). Salt stress affects 20% of the world's cultivable soil and is steadily growing as an outcome of climate change and human activities (Arora *et al*., 2019). Soil salinization is expanding at a pace of 1–2 million hectares per year over the world (Khatar *et al*., 2018). There are numerous effective methods for improving salt-affected land, including the use of organic and inorganic amendments, water leaching, and phytoremediation, as well as proper agronomic practices such as crop diversification and sowing of tolerant cultivars (El-Hendawy *et al*., 2017). Wheat is an important source of food and outnumbers all other grain crops in terms of acreage and production (Abbas *et al*., 2013). It is the world's most important staple food and cereal grain crop and covers all of the major dietary requirements (Anwar *et al*., 2016). High salt concentrations have a negative impact on seed germination and plant development of wheat (Munns & Tester, 2008). Salinity drastically reduces the No. of spikes m^{-2}, No. of kernels spike-1, weight of a thousand kernels and grain yield of wheat crop (Osakabe *et al*., 2016). Several solutions to mitigate the effect of soil salinity on wheat growth and development have been proposed (Chabot & Tonde, 2011). Organic fertilizer and humic acid are presented as alternatives to artificial fertilization and quick supply of nitrogen. It in addition boosts plant growth and yield, stress tolerance, and soil physical characteristics (Khan *et al*., 2018). Humic acid treatment promotes root development, which is closely associated to improve absorption of macro and micronutrients (Araus *et al*., 2013).

Review of literature

Humic substances have an indirect and direct effect on plant development. Positive relationships between soil humus levels, plant yields, and product quality have been reported in several scientific journals. Indirect impacts include those that offer energy to beneficial organisms in the soil, influence the soil's water holding capacity, influence the soil's structure, release of plant nutrients from soft minerals, enhanced availability of trace minerals, and overall improved soil fertility. Changes in plant metabolism that occur as a result of the absorption of organic macromolecules such as humic acids and fulvic acids are examples of direct impacts. Taking into account the benefits of HSs and their potential impact on plant growth and development is reviewed in the following

Influence of HSs on seed germination

Humic compounds and their structural features can have a major impact on seed germination and plant development in the early stages. The absorption of humic compounds into seeds promotes seed germination and seedling growth (Ayuso *et al*. 1996). According to Adetumbi *et al*. (2019), priming rice seed with humic material can improve seedling growth and vigour in both highland and lowland rice cultivation. Fulvic acid had a substantial effect in promoting wheat seed germination. The effect of HSs on seed development due to regulating the amylase activity, which is related to respiration (Qin *et al*. 2016). Braziene *et al*. (2021)made the following observation: The application of fulvic acids for seed dressing consistently increased the eventual germination percentage while shortening the mean germination time in spring wheat, spring barley, and sugar beet. When planting treated seeds in soil, pre-sowing treatment with humic preparations has a notable stimulating impact on germination (Shoba *et al*. 2019). Nandhini *et al*. (2018) revealed that soaking of seeds with humic acid records

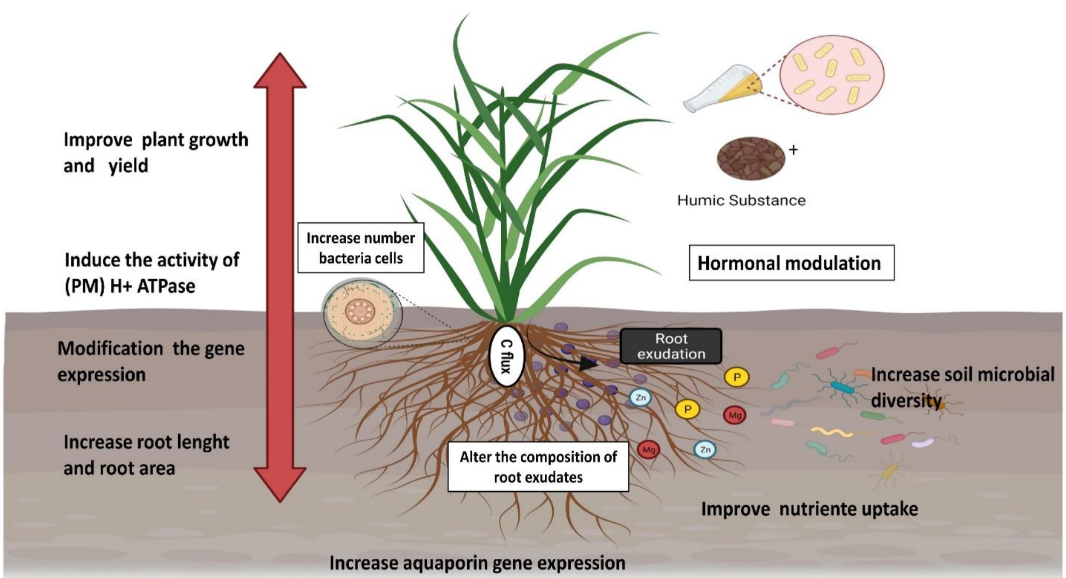

Fig. 1: Role of HSs in Plant development (Silva *et al*., 2021)

