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HUMIC EXTRACT AS A BIOSTIMULANT IN CROPS SUBJECTED TO ABIOTIC STRESS

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ABSTRACT

Because abiotic stresses pose significant challenges to the growth and productivity of crops, the development of plants with greater survival and growth when exposed to unfavorable situations is an objective of several research groups. Biostimulants are substances or microorganisms that, when applied to crops through the foliar or root pathways, serve to enhance various processes related to growth and development. These processes include nutrient absorption, tolerance to environmental stress, and overall harvest quality. Interestingly, biostimulants can achieve these effects even when they possess a low concentration of nutrient elements in their composition. Among the main and most often used biostimulants are plant hormones, algae extract, mycorrhizal fungi, protein hydrolysates, and humic substances (humic and fulvic acids). This review sheds light on the biostimulant effects of applying humic substances to plants experiencing hydric stress conditions and low rainfed cultivation. Additionally, it aims to identify gaps in the current research and highlights areas that require further investigation. Furthermore, the review provides a concise overview of the origin and progression of research on humic substances (HS), including their extraction and obtaining process, as well as their structural characteristics and the relationship between structure, properties, and functions. The review also presents the research findings that support the potential of humic substances influencing crops affected by abiotic stress. These findings highlight the beneficial effects of humic substances in enhancing the resilience and performance of crops facing challenges such as drought, salinity, temperature extremes, and other non-biological stressors. The evidence underscores the significance of humic substances as a valuable tool in mitigating the detrimental impacts of abiotic stress on crop productivity and overall plant health.

Keywords: physiological effects, yield, eustress.

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INTRODUCTION

Plants possess a moderate level of mobility, which means that throughout their vegetative cycle, they encounter unfavorable conditions that hinder the optimal development of their vegetative functions (Zhang *et al.,* 2022). Among the main types of abiotic stress affecting crop development are drought, high salinity, cold, and heat, which can reduce biomass production and performance by 70 % (Liliane and Charles, 2020). These are especially significant in arid and semi-arid regions due to the impact of climate change, disrupting precipitation patterns and altering temperature values in various geographical areas across the globe. This has a severe impact on plants in these regions, exacerbating the difficulties they face in maintaining their vegetative functions (Malhi *et al.,* 2021).

Numerous studies have focused on horticulture and agronomy, to enhance productivity and increase the resistance of various crops to abiotic stresses. The research efforts are directed toward developing strategies and techniques that can improve crop performance under adverse environmental conditions. These studies contribute valuable insights into mitigating the effects of abiotic stresses and promoting sustainable agricultural practices (Drobek *et al*., 2019).

Genetic enhancement is employed as part of a range of strategies aimed at boosting yield and improving crop productivity, particularly in challenging environments. This technique may include the incorporation of genes associated with tolerance, the expression of which modifies the main biochemical and physiological mechanisms involved in tolerance. Some of these genes are related to the transport of ions and water, the synthesis of osmoprotective substances such as glycine-betaine, mannitol, and proline, ion exclusion transporters, mechanisms for the detoxification of reactive oxygen species (ROS), production of chaperone molecules, in addition to the control of transcriptional regulation (Carvajal-Campos and Jiménez, 2021).

In addition to the above, modern agriculture increasingly uses biostimulants as an agronomic tool and an environmentally friendly alternative. Biostimulants are recognized for their ability to help crops cope with frequent drought periods and improve their yields under adverse conditions (Bulgari *et al.,* 2019).

The definition and concept of plant or agricultural biostimulants are continually evolving, mainly due to the wide range of materials that can be used to produce biostimulants (Rouphael and Colla, 2020). From an industrial point of view, these are referred to as compounds that contain both substances and microorganisms, whose function, once they have been applied to crops via foliar or root, is to stimulate processes related to nutrient absorption, tolerance to abiotic stress, and crop quality. Biostimulants, despite their beneficial effects on plants, do not possess direct pesticidal properties. Consequently, they are not categorized or regulated as pesticides. Their primary function is to enhance plant growth, development, and stress tolerance, rather than directly combatting pests (Van Oosten *et al*., 2017). Plant hormones (Zakeel and Safeena, 2019), algae extracts (Hassan *et al*., 2021), protein hydrolysates (Carillo *et al*., 2019), and humic and fulvic acids (Bayat *et al*., 2021) are the main and most used biostimulants in modern agriculture.

Humic substances (HS), from which humic (HA) and fulvic (AF) acids are derived, are recognized for their nutrient absorption and anti-stress effects (Van Oosten *et al.*, 2017). The effects of biostimulants on cultivated plants have indeed been more commonly reported on the root system rather than the aerial (above-ground) parts. This is because biostimulants often interact directly with the root system, influencing root growth, nutrient uptake, and overall root health. These effects can be divided into direct and indirect. Direct effects are understood to be those that do not depend on the characteristics of the complex soil or the availability of micro- and macro-elements, although they do involve the regulation of cell activity, variation in metabolism, as well as changes in gene expression and present hormonal action (du Jardin *et al.*, 2015). One of the indirect effects of biostimulants is the improvement of the granular structure of the soil, leading to enhanced air-water relationships within the soil. Biostimulants can positively influence soil structure by promoting the aggregation of soil particles, which results in the formation of stable soil aggregates. These aggregates create pore spaces within the soil, improving its aeration and water-holding capacity (Chen *et al.*, 2004).

