Chapter

Properties of Humic Acid Substances and Their Effect in Soil Quality and Plant Health

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Abstract

During aerobic and anaerobic decomposition of plant and animal reside a complex aggregate of brown to dark coloured amorphous substances is obtained which is called as Humus. It includes humic substances and resynthesizes products of microorganisms. These products are stable and a part of the soil. Humus is categorised according to their molecular weights and solubility into humus, humic acids and fulvic acids. Humic substances are the organic material naturally present in soil. Humic substances positively effect's soil quality and fertility by increasing its water holding capacity, stabilisation of soil structure, soil microbial activity, plant physiology. It also influence nutrient uptake and root architecture act like phytohormones for phosphorus acquisition, and improving plant adaptation to saline condition. Humus is the primary microhabitat for microorganism such as dictyostelids, myxomycetes, some species of protostelids, members of the genus Copromyxella etc. Other than that auxin like activity of Humic Substances has also been demonstrated in recent studies. The research suggested that it could be the main biological factor that exhibits positive effect on plant physiology. Based on that fertiliser factory also trying to produce are bio- stimulants, based on humic substances and other organic compounds.

Keywords: amorphous, biostimulants, microorganism, nutrient uptake

1. Introduction

The word humus comes from the Latin word meaning soil or soil which refers to living things in the soil. Humus is a black organic matter found in the topsoil that is formed by decomposition by the action of soil microbes such as bacteria and fungi, which divide animal and vegetable material into smaller inorganic particles that can be used to grow plants and plants as well. It is an integral part of the biological life cycle of fertile soil. Technically, humus as a final result of this process is less important for plant growth than products produced during active rot. Humus has a high carbon content and is usually acidic due to its humic acid content. It increases the water storage capacity of the soil and produces carbonic acid, which transports minerals. Regardless of the soil group, the most important indicator of soil fertility is humus content; both in the topsoil and in the entire soil profile [1, 2] because all the essential elements in organic material exist. In humus. It contains a lot of carbon so it is still organic, but micro-organisms can decompose further. Humus is so stable that it can withstand the soil for hundreds of years.

Humification is the formation of humus and affects the development of the soil by determining its colour. Dark brown to dark brown is usually high in humus content. The dark colour of humus helps to warm the cold soil in the spring. Humus plays an important role in sustaining living organisms in the soil which is essential for healthy plant growth. There are so many benefits of humus such as:

- The conversion of organic matter into compost provides micro-life food.
- Mineral storage: Solid humus absorbs minerals and prevents loss.
- Humus is important for storing and binding ionic forms of nutrients and keeps it safe from water extraction and provided for plant absorption.
- Humus helps hold water up to 80 to 90% of its weight. So it can prevent drought stress.
- Humus plays an important role in maintaining a good pH by adding high acidity or alkaline soils.

2. Soil organic matter (SOM)

Bacteria, fungus, algae, actinomycetes, earthworms, nematodes, and arthropods are just a few of the living things that live in soil. All are important in the biological and microbiological activity that results in the decomposition of plant material, roots, dead creatures, and other organic residues that build up in soils. Soil organic matter is created as this organic material decomposes. Soil organic matter has a wide range of chemicals originating from algal, plant, and microbial sources, each with a unique structure and set of properties [3]. The major functional groups in SOM are O-and N-containing groups that donate electrons, fix nitrogen, and go through processes to generate humic substances (HS), a subclass of SOM made up of high molecular weight biopolymers that react strongly with both inorganic and organic pollutants. Soil organic matter makes up only 1–5% of the total soil mass, but it plays a vital role in soil health and fertility because it has an energetic effect on the characteristics, texture, and function of the soil.

SOM is an important component in the creation and stability of aggregates, as well as a wonderful storage for all necessary nutrients [4]. Soil organic matter absorbs nearly all of the nitrogen in the soil and, in most cases, creates it. Apart from that, SOM can remove hazardous substances from the soil, such as Al ions, lowering soil toxicity and providing numerous benefits to plants [5]. Thus, soils with a high SOM content are of higher quality and fertility than soils with a low SOM content, and they provide a wide range of positive benefits to plants and crops, such as improved rooting conditions, critical nutrients for plant growth, and increased soil water holding capacity. Even if the soil has a low pH, it can still deliver enough levels of N, P, and other nutrients if it has a lot of SOM. Overall, SOM has integrated a number of practical, chemical and biological activities that contribute to the cation exchange network, soil conservation, water flow and ventilation and productive forests. Soil organic matter plays an important role in the functioning of the ecosystem by preserving the natural and chemical properties of the soil by incorporating nutrients [6]. Soil organic matter is protected from the soil by three main processes: biochemical recalcitrance, chemical stabilisation and physical protection.