maximum seed germination, optimum shoot and root length of maize. Application rates of humic acids (HAs) or fulvic acids (FAs), required for improved seed germination, range from 20 to 100 mg/liter of seed. Humic compounds must be present within the cells of seeds in order for better germination to occur. As the humic substance enter the seed cells, respiration rate increases, and cell division processes are accelerated. These same respiratory processes enhance root meristem development and activate other growing points within the seedlings. In well-regulated tests, humic compounds have been shown to increase mitotic activity during cell division. The application of these humic compounds to seeds (seed treatment) or within the seed furrow improves seed germination and seedling development dramatically.

Humic substances have a strong impact on plant root development. When humic acids (HAs) and/or fulvic acids (FAs) are added to soil, root initiation and growth are improved. Humic acid, according to Mora *et al.* (2012), increased the quantity of secondary roots as well as root growth in cucumber. Humic substance application to roots enhances plasma membrane H+-ATPase activity, shoot mineral nutrient concentration, and ABA concentrations in roots (Nunes *et al*. 2019). Plants treated with HA had an increase in the quantity, diameter, and length of maize roots. This total improvement might be attributed to the activation of PM H+-ATPase, which is critical in ion absorption and the development of an electrochemical gradient, both of which are required in the growth process under acidic circumstances (De Hita *et al*. 2020). This enzyme combines ATP hydrolysis with H+ transport across the cell membrane, acidifying the apoplasts, loosening the cell walls, and lengthening cells, resulting in increased root growth (Hager *et al*. 1991). Plant root development is promoted to a larger extent in most experimental experiments than above ground plant components. The sort of humic material used had a substantial impact on the percentage of increase. In repeated trials, treated root weights ranged from 20 to 50 percent higher than non-treated root weights. When the smaller molecular components of fulvic acid (FA) are present at concentrations ranging from 10 to 100 mg/liter of solution, root stimulation occurs. Fulvic acids (FAs) boost growth even more when combined with humic acids (HAs) and other essential plant nutrients. Humic acids (HAs) and fulvic acids (FAs) should be used at low doses to promote plant development.

Humic Substances and their role in plant nutrition

The presence of HS in soil encourages root and shoot development by improving mineral nutrition under the soil surface. The activity of these compounds can be measured in terms of plant yield and active growth (Zandonadi *et al*. 2016). HS regulate plant growth and mineral absorption through complimentary and possibly different activities. These impacts are categorized as either direct or indirect (Vaughan and Malcom, 1985; Zandonadi *et al*. 2013). The activities of HS are primarily determined by their structural properties, functional groups, and tendency to interact with inorganic and organic ions and molecules found in the soil substrate (Garcia-Mina *et al*. 2004). Furthermore, the ability of HS to form complexes with metallic ions improves the availability of micronutrients (zinc, manganese, copper, and iron) and macronutrients (phosphorus), particularly when these nutrients are scarce in the soil (Garcia et al. 2016). Humic substances bind nutrients in a molecular form, reducing their solubility in water. These binding processes reduce nitrogen leaching into the subsoil and aid in preventing volatilization into the atmosphere. Other studies have found that increased uptake of calcium (Ca), and magnesium (Mg) when plants are irrigated with humic acids (HAs) or fulvic acids (FAs). Direct action of HS, on the other hand, is associated with their localised targeted and non-targeted actions at plant cell membranes, which can activate biochemical and molecular processes at the posttranscriptional levels in roots and shoots (Van Oosten *et al*. 2017).