The direct effects are mainly associated with the entry of HS, which are low molecular weight compounds, into the plant tissue (at the cellular level) through the epidermis (Kulikova *et al.*, 2016) or due to the existence of physical-chemical interactions (Asli and Neumann, 2010), causing marked stimulations in the different metabolic processes in different plants, as in maize (*Zea mays* L.) (Canellas *et al.*, 2002) and rice (*Oryza sativa* L.) (García *et al.*, 2016; Tavares *et al.*, 2017).

This review aims to provide an overview of the biostimulant effects of HS on crops facing stress and rainfed cultivation conditions. It also highlights the existing gaps in research and identifies areas that require further investigation. Additionally, the review briefly discusses the origin and evolution of research related to HS, the processes involved in obtaining HS, and the relationship between its structure, properties, and functions. The research findings that support the potential of HS in positively influencing crops affected by abiotic stress are also shown.

HUMIC SUBSTANCES

Research related to the HS theme dates back more than 250 years, with the term humus being referenced for the first time in Agriculturae Fundamenta Chemica. Since then, the field of humic substances (HS) experienced a significant surge in research and interest, particularly in the context of agricultural sciences. As agricultural practices advanced, there was a realization that HS could serve as a valuable resource for improving crop production. This realization prompted a greater focus on studying the chemistry and properties of these molecules (Senn and Kingman, 1973).

As natural organic matter, HS is defined as a set of molecules widely distributed in nature, encompassing the entire terrestrial ecosystem, as well as rivers, lakes, and oceans (Yang and Antonietti, 2020). Piccolo (2002) defined them as supramolecular associations of relatively small, self-assembled, heterogeneous molecules held together predominantly by non-covalent forces.

HS are formed through the complex process of decomposition and resynthesis in the soil, primarily driven by the action of microbiota. They originate from the breakdown and transformation of plant and animal residues. Microbiota, including bacteria, fungi, and other microorganisms, play a vital role in the degradation and subsequent synthesis of organic matter, leading to the formation of HS (García *et al.*, 2019; Hernández *et al.*, 2021). The main sources for obtaining HS are soils, peat, coal, manure, compost, and vermicompost, as well as minerals such as leonardite, and others (Hernández *et al.*, 2021).

The wide range of sources available for obtaining HS contributes to the significant structural diversity found in HS molecules, which are the primary constituents of soil organic matter. The fundamental role of the structures and properties of HS has been documented, together with its relationship with physiological effects, both on the aerial part and the root part in plants, as well as its incidence on the main physicochemical properties of the soil (du Jardin *et al.*, 2015). Canellas *et al.* (2005) have proposed a model that is in correspondence with the structural characteristics present in HS.

The primary impacts observed from the application of HS (hormonal signaling) in plants predominantly manifest in the root system. These effects can be categorized into direct and indirect effects, as discussed in the introduction section. These effects significantly enhance various metabolic processes in plants, as demonstrated in previous studies conducted on corn (Z. *mays*) (Canellas *et al.*, 2002), rice (*O. sativa*) (Tavares *et al.*, 2017), and other species (Chen *et al.*, 2004).

STRUCTURE AND OBTAINING OF HUMIC SUBSTANCES

Multiple interpretations exist regarding the structure of humic substances (HS). One of the most widely accepted models was presented by Piccolo (2002). This model encompasses two key aspects: i) the supramolecular structure, and ii) an updated comprehension of the supramolecular arrangement of HS. These propositions have gained significant recognition within the scientific community and are frequently referenced in the literature about HS. According to this model, HS is described as supramolecular assemblies composed of diverse, relatively small organic molecules with varying characteristics (Canellas *et al.*, 2015). This indicates that the HS (Figure 1),

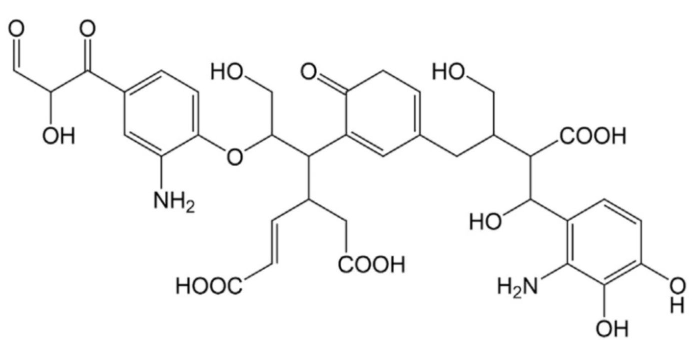

Figure 1. Structural model of a humic acid unit (molecular weight 731 g mol^{-1}).

when they are part of the soil complex, acquire a strong assemblage, as well as greater stability, caused by the interactions of weak forces (van der Waals, π – π, CH – π), being the links by hydrogen bonds, in addition to hydrophobic forces, the main causes of the large molecular size (Canellas *et al.,* 2015).