3. Sources of soil organic matter

Soil organic matter is a major source of carbon in the soil [7], and it can improve chemical, physical, and biological soil properties, as well as serve as a source of carbon and energy for microorganisms and have an impact on greenhouse gas emissions [8]. SOM is a mixture of molecules from plants, animals, and microorganisms with varying compositions, levels of availability, and environmental activities [1]. Plants are the principal source of organic matter in the soil. Organic matter is mostly added to the soil in the form of fallen leaves and plant parts that have died [9]. The crowns and roots of trees, shrubs, grasses, and other native plants provide a high volume of organic matter during the natural phase. Following harvest, a substantial quantity of crop plant parts are left in the soil, which acts as a source of organic matter in the same way as primary minerals serve as the parent materials of soil mineral components. The cytoplasm, cell membranes, and cell walls are the three elements of a plant that can be divided into three categories based on their chemical composition. The cytoplasm is rich in simple sugars, organic acids, amino acids, and enzymes, all of which are necessary for metabolic activity to continue. Proteins are sandwiched between two lipid bilayers in cell membranes. Hemicelluloses are abundant in plant cell walls. Cellulose, lignin, proteins, cuticle, and root wax are all components of the plant. Lignin is the second most prevalent organic compound in plant residues, accounting for about 5Vo of the mass of grasses and up to 6Vo of the mass of hardwood forest species. This component transforms into simple nutritional forms and increases soil organic matter. Animals are also regarded as secondary organic matter suppliers. Insects, millipedes, nematodes, and arthropods are among the soil creatures that contribute to the rise of organic matter. After they die, soil microorganisms contribute a significant amount of organic matter to the soil [10]. The dead bodies of soil organisms will be attacked by other organisms, which will decompose them into simpler products such as carbohydrates converted to simple sugar and proteins converted to simple amino acids. Sugar, starches, and protein decompose quickly, while fat, waxes, and resins decompose slowly. Because they are confined by microbial attack, a fraction of organic elements will survive in the soil for prolonged durations after all decomposable organic molecules have vanished. Decomposition is primarily carried out by microorganisms. Because of their ability to create specialised enzymes and the ability to access novel substrates via hyphae, fungi are regarded as key contributors [11].

Lignin, fat, waxes, and other materials make up the majority of this section. This proportion makes up the majority of humus. For instance, consider manure. Farmyard manure, compost, green manure, fish, meal, oil cake, and other organic matter sources are essential sources of organic matter in the soil, and their addition enhances the mineral content and other nutrients in the soil. Microbe-produced oil enzymes are essential for the biochemical processes of organic matter breakdown. SOM quality regulates SOM degradation and the release of carbon, nitrogen, phosphorus, and other macro and microelements (**Table 1**) [12].

SOM component	Description
Living Component	
Phytomas	Dead trees are also considered as phytomass
Microbial Biomass	Organic matter associated with cells of living soil microorganism
Faunal Biomas	Organic matter associated with living soil fauna
Non-living Component	
Particulate organic matte	usually dominated by plant derived materials
Litter	Organic materials devoid of mineral residues located on the soil surface
Macro-organic matter	Fragments of organic matter >20 pm or > 50 pm {i.e., greater than the lower size limit of the sand fraction) contained within the mineral soil matrix and typically isolated by sieving a dispersed soil

Table 1.
Component of SOM with their description.

4. Characteristics of humic substances

Humic substances are a complex mixture of biotic-derived organic molecules that have undergone extensive alteration since they were first created by plants. Humic substances, often known as "gelbstoff" or "yellow compounds," are the end products of organic decomposition in marine, freshwater, and soil environments. They are highly tranformable in their combinations of organic molecules (carbohydrates, amino acids, and fatty acids) as polymer compounds, and they are also highly resistant to further microbial degradation. Humic substances can be divided into humic acid (insoluble below pH 2), fulvic acids (soluble at any pH), and humin based on their solubility (insoluble in water) [13]. We also know that humic compounds are formed by the breakdown of plant material and can originate on land or in the sea (e.g., phytoplankton), and are a major source of organic C and N in aquatic systems.