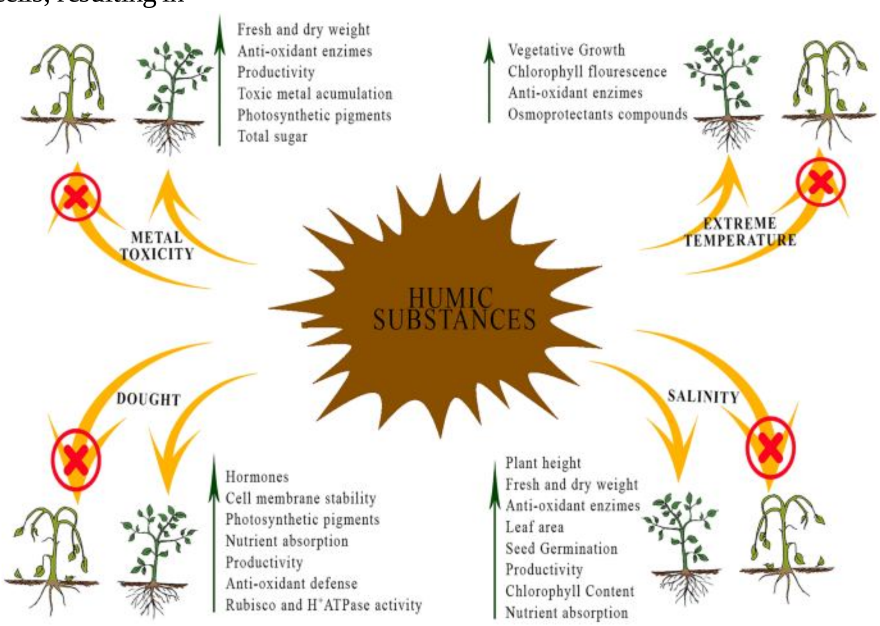

Fig. 2: Role of Humic Substances in Plants (Canellas *et al*., 2024)

In general, targeted actions of HS tend to increase plant absorption of macronutrients and micronutrients. Only a small percentage of 14-C labelled HS, particularly those with lower molecular weight, penetrate the root apoplastic pathway, according to Vaughan and Malcom (1985). As a result, this property can regulate the activity of HS in increasing nutrient absorption through molecular systems and signalling pathways found in root cell membranes (Asli and Neumann, 2010; Nardi *et al*. 2016). The full benefit of this type of direct influence has yet to be determined. However, it is possible that the non-specific action of HS on the leaf or root surface could influence molecular and biochemical processes by influencing events at both the transcriptional and post-transcriptional stages.

Promoting plant growth by Humic substances and plant growth-promoting Bacteria

Plants are highly plastic in development, which affords them sensitivity to respond to the most diverse environmental conditions. The presence of beneficial microorganisms, such as PGPB, and bioactive compounds, such as HSs, has provided the most favorable conditions for various agricultural systems. Some of the significant impacts of PGPB and HSs are improved nutrient acquisition, stimulation of root systems, and greater tolerance to stress (Olivares *et al*., 2017). Already, Til'ba and Sinegovskaya (2012) observed in the field that application of Na-humate to soybean seeds inoculated with *Bradyrhizobium* in the presence of molybdate, together with foliar application of Na-humate, was able to improve soybean yield in the field; higher values were found for number of nodules and BNF efficiency. The greater efficiency of nodulation in the presence of HSs may be linked to the ability of these substances to regulate quorum sensing (QS) in rhizobia. QS plays an essential role in the growth and development of legume symbiosis (Bogino *et al*., 2015; Koul *et al*., 2016).

Humic substances have already been reported to increase microbial growth, affecting the regulation of cellular metabolism (Table 1 and Fig. 1) (Kirschner *et al*., 1999; Tikhonov *et al*., 2010). One study evaluated the role of water-soluble humic materials in *Bradyrhizobium liaoningense*; under this condition, *B. liaoningense* showed a gene profile similar to that found for the same strain in the presence of flavonoids (Gao *et al*., 2015). Flavonoids are molecules responsible for activating the expression of genes in rhizobia that are essential for initiating the symbiosis process (Oldroyd *et al*., 2011). In addition, greater expression of nod and nif genes and nitrogenase complex formation were observed in free-living bacteria when in contact with this substance. Greenhouse experiments confirmed a greater increase

Table 1: Studies examining bacteria associated with humic substances.

