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S Pazhanisamy

Department of Agronomy,
Dr. Rajendra Prasad Central
Agricultural University, Pusa,
Bihar, India

Sai Linga Reddy

Department of Agronomy,
Dr. Rajendra Prasad Central
Agricultural University, Pusa,
Bihar, India

Rodda Chandana Devi

Department of Soil Science,
Dr. Rajendra Prasad Central
Agricultural University, Pusa,
Bihar, India

Correspondence

S Pazhanisamy

Department of Agronomy,
Dr. Rajendra Prasad Central
Agricultural University, Pusa,
Bihar, India

Effect of humic substance in soil and plant system: A review

S Pazhanisamy, Sai Linga Reddy and Rodda Chandana Devi

Abstract

Humic substances (HS) are the humified organic matter fraction in soils and play a key role in various soil and plant functions. HS influence the adaptation of plants to conditions of environmental stress by increasing the nutrient availability. They also have a direct effect on plant metabolic processes related to growth and development. The biochemical and molecular mechanisms underlying these events are only partially known. HS have been shown to contain auxin and an "auxin-like" activity of humic substances has been proposed, but support to this hypothesis is fragmentary. In this review article, giving an overview Effect of humic substance on soil and plant system, with special emphasis on their hormone activities.

Keywords: Triclosan, TCS, determination, detection, sensor

Introduction

Humic substances (HS) are the largest constituent of soil organic matter (~60%) and are considered as a key component of the terrestrial ecosystem, being responsible for many complex chemical reactions in soil. Composition of humic substances also varies depending on conditions of plant residue decomposition, e.g. temperature, pH, redox (Stevenson, 1994) [26]. They cannot be decomposed readily because of their intimate interactions with soil mineral phases and are chemically too complex to be used by microorganisms (Sara *et al.*, 2010) [21]. As far as soil is concerned, one of the most striking characteristics of HS is their ability to interact with metal ions, oxides, hydroxides, mineral and organic compounds, including toxic pollutants, to form water-soluble and water-insoluble complexes. Through the formation of these complexes, can dissolve, mobilize and transport metals and organics in soils and waters or accumulate in soil horizons. Accumulation of such complexes can contribute to a reduction of toxicity, e.g., of aluminum (Al) or to remove Cr (VI) from aqueous solutions. Recently Wang and Mulligan (2009) [32] have used HS in the arsenic and heavy metal remediation, indicating the HS as a possible remedial to reduce and avoid further contamination. Moreover HS can interact with xenobiotic organic molecules such as pesticides and influence nutrient availability (N, S, P), especially of those nutrients present at a very low concentration. Moreover, these are able to produce various morphological, physiological and biochemical effects on higher plants. Numerous studies have shown that HS enhance root, leaf and shoot growth but also stimulate the germination of various crop species. These positive effects are explained as an interaction between HS and physiological and metabolic processes. The addition of HS stimulate nutrient uptake, cell permeability and seems to regulate mechanisms involved in plant growth stimulation. It is not easy to distinguish between the direct and indirect effects of these substances. In fact, some of their positive effects may be ascribed to a general improvement of soil fertility, leading to a higher nutrient availability for plants. Whilst, in other cases, HS seem to positively influence metabolic and signaling pathways involved in the plant development, by acting directly on specific physiological targets. For this reason, understanding HS biological activity and the molecular mechanisms through which they exert their functions is becoming an important ecological task and a valid tool in facing environmental problems. This review is aimed at clarifying the main signaling events governing the physiological effects of HS on plant metabolism, in order to shed more light on nature, properties, dynamics and functions of HS as part of soil and agricultural ecosystems (Sara, 2010) [21].

Molecular Structure of Humic Substances

Humic substances are often described as coiled, long-chain molecules of two- or three-dimensional cross-linked macromolecules which vary in molecular weight from 500 to 10,00,000 Da depending on humic acids and method of measurement (Stevenson, 1994) [26]. One of the many physico-chemical properties of humic substances is the ability to complex metal-ions, which is usually attributed to the high content of oxygen-containing functional groups including COOH, phenolic- alcohol, enolic- OH and C=O. Nitrogen and S containing functional groups may also be involved in metal complexation. However, the latter two functional groups are present in small amounts and for this reason, their contribution to metal-humic substance complexes is less pronounced by Stevenson (1994) [26].