5. Humic substance calcification

5.1 Fulvic acids

Carbon, hydrogen, oxygen, and nitrogen make up the majority of fulvic acid. Fulvic acid has a much lower molecular size than humic acids, which have molecular sizes ranging from 1000 to 10,000. They are water soluble in all pH ranges. The dissolution of protons in fulvic acids causes a progressive array of negative charges with rising pH. Over the lower pH range, say below pH 7, it is now well established that carboxylic type groups are mostly responsible for this behaviour. Polysaccharides and low molecular weight fatty acids are the main sources of fulvic acid.

5.2 Humic acids

Humic acid has a molecular size of 10,000 to 1,000,000, which is larger than other humic substances. They are dark in colour, ranging from brown to black, and are made up of a mix of carbon chains and rings. In an acidic environment, humic acid is not soluble in water, but it is soluble in an alkaline solution.

5.3 Humin

Because it is strongly bound to inorganic soil colloids, humin is an insoluble component of humic material. Humins are humic material fractions that are insoluble at all pH levels. Because of their molecular weight, humin complexes are classified as macro organic compounds. The humin fraction increases the capacity of the soil to store water.

6. Mechanisms governing humus formation

One of the least understood features of humus is the generation of humic compounds. Decomposition and synthesis are two types of chemical reactions that have resulted in the production of humus. Plant and animal bio-chemicals are broken down by soil microbes during decomposition. The second of the humus-forming processes involves the breakdown of other basic organic compounds. Following that, the humus synthesis process begins with the polymerisation of monomers, resulting in the formation of polyphenols and polyquinones.

These high-molecular-weight molecules interact with N-containing amino acids and make up a large part of resistant humus. The synthesis of these polymers is aided by colloidal clays. The humic group and the nonhumic group are the two main groupings of chemicals that make up humus. According to humus formation theories, there are numerous avenues for the synthesis of humic compounds during the decomposition of plant and animal leftovers in soil. All of these processes, including sugar amine condensation, must be evaluated as possible mechanisms for the production of humic and fulvic acids in nature (**Figure 1**).

6.1 Lignin theory

According to this theory, the residue of incomplete lignin breakdown by microorganisms becomes part of the soil humus. Loss of methoxyl (OCH3) groups with the formation of o-hydroxyphenols and oxidation of aliphatic side chains to create COOH groups are two examples of lignin modification. The changed product undergoes unknown alterations in order to create humic and fulvic acids. According to this notion, humic chemicals were transformed to lignin breakdown earlier by cellulose

a b c d

Figure 1.
Physiological changes in humic acid formation.

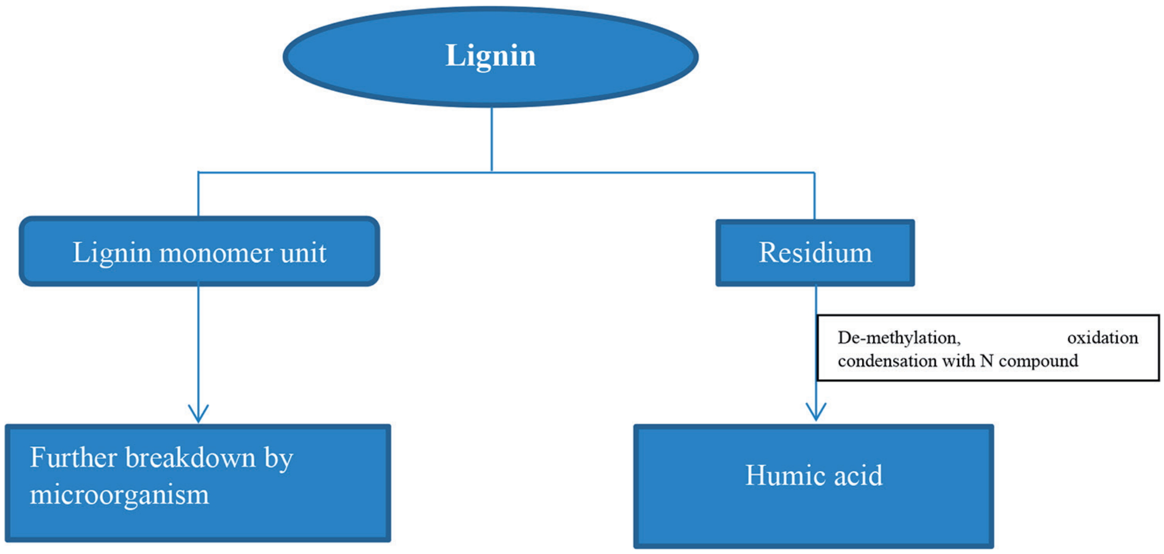

Figure 2.
Lignin theory.

degrading mycobacteria. Lignin can be broken down into low molecular weight compounds in usually aerobic soils (**Figure 2**).