Microorganisms	Humic substance	Biological effect	References
Azotobacter chroococcum	Na-humate and fulvic acid	Na-humate and fulvic acid increase cell growth in Azotobacter chroococcum.	Gaur and Bhardwaj, 1971
Pseudomonas sp	Fulvic acid	Fulvic acid increased cell yield and cell yield per µl O_2 taken up by Pseudomonas.	De Haan, 1974
Klebsiella aerogenes	Humic acid	increased the survival of Klebsiella aerogenes exposed to ultraviolet irradiation (UV).	Bitton *et al*., 1972
Mycobacterium avium	Humic acid and fulvic acid	Soil Humic acid and fulvic acid stimulated cell growth of Mycobacterium avium.	Kirschner *et al*., 1999
Bacillus subtilis	Humic acid	Humic acid increased the number of B. subtilis immobilized in alginate beads.	Young *et al*., 2006
Bacteria in soil and in the digestive tracts of earth worms	Humic acid	Humic acid stimulated bacterial growth.	Tikhonov *et al*., 2010
Bradyrhizobium liaoningense	Water-soluble humic materials (WSHM)	WSHM stimulated cell growth and metabolism, including nodulation-related proteins and BFN	Gao *et al*., 2015
Streptomyces sp.	Humic acid	Humic acid stimulated both growth and the ability of Streptomyces sp. to solubilize rock phosphate.	Farhat *et al*., 2015
Sinorhizobium meliloti	Water-soluble humic materials	WSHM regulate the quorum sensing and increased cell density of Sinorhizobium meliloti.	Xu *et al*., 2018
Sinorhizobium meliloti	Commercial fulvic acid	Fulvic acid stimulated cell growth of Sinorhizobium meliloti	Capstaff *et al*., 2020
Bradyrhizobium sp.	K-humate from leonardite	Increased Bradyrhizobium spp. survival in soybean seeds.	da Silva *et al*., 2021b

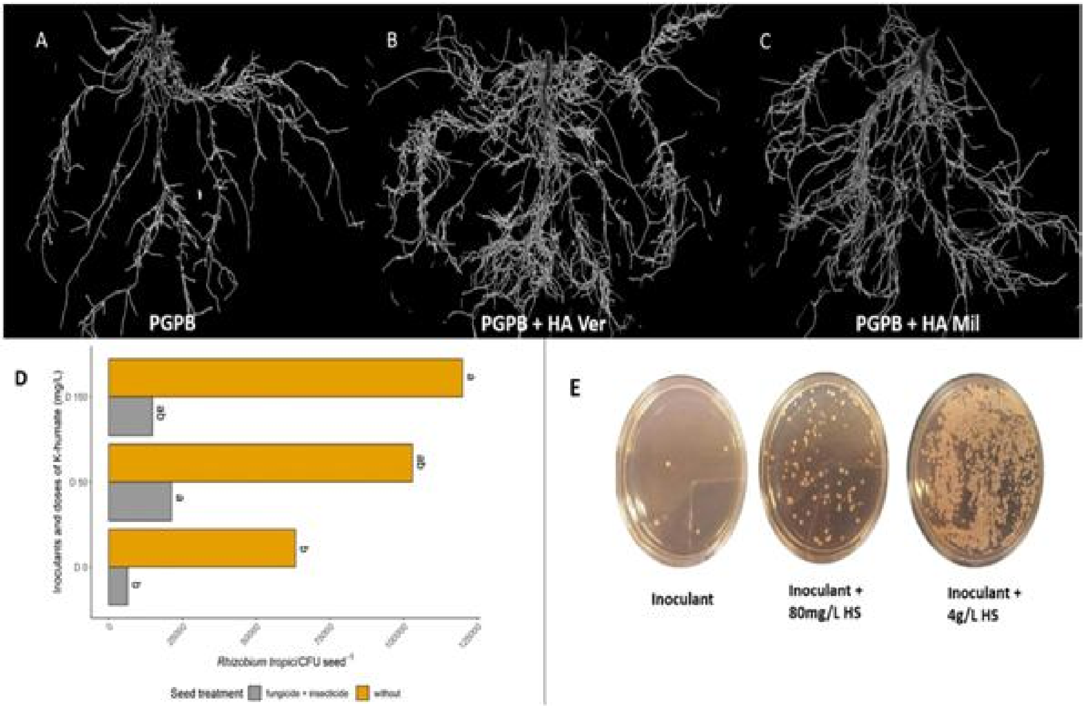

Fig. 3: Effects related to the combined use of plant growth-promoting bacteria (PGPB) and humic substances (HSs). (A–C) Show the positive impact on the branching and biomass of soybean roots inoculated with *Bradyrhizobium* (A) and treated with a combination of humic acid from vermicompost (B) and millicompost (C) in the soil. (D) Shows the increased survival of *Bradyrhizobium* (D0) in soybean seeds in the presence of 50 (D50) and 150 (D150) mgL-1 K-humate in both "raw" seeds (yellow bars) and seeds treated with fungicide and insecticide (gray bars). The values are expressed in colony-forming units (CFU) of *Bradyrhizobium* per gram of seed. (E) Increased growth of *Rhizobium tropici* (inoculant) with the application of K-humate (unpublished data). Figure created using BioRender (https://biorender.com/).