Alvarez Puebla *et al.* (2005) [1] proposed a macromolecular model of HS in which simple (though heterogeneous) monomeric units progressively build up into high molecular weight polymers by random condensation and oxidation processes. In this model is demonstrated that linear or branched polymeric chain, assuming several conformational folding that would give more resistant to microbial degradation and lengthen their turnover in the soil (Insam, 1996) [11]. A supramolecular association of heterogeneous molecules held together by hydrophobic interactions (van der Waals, π - π , ion-dipole) and hydrogen bonds has been proposed by Simpson *et al.* (2002) [24] and Schaumann (2006). The whole of these forces stabilizes the structure of molecular aggregates.

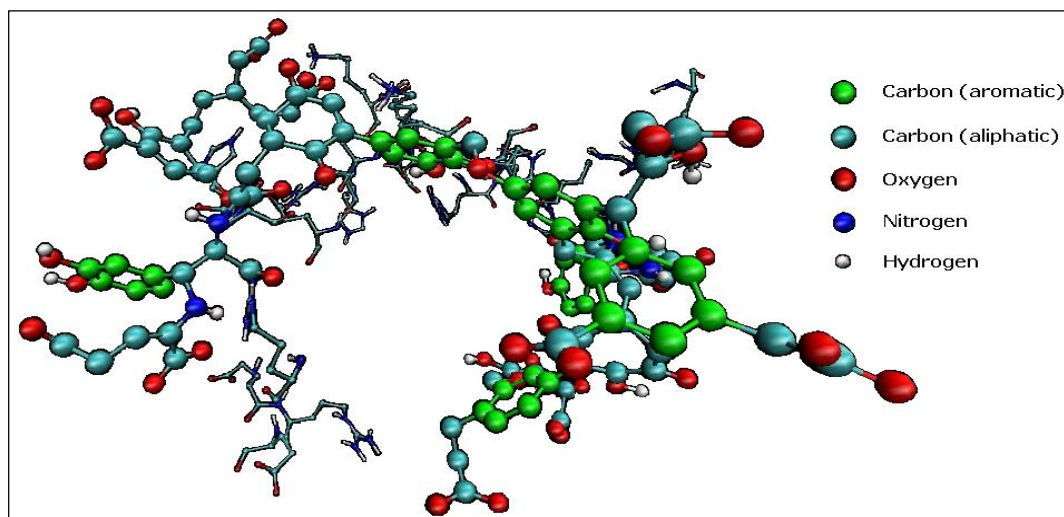


Fig 1: Schematic structure of humic acids (HAs)

Moreover, the stability of HS aggregates in solution seems to be of a dynamic nature and influenced by the solution ionic strength and pH (Kucerik 2007) [12]. At the alkaline pH the HS are in disperse form because intramolecular hydrogen bondings are completely disrupted. Acidification of HS with weak acids (i.e., acetic acid) solutions produces a significant decrease of molecular sizes or disaggregation for disruption of weak non covalent interactions, such as van der Waals, π - π , and CH- π (Smejkalova and Piccolo, 2008) [25]. At lowest pH values (<3) the HS collapse into smaller aggregated. This phenomenon is mainly due to the protonation of carboxylate groups that favor an increase in the number of intra and intermolecular hydrogen bonds inside the humic macromolecules. From a chemical point of view the HS are molecular aggregates consisting of sugar, fatty acids, polypeptides, aliphatic chains and aromatic rings by Simbson *et al.* (2002). HS are operationally classified into humin and humic and fulvic acids in relation to different solubility at acid and alkaline pH. This classification was based only on superficial criteria and no indication on chemical behavior or structures was deduced (Stevenson, 1994) [26]

Biological Activities

Different authors hypothesized that HS may be adsorbed by plant roots, even if high molecular weight (HMW) and low molecular weight (LMW) fractions seem to behave differently (Vaughan, 1967) [31]. Up to today it has not been yet completely clarified the mechanisms through which HS interact with the root cells and may subsequently influence plant physiology and growth. Among the modifications

induced by HS on treated plants, changes in size and development were the first to be studied analytically. Under particular conditions, HS can stimulate plant growth in terms of increase in plant length and dry or fresh weight, these effects seem to depend on the concentration and source of the substance, on the plant species and age, as well as on the culture conditions of the trial it proposed by Guminski (1968) [9] and Hernando (1977) [10]. Recently, many studies have confirmed the hypothesis of a direct effect of HS on plant physiology, in particular concerning root hair formation and lateral root development. The effects of HS on plant metabolic processes have been extensively reviewed. For instance, many reports showed that HS influence respiration, protein synthesis and enzyme activity in higher plants (Nardi *et al.*, 2002) [16]. As far as the photosynthesis process is concerned, few reports, focusing on the chlorophyll content and electrons transport are available (Pflugmacher *et al.*, 2006) [17]. Positive effect of HS on nutrient uptake has been reported for the major inorganic elements, such as nitrogen, phosphorus, potassium (Mylonas, 1980) and sulphur, (Guminski, 1968) [9] but different HS fractions seem to differently affect their uptake. A recent study focused on iron uptake and assimilation in cucumber plants treated with purified leonardite humic acid (PHA), evidenced an induction of the expression of *CsFRO1* and *CsIRT1*, encoding a Fe (III) chelate-reductase and a Fe (II) root transporter respectively (Elena *et al.*, 2008). These results strongly support the hypothesis that beneficial effects of HS on plant development may, at least in part, depend on their capacity to improve Fe availability for plant uptake under Fe-deficient conditions