6.2 Polyphenol theory

According to this theory, the condensation of phenolic compounds produces humic chemicals. Lignin polyphenols are oxidised to quinones. To generate humic molecules, these quinones are condensed with low molecular weight microbial products. Amino acids, nucleic acids, and phospholipids are among the microbial products. Enzymatic conversion of phenolic aldehydes and acids released from lignin during microbiological attack to quinones, which polymerise in the presence or absence of amino compounds to create humic-like polymers. The synthesis of brown-coloured compounds by reactions involving quinones is a well-known phenomenon that occurs during the production of melanine. Lignin, microbes, and lignin decomposition are all possible sources of phenols for humus production. The phenols that are generated during decay are also used to make humus.

6.3 Sugar-amine condensation theory

According to this concept nonenzymatic polymerisation of reducing sugars and amino acids created as by-products of microbial metabolism results in brown nitrogenous polymers similar to those produced after dehydration of certain food products at moderate temperatures. The amine is added to the sugar's aldehyde group to generate the n-substituted glycosylamine in the first step of the sugar-amine condensation reaction. The N-substituted-1-amino-deoxy-2-ketose is then formed from the glycosylamine. Dehydration and creation of reductones and hydroxymethyl furfurals; fragmentation and formation of 3-carbon chain aldehydes and ketones, such as acetol, diacetyl, etc. All of these molecules are very reactive, and in the presence of amino acids, they quickly polymerise to generate brown-coloured products.

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7. Effects of humus on soil invertebrates

Invertebrates play a crucial part in the soil's physical, chemical, and biological activities, although they are often overlooked. According to studies, invertebrates have self-organising systems and perform various types of ecosystem engineering [14]. Nematodes, collembola, and mites are major soil invertebrates in coniferous forests, while earthworms and termites are prevalent in temperate deciduous and tropical forests [15]. Invertebrates Griffiths *et al*. [16] have shown that both trophic (decomposition) and non-trophic (moment) effects are involved in the production of soil humus. [17] examined soil invertebrates' internal (ingestion and associated transformations) and outward (defecation, construction) regulatory mechanisms, concluding that these systems contribute to the stabilisation and destabilisation of soil organic matter. Microbial decomposition is aided by invertebrates, which also operate as real decomposers, breaking down dead organic waste with their own endogenic enzymes, which has an impact on the soil environment and plants. While macro invertebrates consume humus and redox active components (iron minerals and humic compounds), they change soil organic matter [18, 19]. In the case of arthropods, species richness is around 85%, and they make up a major component of the soil's meso and macrofauna. Arthropod excrement is the basis for the production of soil aggregates and humus. It physically stabilises the soil, increasing its nutrient storage capacity. Culliney, [20] During intestinal transit, macro invertebrates like soil-feeding termites virtually totally decrease acid soluble Fe (III) and humic acids. In the alkaline intestines of scarab beetle larvae, the same process occurs. The gut homogenates of Pachnoda ephippiata no longer converted Fe (III) to Fe (II) after sterilisation, suggesting the importance of the gut microbiota in the process. At pH 7 and 10, the PeC11 strain demonstrated a significant ability to reduce dissimilatory Fe (III) [15]. The full polymerisation of phenols and proteins is aided by some fly (Bibio marci) larvae and earthworms (Aporrectodea caliginosa). Using 14C-labelled phenols and proteins, invertebrates were able to understand the biodegradation of organic materials and the creation of humic compounds in lab conditions. The same organic chemicals were employed as a control without the impact of invertebrates (phenols and proteins). In the excrements of *Bibio marci*, the fraction of the 14C isotope in the humic acids was higher than in the control substances. The earthworm A. caliginosa excrement showed a substantial favourable response to proteins [19].