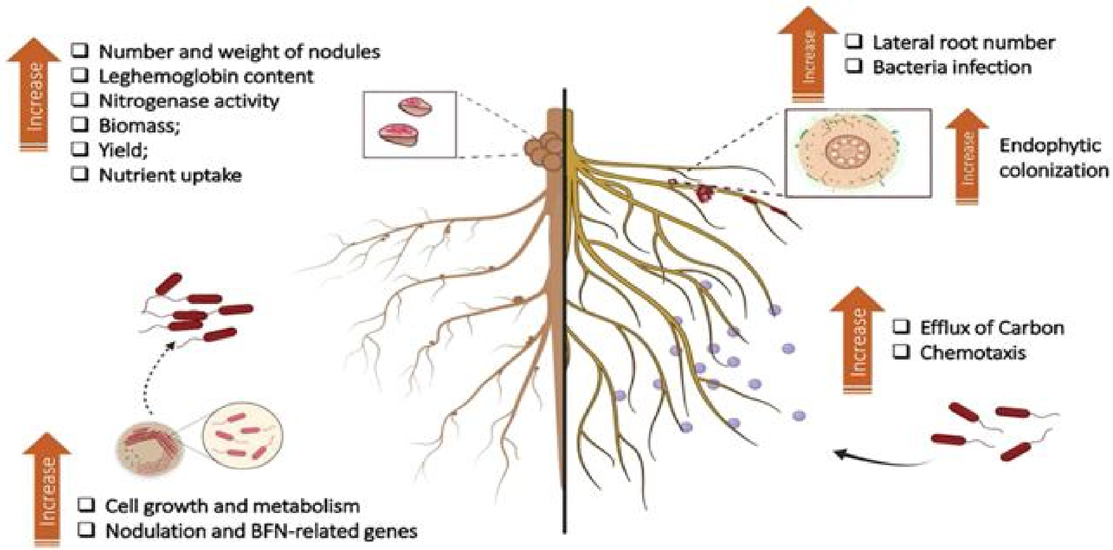

Fig. 4: Summary of results found in the literature on the effects of humic substances (HSs) on plant growth-promoting bacteria (PGBP) and interaction with plant root systems. The application of HSs to culture medium affects the growth and bacterial metabolism of rhizobia and induces the expression of genes related to nodulation and nitrogen fixation processes. Experiments in a greenhouse revealed that the application of HSs + rhizobia to legumes provided an increase in the number and weight of nodules, N levels, nitrogenase activity, and leghemoglobin contents. In nonleguminous plants, the combination of HSs + PGPB has been linked to increased endophytic colonization. The increase in root branching due to HSs provides more infection points for bacterial entry and, therefore, for colonization of plant tissue. The increase in carbon efflux through the roots stimulates microbial chemotaxis from the soil to the rhizosphere. Adapted from Olivares *et al*. (2017). Figure created using BioRender (https://biorender.com/).

Table 2: Regulating activities of humic substances in Wheat

Parameter	Mechanism of Humic substances	References
Seed germination and seedling growth	As the humic material enters the seed cells, the rate of respiration rises and cell division processes speed up. These same respiratory mechanisms promote root meristem growth and the activation of additional growing sites in seedlings.	Qin *et al.* 2016; Litvin *et al.* 2020
Root growth	Humic substance application to roots enhances plasma membrane H+-ATPase activity, shoot mineral nutrient concentration, and ABA concentrations in roots. Plants treated with HA had an increase in the quantity, diameter, and length of maize roots	Ali *et al.* 2014
Plant nutrition	The presence of HS in soil promotes root and shoot development by enhancing mineral nutrition under the soil surface	Bezuglova *et al.* 2017
Plant metabolism	HSs stimulates numerous metabolic pathways in plants, namely photosynthesis and respiration etc.,	El-Bassiouny *et al.* 2014
Growth and development	Increased vegetative growth attributed to increasing microbial activity and improving soil effectiveness in nutrient uptake as a chelating agent, as well as bio-stimulation of plant growth, resulting in a rise in the carbohydrates content of the leaves and stems. Enhanced carbohydrate synthesis can result in either higher product quality or higher yields	Baris *et al.* 2009