The introduction of the supramolecular concept in understanding the structure of HS (Figure 2) brought about a significant shift in the previously employed classifications. This novel approach revolutionized our comprehension of HS by providing insights into their interactions with soil minerals, as well as their capacity for the adsorption

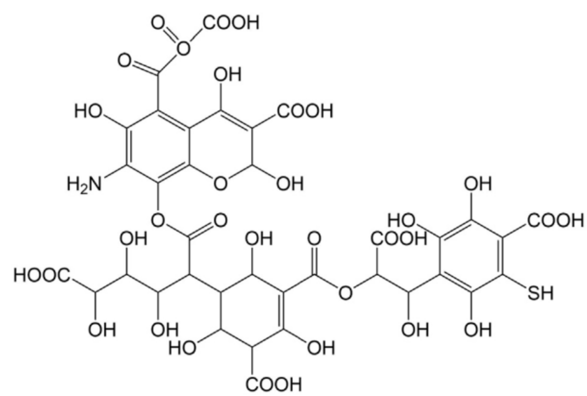

Figure 2. Structural model of a fulvic acid unit (molecular weight 1051 g mol^{-1}).

and complexation of ions. The adoption of the supramolecular perspective allowed for a more comprehensive understanding of HS and paved the way for advancements in studying their intricate behaviors and functionalities. It allowed a better practical interpretation of the humification process, understood as a fluid degradation process of organic waste from animals and plants, which, through the action of microorganisms, start to form substances with structural patterns that coincide with the HS (Piccolo, 2016; Hayes and Swift, 2018; García *et al.,* 2019).

After obtaining HS, the next step in the process involves purification, typically achieved through dialysis. Fractionation is the most widely used method to reduce the heterogeneity of substances, which is based on the separation of its components taking advantage of the properties related to its molecular structure. The most widely used fractionation technique of HS is based on the variation of its solubility at different pH values (Figure 3) (Nebbioso and Piccolo, 2012).

Depending on their solubility at different pH values, three main fractions that make up HS are recognized: humin (H), an insoluble fraction in an aqueous medium at any pH value; fulvic acids (FA) (Figure 2), a fraction soluble in an aqueous medium at any pH value; and humic acids (HA) (Figure 1), which are soluble in a basic medium and insoluble at pH close to 2 (Stevenson, 1994). In the light of more recent studies, both quantitative and qualitative information has been found regarding the main

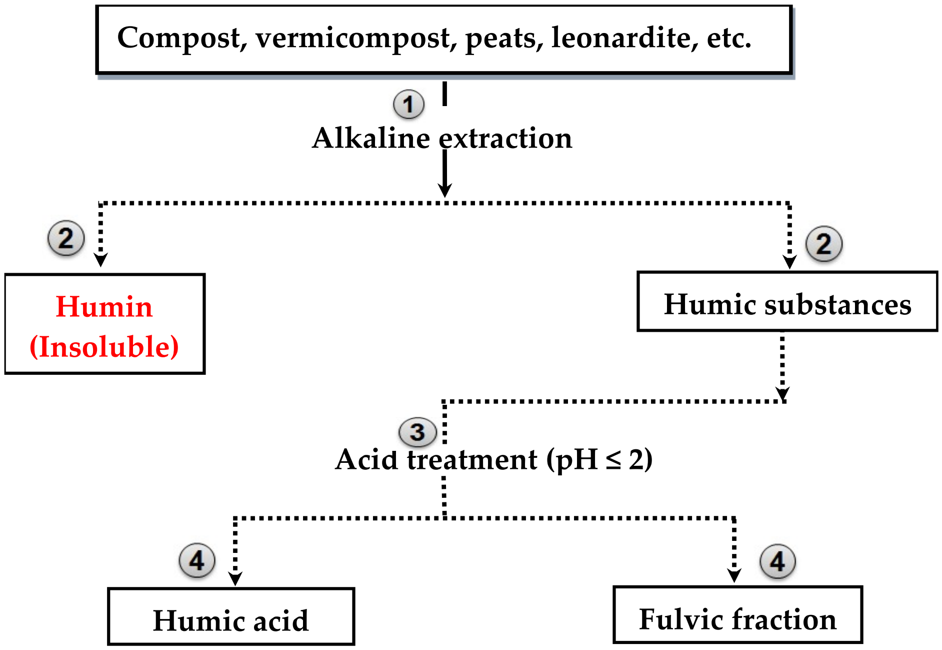

Figure 3. Diagram of the pH-based fractionation process of the humic substances (Stevenson, 1994).

functional groups found in the structure of HS (García *et al.,* 2019). In reports that include spectroscopic studies, HS generally presents aromatic structures (benzenes and polysubstituted phenols), as well as phenolic and alcohol-COOH, carboxylic acid-COOH, esters, quinones, among others (Schulten and Schnitzer, 1993).