identified by Chen *et al.*, 2004^[4]. Schmidt *et al.* (2005)^[22] evidenced the capacity of a specific fraction of HS (water extracted fraction WEHS) to stimulate some of the typical plant responses to Fe deficiency, such as the induction of Fe (III) chelate-reductase activity. This action of WEHS was also associated to significant increase in rhizosphere acidification. Furthermore, as a consequence of the environmental impact that the nitrogen fertilization has assumed during the last century. Sessi *et al.* (2002) demonstrated a strong positive effect of the LMW fractions on nitrate uptake and assimilation, whereas HMW fractions induced only weakly the same pathways, Nardi *et al.* (2002)^[16] demonstrated that the increment of nitrate influx measured in response to HS depends at least partially on a transcriptional activation of a gene encoding a major H⁺-ATPase of maize (*Mha2*), likely leading to the generation of a more favorable electrochemical gradient. In fact, nitrate influx across the plasmalemma of root cells is coupled to the favorable H⁺ electrochemical gradient created by the plasma membrane H⁺-ATPase (Thibaud and Grignon, 1981; Meharg and Blatt, 1995)^[27, 14]. Trevisan, 2009^[28] studied a wide transcriptomic approach to study the biological processes involved in the *Arabidopsis thaliana* response to HS. This study represents the first report concerning the global molecular mechanisms governing the HS-plant interaction.

Hormone-Like Activities

A putative HS hormone like activity is not surprising as it is known that soils vary in their native auxin content and fertile soils contain greater amounts of auxin than less fertile ones (Dahm *et al.*, 1977)^[5]. Auxin and gibberellin levels are usually higher in the rhizosphere than in the bulk soil, probably as a consequence of increased microbial populations or of an accelerated metabolism owing to the presence of root exudates. Although numerous soil and rhizosphere microorganisms, as well as the root systems of higher plants, have been reported as producing auxin (Lebuhn *et al.*, 1993; Rademacher, 1992)^[13, 19] there is little information about their stability and only indirect conclusions have been made about their presence in amounts high enough to be biologically active. In addition, the hypothesis of a HS auxin-like activity was also supported by reports showing a positive effect of such substances on specific targets of auxin action. *Mha2*, a major maize isoform of H⁺-ATPase that is preferentially expressed in guard cells, phloem and root epidermal cells and that appears to be strongly stimulated at the transcriptional level in response to auxin. Frias *et al.* (1996)^[7] evidenced a significant up regulation of its mRNA abundance in roots of maize seedlings treated for 48 hours with earthworm low molecular size HS (Quaggiotti *et al.*, 2004)^[18]. Furthermore, Russel *et al.* (2006)^[20] studying the effects of two different molecular weight fractions of HS on pea, evidenced an auxin-like effect of both fractions on stomatal opening as influenced by phospholipase A₂, that is considered to be involved in auxin signaling. In former experiments, Muscolo *et al.* (1996)^[20] showed a morphogenetic effect of HS on *Nicotiana plumbaginifolia* leaf explants, probably triggered by a modification of peroxidase and esterase activities. These effects, peculiar to humic fraction with a low relative molecular mass (<3,500 Da), were similar to those produced by IAA. A subsequent study on homogeneous carrot (*Daucus carota*) cell cultures compared the effects of the low relative molecular mass humic fraction to different auxins (Muscolo *et al.*, 1999)^[15]. This humic fraction caused an increase in carrot cell growth similar to that induced by 2,4-

dichlorophenoxyacetic acid (2,4-D) and promoted morphological changes similar to those induced by IAA. In addition, Muscolo *et al.* (1999)^[15] demonstrated that IAA and LMW fractions richer in carboxylic groups bind in the same way with carrot cell membranes. Zandonadi *et al.* (2007)^[33] comparatively evaluate the effects of indole-3-acetic acid and humic acids (HA) isolated from different soils substances on maize root development and on activities of plasmalemma and tonoplast H⁺ pumps. They observed that HA as well as low IAA concentrations (10⁻¹⁰ and 10⁻¹⁵ M) stimulated root growth by inducing the proliferation of lateral roots along with a differential activation not only of the plasma membrane, but also of vacuolar H⁺-ATPases and H⁺-pyrophosphatase.