in BNF in plants inoculated with B. liaoningense in the presence of water-soluble humic materials. These results elucidated the direct effect of HSs on bacteria and how they may be related to the improvement of symbiosis with the host plant (Gao *et al*., 2015). FA has also been found to induce the growth of *Sinorhizobium meliloti*. In addition, this combination was shown to provide an increase in active nodules and yield in *Medicago sativa* (Capstaff *et al*., 2020). These FA-treated plants showed expression in root genes related to various processes, such as defense, oxidoreduction and C and N metabolism, in addition to specific nodulation genes. These data suggest that HSs act on the plant (Capstaff *et al*., 2020), inducing early nodulation and regulating the expression of BNF-related genes in microsymbionts (Gao *et al*., 2015).

The application of K-humate and *Bradyrhizobium* to soybean seeds was found to result in morphological changes in plant roots compared with the inoculated control (Fig. 1). In a greenhouse experiment, better values were observed for nodulation and N increase in the shoots of plants inoculated with *Bradyrhizobium* with 50 mg/L K-humate via seeds than in shoots of plants inoculated only with the control (da Silva *et al*., 2021). The application of K-humate to chicory generated gains in plant growth and variations in the number of bacterial autotrophic and heterotrophic nitrifiers in the soil. This study suggested that the effect of plant growth and microorganism variation may be related to increased nutrient permeability of the plant membrane. This same work isolated the effect of K on these parameters, stating that the gains obtained came from HSs (Valdrighi *et al*., 1996). In addition, HSs appear to increase the survival of rhizobia in soybean seeds (da Silva *et al*., 2021), to protect bacteria against irradiation (Bitton *et al*., 1972), and to increase the viability of the inoculant during storage when applied together with alginate (Young *et al*., 2006). These characteristics indicate that in addition to improving communication between microorganisms and plants, this combination can protect the inoculant against harmful effects of the environment. Another study used a phosphorus solubilizing bacterium, *Pseudomonas putida*, together with HA in soybean plants. Despite increasing pH and phosphorus (P) in the soil, the combination was not able to increase crop yield (Winarso *et al*., 2021). In an experiment using pea as a host plant, it was observed that the application of vermicompost enriched with HA (HARV) was able to provide soil health and plant growth, as well as root nodulation and colonization by *arbuscular mycorrhizal* fungi (AMF). The authors suggested that AMF and rhizobia act synergistically with HARV on soil and plant improvement (Maji *et al*., 2017). AMF play an important role in the supply of P to plants (Smith et al., 2011). P is one of the most limiting nutrients for agricultural production due to its low availability in the soil and the high demand of plants for this nutrient in their early stages of growth (Chien *et al*., 2011). A study using P-solubilizing microorganisms (PSM) and HSs attempted to evaluate the effect of these biostimulants on the P solubility of natural rock phosphate. The results showed an increase in the shoot and root weight of plants compared with the non-inoculated treatment. The findings suggested an increase in the efficiency of P use and that the application of PSM and HSs can become an alternative to reduce the use of soluble P fertilizers without harming plant yield (Giro *et al*., 2016). A recent study also reported that the combined use of HA and *Pseudomonas* spp. and *Bacillus amyloliquefaciens* in maize provided superior effects on P absorption compared with the isolated inoculation of each bacterial strain (Cozzolino *et al*., 2021). The greatest increase in P uptake was obtained when B. *amyloliquefaciens* was applied in combination with HA and AMF and when *Pseudomonas* spp. was used together with HA. This same work observed significant changes in bacterial and fungal diversity upon inoculation of the strains alone or in combination with HA and AMF. Thus, combinations of biostimulants can promote greater plant growth along with changes in soil microbiota.

Conclusion

The use of HS as a bio-stimulant for plant growth is a helpful and environmentally sustainable method. Plant morphological and biochemical changes in the root system caused by HS are the primary variables responsible for increased nutrient absorption, however an increase in nutrient availability caused by chelation and partially by auxin-like effects is another way HS contributes to plant development. HS may have a favourable effect on higher plant metabolism, resulting in increased cell division and plant development. LMS humic fractions appear to do this job more easily because they can penetrate the plasma membrane of root cells and subsequently be translocated. As a result, the use of HS not only promotes plant growth and development but also improves soil conditions and contributes to sustainable agricultural production Author

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