An approximate representation of the HS structure (Figure 4) was proposed by Schulten and Schnitzer (1993), in which the main functional groups are observed, as well as the aliphatic and aromatic parts, in addition to the outer hydrophilic domain, which consists of polar groups (for example, carboxylic residues), and the interior hydrophobic domain, which is composed of macromolecules of plant origin. According to Muscolo *et al.* (2007), both domains HS and the variability of functional groups within them play crucial roles in determining the biological effects of these compounds on plants because of the solubility and biological reactivity that they generate when applied to plants. HS are formed irregularly depending on the hydrophobicity present in the components of the humic molecules, although they are also the result of their size, shape, and chemical interactions (Nebbioso and Piccolo, 2012).

Recent advances in technology and analytical techniques have significantly contributed to our understanding of the structures of HS. One area of particular interest is the determination of the maturity index of HS derived from various sources. This analysis is valuable as it enables the tracking of organic residue degradation and facilitates the description of biochemical changes that occur during processes such as vermicomposting. By assessing the maturity index, researchers can gauge the extent of

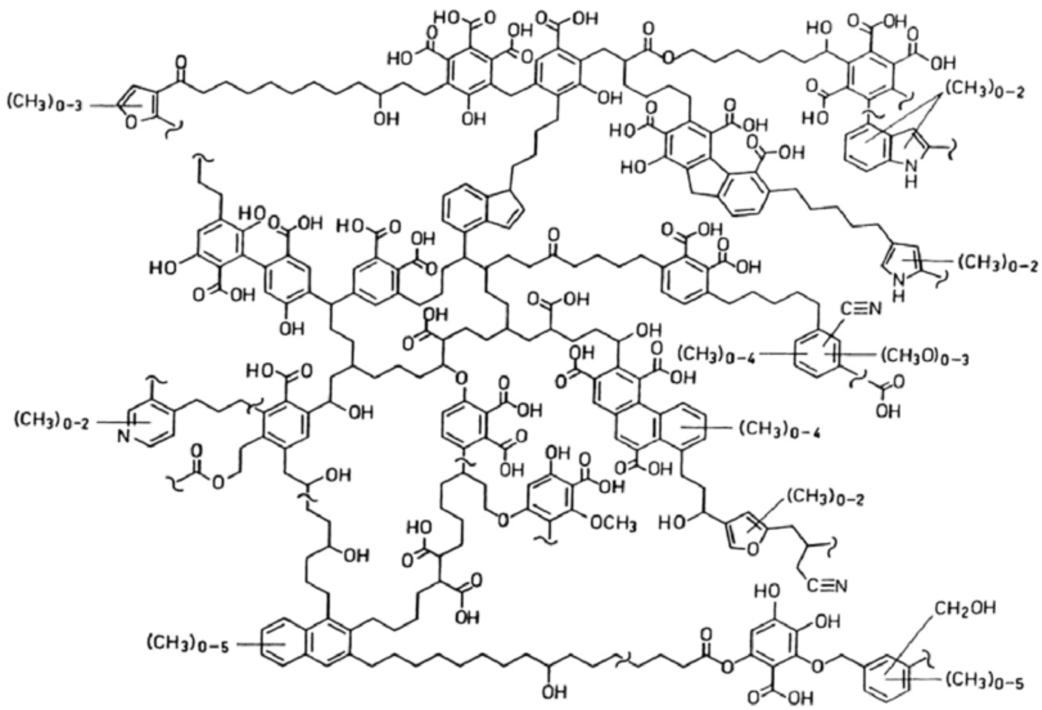

Figure 4. The approximate structure model of a humic acid molecule, developed from a tentative chemical network of humic substances ($C_{308}H_{328090}N_5$) proposed by Schulten and Schnitzer (1993).

mineralization and decomposition of the raw materials used. The information is crucial for evaluating the effectiveness and efficiency of composting processes, providing insights into the rate of organic matter breakdown and nutrient release. The ability to monitor and quantify these changes aids in optimizing composting practices and using organic residues more effectively in agricultural and environmental applications (Bhat *et al.,* 2017). These techniques are as follows: FT-IR (Fourier Transform Infrared Spectroscopy), UV/Vis (Ultraviolet-Visible Spectroscopy), Py-GC/MS (Gas-liquid Chromatography coupled with Pyrolysis and Mass Spectroscopy), 13C CP/ MAS - NMR (Carbon 13 Nuclear Magnetic Resonance) (Canellas *et al.*, 2005).
The variability of the HS structure during the vermicomposting process has been reported and more than 250 compounds derived from different sources such as lignins, carbohydrates, proteins, alcohols, and fatty acids have been identified, the presence of which varies according to the progress of the stabilization of organic matter.