8. Effect of humus on soil fertility

Because of their complex and heterogeneous structures, soil organic matter, which is made up of 80% humic acid and the remainder un-decomposed matter, plays a vital function and has a direct impact on soil fertility and textures. Carbon, oxygen, hydrogen, nitrogen, and sulphur are all significant elements found in humic compounds. It is composed of 80% organic matter derived from the soil [7, 21]. Fulvic acid and humic acid, which are both soluble in sodium hydroxide, are categorised as humic compounds. Humin, on the other hand, is insoluble in sodium hydroxide. [7, 21]. After solubilising in sodium hydroxide, humic compounds are treated with acid, which dissolves the fulvic acid portion while precipitating the humic acid portion [7].

Decomposition of corpses and debris produces humic chemicals. Total soil nitrogen, available nitrogen, total phosphorus, available phosphorus, total potassium, available potassium, and organic matter levels were all improved, as were enzymes like urease, sucrase, and phosphatase, as well as bacteria and fungi. Granular maize straw was added to soil in an experiment to improve soil humus carbon, fulvic acid carbon (FAC), and humic acid carbon (HAC). After applying granular straw to the soil, HEC, FAC, and HAC all increased by 17.59%, 8.32%, and 26.51%, respectively. The application of straw makes the structure of humic acid aliphatic, simple, and younger, as well as promotes the continuous renewal of humus and making soil younger and more fertile, according to techniques like infrared spectrum and fluorescence spectrum used to analyse soil humic acid and principal components analysis (PCA) [22]. Zhang et al. [23] investigated HA molecular structure variation after a 35-year long-term experiment in black soil. The researchers used methods such as Fourier transform infrared spectroscopy (FTIR), 13C nuclear magnetic resonance spectroscopy (NMR), and fluorescence spectroscopy. The findings revealed that the molecular structure of HA in black soil is more simple, aliphatic, and younger as a result of manure application, but inorganic fertilisers make the HA structure more complicated due to increased condensation. Manure can be used alone or in combination with inorganic fertilisers to increase crop yield and improve soil organic matter structure. The use of humus in the soil was also found to be statistically significant in increasing corn N uptake in salty land, although the use of foliar humic acids did not increase P, K, Mg, Na, Cu, and Zn uptake but did increase P, K, Mg, Na, Cu, and Zn intake. Wheat has been subjected to similar findings. Under salt stress, the first dosages of humic compounds applied to the soil and foliarly boosted nutrient absorption in corn and wheat [24, 25].

9. Effect of humus on toxic pollutants in soil

In aqueous solutions, humic substances (HS) demonstrate the property of detoxifying agents. It has the ability to eliminate free contaminants (indirect bio-effects) as well as trigger organisms' protective responses (direct bio-effects). Bondareva and Kudryasheva will get married in 2021 [26]. Bacteria, such as Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes, as well as fungi from the phyla Basidiomycota and Ascomycota, were discovered to be the predominant degraders in the interactions between microbes and humic compounds. Laccase, peroxidase, and dehydrogenase enzymes in the soil were also found to be positively influenced. In the presence of sufficient nitrogen, humic acid and fulvic acid promote microbial activity. The breakdown of oils into fatty acids and sugars by humic substances includes enzyme production, chemical reactions, and microbial degradation, as well as some biological technologies. The oxidative binding of organic contaminants to humic compounds can be catalysed by these enzymes. Humic compounds and microorganisms are employed as terminal electron acceptors to promote organic pollutant biodegradation or lower metal bioavailability by suppressing electron transfer to CO2, reducing CH4 production in anoxic settings. On the other hand, fulvic acid was discovered to be a powerful chelating agent capable of extracting metal ions from salt molecules. The use of humates as a remediation strategy has a number of advantages over the use of microorganisms, including increased soil water wetability, lower oxygen requirements, simplicity of application, and lower cost. [27–29]. During short-term (45-day) experiments in mining and metallurgical places in the Kola Peninsula, Russia, the effects of exogenous coal humates, peat humates, and their combinations with nitrogen fixers and mycorrhizae forming fungi showed promising effects on environmentally friendly and effective solutions for soil health restoration caused by long-term air pollution with sulphur dioxide and heavy metals. pH, HM mobility, dissolved organic carbon content, and microbial activity all improve when coal is present. It reduces HM mobility (from 4 mg/kg to 12 mg/kg for Ni and Cu, respectively, to 1 mg/kg and 2 mg/kg) and raises pH from 4.1–5.0 to 5.5–6.0. It does not necessitate the use of lime and has a beneficial impact on the growth of test cultures and microbiological activity. Innoculation of humates with nitrogen fixers has no effect, whereas mycorhizae-forming fungi operate well with coal humates and promote the development of the test culture's root system [30]. The number of bacteria, rate of oxygen consumption, and generation of carbon dioxide in soils supplemented with fulvic acids followed a normal sigmoid curve, showing that mineralisation occurred promptly with no lag phase. The ability to oxidise aromatic chemicals, such as vanillio and phydroxybenzoic acid, increases as fulvic acids and microorganisms decompose. The structural study of humic acids and aromatic compounds is done using IR spectra and chromatographic examinations of their hydrolysates. The findings revealed that soil samples pre-incubated with glucose had a higher rate of fulvic acid decomposition than untreated samples, as well as a link between fulvic acid metabolism and aromatic chemical metabolism Kunc et al. [31]. In the treatment of arsenic (As)-contaminated soil, humus can be employed as a green agent to improve EKR (electrokinetic) technology. During electrokinetic remediation, electromigration controlled the distribution of As in the soil, and the addition of humus considerably increased the release of As from the soil (EKR). When 4 g of humus was added to the mix, the efficiency of the cleanup was increased by 20%. The presence of multiple oxygen-containing functional groups in humus was revealed by functional group analysis, which desorbed as from soil particles under both acidic and basic conditions. At pH 10, approximately 11.63% of bound as was desorbed as free as, indicating that EKR [32] was correct.