Interactions between Plant Root Systems and HS

The interactions that occur between HS and plant root systems are of great importance for understanding the modes of action of HS. Two fundamental issues are important for the studies of the HS-root relationship. The first issue is about understanding what happens in plant's environment where HS are present, and the second issue is related to the fact that most experiments examining the effects of HS in plants use root application methods. HS fractions interact directly with root structures. Studies of HA and FA marked with ¹⁴C isotopes have shown that these HS fractions are associated in greater quantities with the cell wall within the first few hours of HS-root interaction (3 h) and subsequently (18 h) become part of the soluble component of the cells. Of the different HS fractions, HA are associated in higher numbers with the cell wall at the roots, while more FA are incorporated into the cell (Vaughan and Ord 1981)^[30]. Observing HS-root interactions allow us to understand how these substances are assimilated and how they affect plant processes at the leaf level. Experiments with wheat plants have shown that of the total ¹⁴C associated with HS that is assimilated by plants through roots, only 5 % is transported to leaf tissues (Vaughan and Linehan 1976). Additionally, with regard to HS-root interactions, there are reports in the literature of physical interactions (specifically with HA) that change the functionality of cell membranes. HA supramolecular colloidal clusters in solution can migrate to the surface of the roots and cause the clogging of pores and root transpiration sites. These HA-root interactions and the formation of layers of agglomerates may be governed by electrostatic and Van der Waal interactions. This phenomenon causes reductions in hydraulic conductivity, leaf organ growth, transpiration, and resistance to hydric stress and the mechanism of action is known as colloidal stress (Asli and Neumann 2010)^[2]. Evidence of the agglomeration phenomenon of HA in roots was recently captured by light microscopy, as shown in Fig.2 HA-root interactions have also been demonstrated using ¹³C-NMR spectroscopy, which showed that HA that formed agglomerates on root surfaces had lower structural complexity than exogenously applied HA. At the same time, it was determined that these HA interaction and agglomeration events are detected by ROS generation mechanism in the leaf and root tissues, triggering the activity of antioxidative metabolic enzymes. For the first time, it was observed that this type of interaction could be related to the modes of action of HS in plants and that growth and development could be controlled through the regulation of REDOX homeostasis and other metabolic processes that are stimulated by HS (García *et al.*, 2012a ; Barbera and García, 2014)^[8, 3].

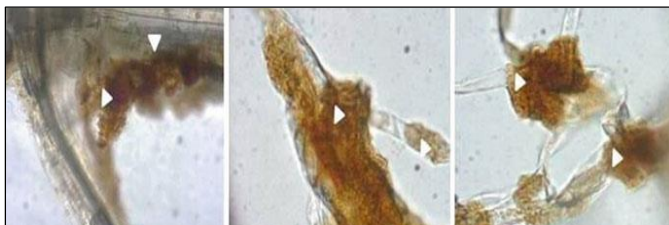


Fig 2: Agglomeration effects of HA isolated from cattle manure vermicomposting on the roots of rice plants grown in nutrient solution ($\times 100$ magnification). Layers of HA agglomerates on the root surface and emerging root hairs can be observed.

Conclusions and Perspectives

Humic substance, as the major component of soil organic matter, have been widely studied in various areas of agriculture, such as soil chemistry, fertility and plant physiology. HS plays an important role in controlling the behavior and mobility of pollutants in the environment and contribute substantially in improving the global soil fertility status. These features together with a major demand of safe food and sustainable agriculture have contributed to enlarge the environmental significance of humic substance, which have been recently recognized as a possible tool in facing environmental problems. Many of their positive effects on soil and plant growth have been demonstrated to rely on their chemical composition, but progress in research on HS has been known to be under the control of auxin. The structure, function and properties relationship should be the basis of future studies of HS and their effects on the environment. Due to the heterogeneous structural characteristics and numerous sources of origin of HS, every study, whether dealing with living organisms or natural systems, must include the structural characterization of HS as its premise. Regarding the mode of action of HS in plants, we observed that in spite of experimental design, HS have effects on several different metabolic processes in plants, as demonstrated through the use of large-scale gene sequencing techniques. The main actions of HS can potentially be observed in each specific metabolic pathway. However, we still lack a complete explanation of how these actions create more effective plant responses that protect plants against possible stress.

Finally, the results discussed in this study and the proposed framework for future studies should aid in the development of new ideas about the application of HS that will guide future studies on the use of humified organic matter in agricultural ecosystems.

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