RELATIONSHIP BETWEEN STRUCTURE-PROPERTY AND FUNCTION OF HS

The relationship between the structure and properties of HS (Figure 5) is crucial for comprehending their functional characteristics (García *et al.,* 2016; Nardi *et al.,* 2017).

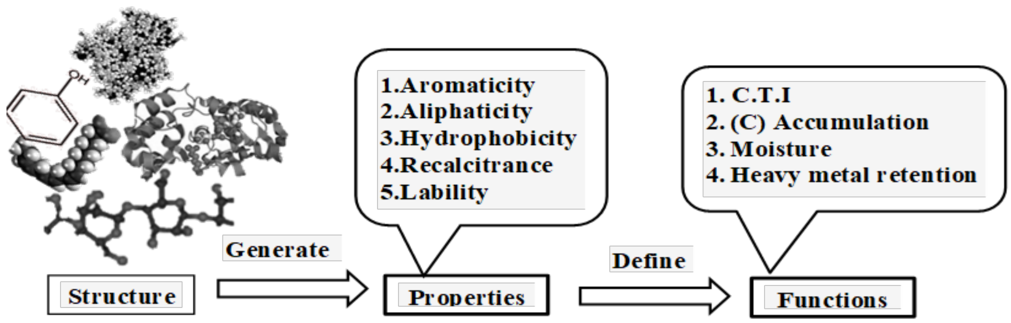

Figure 5. Proposal of the relationship between structure, property, and function of humic substances (García *et al.*, 2019).

Understanding how the structure of HS influences its properties provides valuable insights into how these molecules, derived from the humification process, exert their effects on plants. The properties of HS, such as solubility, chemical reactivity, and complexation capacity, directly impact their interactions with plant systems.

Explaining the relationship between the structure, properties, and effects of HS can be complex, particularly considering the varying effects and intensities observed when HS are applied either foliarly (on leaves) or radially (to the roots) in different plant species (Nardi *et al.*, 2017). Muscolo *et al.* (2007) found differences in the activity of carbon and nitrogen metabolism in Pinus nigra J.F.A plants when applying HS extracted from two different sources, one with an abundant presence of methyl (CH_3) and carboxyl (COOH) groups, and the other with abundant contents of betaine and organic acids, justifying said differences by the structural inequality. García *et al.* (2019) state that HS obtained from vermicompost material in an alkaline medium with an abundant presence of carboxyl groups (COOH) achieved significant increases in the development variable of lateral roots. Nardi *et al*. (2017) state that there is a direct relationship between some specific functional groups and their biological activity. Using volcanic soil as a source of origin, they separated the HS from the original sample into three fractions of different sizes, to be able to observe a relationship between its structure and its biological activity.

During the evaluation, it was observed that both the original sample of HS and its three derived fractions significantly stimulated enzymatic activities associated with the glycolytic pathway and respiratory processes in maize plants. Notably, fraction III, which had the smallest molecular size and a more flexible conformation, yielded the most favorable results. According to Nardi *et al*. (2017), this is because fraction III presents a more flexible conformation than the other fractions, thus allowing a better diffusion of the bioactive humic components when coming into contact with the maize cells. On the other hand, Canellas *et al.* (2010) described contrary results in *Z. mays* crops after carrying out different experiments in which they evaluated the bioactivity of HA extracted from different sources (vermicompost, oxisols, tropical soils with different degrees of weathering).

In a review, García *et al.* (2016) explain the relationship between the biological activity of HA and the structures and properties present in said humic supramolecule. After characterizing a large number of HA from different areas of Brazil and establishing a structural pattern where labile (HS) and recalcitrant (HA) structures predominate, they conclude that this pattern is significantly related to the growth of smaller roots and that the biological activity of such products is directly related to the structure. In a more recent review, García *et al*. (2019) described the relationship structure - property – function of HS with the oxidative metabolism of plants. The authors conclude that: i) regardless of the type of origin of the HS, there is a common structural pattern for this type of humic compound, which makes them different from any other group of compounds present in the complex soil. If there is a reproducible structural pattern, then it follows that HS is seen as a group of substances with their own structural identity; ii) therefore, the fact that how these humic superstructures are formed is unknown but insufficient to deny its existence; iii) humification continues to be the precursor process of HS, which largely determines the total number of components present in the humic supramolecule.

BIOSTIMULATION ACTION OF HS

Humic substances (HS) are used as biologically active materials due to their ability to modify the physical, chemical, and biological properties of soil. When applied to soil, HS can have significant positive impacts on plant growth and development processes (García *et al.,* 2012). Plant development is closely related to good mineral nutrition, availability of water, air, and environmental parameters such as light and temperature. However, the use of organic matter in both its liquid and solid forms has a proven effect on plant development under laboratory conditions and some under field conditions (Canellas *et al.,* 2015; Yoon *et al*., 2020; Nardi *et al*., 2021; Sun *et al*., 2020). Numerous authors (Gerke, 2018; García *et al.,* 2019; Nardi *et al*., 2021; Bondareva and Kudryasheva, 2021) have separated the effects exerted by HS into direct and indirect, as mentioned earlier.