10. Effect of humic substances on agricultural productivity

Humic substances (HS) are known to be natural, effective growth promoters because they induce beneficial local and systemic physiological responses via hormone-like signalling pathways. The dosage, origin, and molecule size, as well as the degree of hydrophobicity and aromaticity, as well as the spatial distribution of hydrophilic and hydrophobic domains, all have a role in defining the effect. Low-molecular-weight HS can enter root cells and evoke intracellular signals directly, but high-molecular-weight HS bind to exterior cell receptors to activate molecular reactions. Nutrient transporters, plasma membrane H^+ -ATPases, hormone pathways, and genes/enzymes involved in nitrogen absorption, cell division, and development are all affected by HS.

Humic acid and humic substances, when used in the soil and in the laboratory, can improve soil structure, increase fertiliser utilisation, and act as growth enhancers by promoting morphogenesis, lateral root formation, and root hair initiation in intact plants, as well as stimulating root and shoot development in treated cell calluses. It improves nutrient utilisation efficiency, macro and microelement assimilation, and carbon, nitrogen, and secondary metabolism induction. Encourage crop growth as a result to increase production and income [33–35]. External application of plant-derived humic acid (PDHA) and coal-derived humic acid (CDHA) to wheat growing in two alkaline calcareous soils in pots resulted in considerable spike weight increases in both clayey and sandy loam soils. Serenella et al. [36] discovered that applying PDHA and CDHA at rates of 50 mg/kg to both soils increased grain yield by 21 and 11 percent, respectively, over control. Physiological and molecular techniques were used to test nitrate assimilation in Zea mays seedlings. The gene expression of Z. mays plants was affected by HS treatment at the transcriptional level, and its regulation was highly influenced by the availability of nitrate in the growing medium [37]. In flowering plant like Gladiolus (*Gladiolus grandiflorus* L.) applications of HA and NPK gives early and uniform sprouting, more foliage growth per plant, greater leaf area, and total leaf chlorophyll contents, earlier spike emergence, greater number of florets per spike, longer stems and spikes, and greater diameter of a spike, higher flower quality, longer vase life, higher number of cormels per clump, and greater cormel diameter and weight applied Iftikhar *et al*., [38]. To counteract the negative effects of salt on flax seed. Opal, Giza-8, and Mayic flax types were grown and treated with humic acid and/or foliar-applied chemical additions. The results revealed that the Giza-8 variety responded positively to these chemical additions, allowing it to more effectively withstand the harmful effects of salinity. Bakry et al. [39] found that foliar spraying of proline improved seed yield, straw yield, and oil yield at humic acid.

11. Conclusions

Crop production productivity is the basis of certain nutrients for human life which depends on amount of available nutrient in soil. This review has revealed that humus and humic acid substances has significant roles in soil quality, plant health, soil environment, plant-soil-microbial interactions and as well as productivity. This review counted a lot of factors that affect humic acid substances performance in soils, plants and also crops. Humic acid substances brings benefits in terms of agricultural yield, improves plant growth and the uptake of nutrients; but its economical application levels should be also determined. More research is needed to optimise the combined effect of different humic acid substances application and soil quality parameters under defined field conditions.

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