When HS are sprayed on plants to evaluate their effects on growth, it has been observed that the impacts are often more pronounced and evident in the root system compared to the aerial part of the plants. The responses to HS, when applied to the root system of plants, have been widely discussed, and different viewpoints exist regarding the mechanisms and effects on plant roots. These differing perspectives aim to elucidate the underlying processes and outcomes of HS application. The first considers an initial effect on the roots of the plants and, later, in the whole plant. Nardi *et al.* (2002) state that this effect is mediated by the presence of significant concentrations of both plant hormones and the presence of molecules with structures similar to plant hormones as components of HS supramolecules, which could trigger actions analogous to those promoted by plant hormones (Canellas *et al.,* 2011). Alternatively, Olaetxea *et al.*

(2016) report that, although hormonal concentrations like those of plant hormones are not detected in the HS structures studied, they promote molecular and biochemical responses that affect the root system of HS-treated plants. The transcriptional regulation of gene networks, reported in some studies, allows us to affirm that the foliar application of HS affects certain transcription factors involved in the absorption of nutrients by the root, and their subsequent metabolism, thus expressing positive effects on plant metabolism and plant root development (Nardi *et al.*, 2021).

Due to their direct effects on plant development, HS can modify the different endogenous concentrations of phytoregulators in the root system, which present a close relationship between auxins, root characteristics, and biological activities (Jindo *et al.*, 2020; Nardi *et al.*, 2021). The main effects caused by HS through indoleacetic acid (IAA) are associated with root morphology and the emission of lateral roots (Canellas *et al.*, 2002; Jindo *et al.*, 2020; Nardi *et al.*, 2021). According to these authors, this change in the morphology of the root and the promotion of growth is associated with the auxin-like effect by HS, managing to stimulate the activity of H^+-ATPases in the root plasma membrane. This coincides with the acid growth theory that said: during the process of cell elongation, the accumulation of auxin in the apoplast can activate the auxin receptor protein ABP1 and then stimulate the activity of H^+-ATPases in the plasma membrane, secreting protons outside the membrane cell, coming to acidify the cell wall whose optimum pH is 5, for which this decrease in pH induces the activation of polysaccharidases. These enzymes that can hydrolyze a series of cell wall bonds and as a consequence, the wall loses its rigidity and it can undergo a viscoelastic extension caused by the pressure potential (Sun *et al.*, 2020).

In the Soil Laboratory of the Interdisciplinary Research Center for Integral Regional Development, Oaxaca Unit (CIIDIR-Oaxaca Unit), significant results have been obtained in different crops of agronomic interest, specifically under rainfed conditions. In addition, arbuscular mycorrhizal fungi (AMF) have been used to increase tolerance to water stress in maize plants under rainfed conditions (Matías *et al.*, 2021). To achieve similar objectives, humic extracts derived from urban solid waste (USW) and vermicompost produced from bovine manure have been utilized. These extracts have been applied to various landraces of native maize and different rice cultivars. Promising outcomes have been observed not only in greenhouse conditions but also in open-field settings, encompassing the vegetative to reproductive stages of plant growth. These findings highlight the potential of utilizing humic extracts derived from USW and vermicompost as effective treatments for enhancing crop performance and development (Hernández *et al.*, 2021).

Various laboratories and research groups at the international level have reported significant results by obtaining HS from diverse sources such as bovine manure vermicompost, urban solid waste, soils from different regions, and minerals like leonardite. These HS are then processed into humic extracts, which are subsequently applied to crops facing non-optimal conditions. The objective behind these studies is to enhance the crops’ responses to different abiotic stresses and increase potential yields under challenging environmental conditions. Among these are the group from the Soil Biological Chemistry Laboratory, UFRRJ, Brasil (García *et al*., 2016; Pinos *et al.*, 2019), the Agricultural Chemistry and Biology Group (BACH) of the University of Navarra, Spain (Olaetxea *et al.,* 2016; Olaetxea *et al.,* 2018), and the Organic Matter and Biostimulants Research Group (MOBI) of the Chemistry Department of the Agrarian University of Havana, Cuba (Galbán-Méndez *et al.,* 2021).

The biostimulant effects of different doses of humic extracts from various sources have been demonstrated in different plants of agricultural interest such as rice (*O. sativa*) and maize (*Z. mays*) (Hernández *et al*., 2021; Galbán-Méndez *et al*., 2021; García *et al.,* 2019), lettuce (*Lactuca sativa* L.) (Hernández *et at*., 2015), garlic (*Allium sativum* L.) (Balmori *et al*., 2019), and common bean (*Phaseolus vulgaris* L.) (Farías *et al.,* 2020). During the physical-chemical characterization of the extracts, the presence of HS (HA, FA), phytohormones, beneficial microorganisms, amino acids, lignin, and cellulose has been detected (García *et al*., 2019; Jindo *et al*., 2020).

The application of HS has been reported to stimulate key enzymes involved in the metabolism of carbon (C) and nitrogen (N) in plants. Several studies conducted under different experimental conditions have shown that certain enzymes associated with N metabolism exhibit increased activity following HS application. These enzymes include glutamate dehydrogenase, phosphoenolpyruvate carboxylase, nitrate reductase, and glutamine synthetase (Canellas and Olivares, 2014; Vaccaro *et al.,* 2015). A significant increase in the activity of the main enzymes involved in the reduction and assimilation of inorganic N has been reported as an effect of the application of HA at different concentrations (Vaccaro *et al*., 2015). The enzymatic activity of the proton pump (H^+-ATPases) in the root plasma membrane is also increased by the application of HS, which induces changes in root morphology and growth promotion and is closely linked to the theory of acid growth (Canellas *et al.,* 2002; Sun *et al.,* 2020). This enzyme, in addition to being associated with the aforementioned processes, promotes an increase in branching or a greater density of root hairs, and it is also related to greater absorption of nutrients and changes in the exudation profile of the root, as well as in primary and secondary metabolism (Canellas and Olivares, 2014).

CROPS UNDER ABIOTIC STRESS CONDITIONS AND BIOSTIMULANT EFFECTS OF HUMIC SUBSTANCES

Unfavorable climatic conditions, including prolonged drought periods, high soil salinity levels, and extreme temperatures, have detrimental effects on crop yields. According to a study by Ibarra-Villareal *et al*. (2021), these adverse conditions can result in yield losses of more than 52 % for crops. These conditions cause oxidative stress, which in turn stimulates the production of extremely harmful reactive oxygen species (ROS) for plants, especially when the balance between production/elimination is affected, causing enzymatic inhibition, in addition to the degradation of chlorophylls, severe damage to biomolecules, and lipid peroxidation mediated by the presence of free radicals such as superoxide (O_2^{2-}), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) (Apel and Hirt, 2004).

The concerning impact of unfavorable climatic conditions on agricultural productivity poses a significant risk to food security worldwide. As highlighted by Rouphael *et al*. (2018), this situation raises concerns regarding the ability to meet the growing global demand for food. To address the challenges posed by unfavorable climatic conditions and enhance agricultural productivity, the use of environmentally friendly biostimulant products has been suggested, such as plant hormones (Zakeel *et al*., 2019), algae extract (Hassan *et al*., 2021), protein hydrolysates, amino acids (Carillo *et al*., 2019), and HS (Bayat *et al*., 2021). All of these are considered agronomic tools with significant potential (Povero *et al*., 2016). This can be corroborated by the increase in scientific publications in this area and, from a practical point of view, by the increase in the number of products formulated with these substances throughout the world (Caradonia *et al*., 2019). According to market estimates, the production of natural compounds, including biostimulants such as plant hormones, algae extracts, protein hydrolysates, amino acids, and HS, is projected to generate significant profits in the coming years. It is anticipated that by the year 2025, the global market for these natural compounds will exceed USD 4.14 billion in revenue (Bulgari *et al*., 2019).

HS, in addition to their roles as regulators of primary and secondary metabolism, have been found to have beneficial effects in attenuating environmental stresses in plants. Studies by Canellas *et al*. (2015), du Jardin (2015), Van Oosten *et al*. (2017), and Veobides-Amador *et al*. (2018) have highlighted the ability of HS to mitigate the negative impacts of soil water deficit, salinization, and exposure to suboptimal temperatures, resulting in improved plant performance and productivity.

One example is the foliar application of humic acid (HA) to pepper plants (*Capsicum annuum* L.) under high salinity conditions (100 mM NaCl). Kaya *et al*. (2018) reported that the foliar application of HA enhanced the plant's tolerance to salt stress. This was observed through several physiological and biochemical changes in the plants. The application of HA resulted in increased plant biomass, enhanced chlorophyll and proline content, and enhanced activity of antioxidant enzymes such as peroxidase, catalase, and superoxide dismutase. These changes indicate an improved ability of the plants to cope with oxidative stress induced by salinity, leading to better plant growth and productivity. Subjected to drought stress, maize seedlings demonstrate enhanced growth and physiological responses upon the application of HA. This includes an increase in the content of photosynthetic pigments, relative water content, root and shoot length, and the diameter of the root metaxylem (Bijanzadeh *et al*., 2019). In rice experiencing water deficit conditions, the application of HA through the roots leads to an elevation in proline content and antioxidant activity, while concurrently reducing the levels of H_2O_2 (García *et al*., 2012). Similar results have been reported for sugar beet (*Beta vulgaris* L.) (Khodadadi *et al*., 2020) and tomato (*Solanum lycopersicum* L.).

Despite evidence for the protective action of HS in plants grown under stressful conditions, as well as its biostimulant effects, many unanswered questions remain. For this reason, future research should consider the protective effects against abiotic types of stress using humic products extracted from cheap and highly available sources (manures, household organic waste, composts, and vermicompost), sprayed both via the foliar and root routes to cultivate plants of agricultural interest, including the seedbed, nursery, and open field stages. Likewise, the development of formulations and products must be validated under commercial cultivation conditions, before being placed on the market for the availability of farmers.

RESEARCH NEEDS AND KNOWLEDGE GAPS

The study of HS has seen continuous advancements, with numerous publications contributing to our understanding of their properties and applications. Several notable studies in the literature have significantly contributed to this field. These include works by Piccolo *et al.* (2016), García *et al*. (2016), García *et al*. (2019), Nardi *et al*. (2017), Nardi *et al*. (2021), and Olaetxea *et al*. (2018). These publications have shed light on various aspects of HS, such as their structure, functions, and effects on plant growth and development. Many studies on HS have indeed focused primarily on the chemical aspects, aiming to elucidate their structure, formation process, and the relationship between structure, properties, and functions. These investigations have provided valuable insights into the complex nature of HS and their potential pathways of action. The knowledge gained from these studies has contributed significantly to our understanding of the role of HS in soil and plant systems.

However, it is important to recognize that the application of HS under controlled laboratory or greenhouse conditions may present limitations when it comes to understanding their effects in real-world open-field conditions. Controlled conditions allow researchers to isolate and study specific variables, but they may not fully represent the complexities and interactions that occur in natural environments. In this context, the studies designed under laboratory conditions consider the effect of HS only between 30 and 45 days (vegetative stage), therefore, only the initial phases of the crops are analyzed, when in real open field conditions, most crops exceed 4 months. The current practice of evaluating HS application at specific doses and in controlled conditions, primarily focusing on the early stages of crop growth, presents limitations in terms of understanding the effectiveness of these application rates in open field conditions. As a result, these investigations, although aimed at demonstrating the product's impact on crop quality, physiology, morphology, and yields, often fail to extend their evaluations to the later stages of crop development.

Open field conditions introduce a multitude of factors that can significantly influence the effectiveness of HS application, such as the interaction of multiple stresses, varying climatic conditions, and changing soil dynamics. It is therefore necessary to expand the scope of research to encompass the entire crop cycle and replicate field conditions more accurately.

This discrepancy between the results obtained under controlled conditions and the application of HS in open-field conditions highlights a significant research gap. It raises doubts about whether the same outcomes observed in controlled environments can be replicated when HS are applied under the complex and dynamic conditions of open-field agriculture, where crops are exposed to multiple simultaneous stresses.

In the agricultural market, there are currently various natural bioproducts available that aim to enhance the entire vegetative cycle of crops, increase agricultural yields, and mitigate specific conditions such as water stress and salinity. These products are designed to address the challenges faced by crops throughout their growth stages, providing comprehensive support and promoting overall plant health and productivity.

In a similar vein, the application of HS in crops aims to achieve similar objectives. By applying HS under open field conditions, the goal is to enhance the resilience and adaptability of crops to various types of stress. HS has the potential to improve nutrient uptake, enhance root development, stimulate plant growth, and modulate plant responses to environmental challenges. However, to fully realize the potential benefits of HS in open-field agriculture, further research is needed. Field trials should be conducted to evaluate the effectiveness of HS in real-world farming scenarios, where crops are exposed to multiple stresses simultaneously. These trials should consider different crop species, soil types, climate conditions, and agronomic practices.

By conducting field studies, researchers can assess the performance of HS in diverse agricultural contexts and determine their effectiveness in increasing agricultural yields and mitigating stress. These studies would provide valuable insights into the practical applications of HS and their potential to contribute to sustainable and resilient agriculture.

CONCLUSION

Humic substances have a complex structure characterized by high variability of functional groups and the presence of small heterogeneous molecules that interact through weak bonds. These compounds offer a wide range of benefits when applied to plants. Notably, they help mitigate the adverse effects caused by abiotic stresses and stimulate plant growth and productivity. Although the exact mechanisms of action are still being extensively studied and remain a topic of discussion in scientific literature, various research efforts have focused on understanding the relationship between the structure, properties, and functions of humic substances. This has led to the elucidation of potential mechanisms, such as their similar activity to auxins, which explain their biostimulant effects.

Humic compounds have demonstrated the ability to modulate secondary metabolism, leading to the stimulation of antioxidant enzymatic complexes in conditions of water stress. This action on plant physiology suggests that humic substances could serve as a viable and environmentally friendly alternative for mitigating the consequences of climate change. In effect, they may enhance the characteristics of plants grown under unfavorable conditions. As research progresses, the efficacy of humic products under field conditions, optimal application rates, and the ability to differentiate genuine products from fraudulent ones will become clearer. This increased understanding will further enhance the utilization of humic products over time.

Overall, the complex structure and diverse functionalities of humic substances make them valuable assets in agriculture and plant science. Their potential to alleviate abiotic stress effects and promote plant growth holds promise for sustainable agricultural practices in the face of climate change. Continued research and exploration of humic compounds will pave the way for their optimal use and integration into agricultural systems.

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