Chapter

Humic Substances: Prospects for Use in Agriculture and Medicine

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Abstract

Humic substances are the naturally occurring biogenic, heterogeneous organic compounds resulting from the decomposition of plant, animal and microbial residues, over many thousands of years. Research into humic substances have found them to be beneficial not only for crop production, but for human and animal health also. Humic substances influence crop yields by affecting soil texture, water retention, nutrient buffering capacity and microbial diversity. Their stimulatory effects on plant growth and physiology can be ascribed to enhanced nutrient uptake, hormonal activity with root growth and proliferation, activation of antioxidant defense under various abiotic stresses. Furthermore, their potential to remove toxic pollutants from soil as well as aquatic systems might resolve the problem of degrading environment. Positive effects of humic substance on soil as well as crop without harming the environment make humic acid a sustainable solution for crop production. The antioxidant, immunomodulatory, anti-inflammatory, detoxifying and nutritional properties of humic substances opens up new avenues for their medical applications. Still, more research is needed to completely explore their potential. This chapter will summarize and review information about the prospects of using humic substances in agriculture and medicine.

Keywords: Humic substances, Fulvic acid, Humic acid, agriculture, medicine

1. Introduction

Humic substances (HS) are soil-derived substances that form one of the vast reservoirs of organic carbon in nature. HS are primarily produced by physical, chemical and microbial degradation and transformation of plant and animal tissues (humification process) over millions of years. They constitute the bulk of organic matter of the soil – humus, peat, lignites and also brown coal. HSs may also be produced as a by-product of the synthetic oxidation reactions of phenolic compounds.

Humic substances contain carbon, hydrogen, oxygen and nitrogen with small amounts of sulfur and phosphorus. They are a mixture of acids that can be fractionated on the basis of differences in their solubility into humic acid (HA), fulvic acid (FvA) and humin fractions, beside ulmic acid, and some microelements. Humic acid includes aggregates of long chain, high molecular weight compounds, soluble in alkali, while FVA is a mixture of short chain, low molecular weight compounds, soluble in both acidic and alkaline solutions. Humin, in contrast, constitutes the non-soluble fraction of the humate.

Owing to their unique structural and chemical properties, humic substances make an excellent soil amendment. They offer beneficial impacts in terms of overall soil structure, water holding capacity and nutrient availability in the soil along with

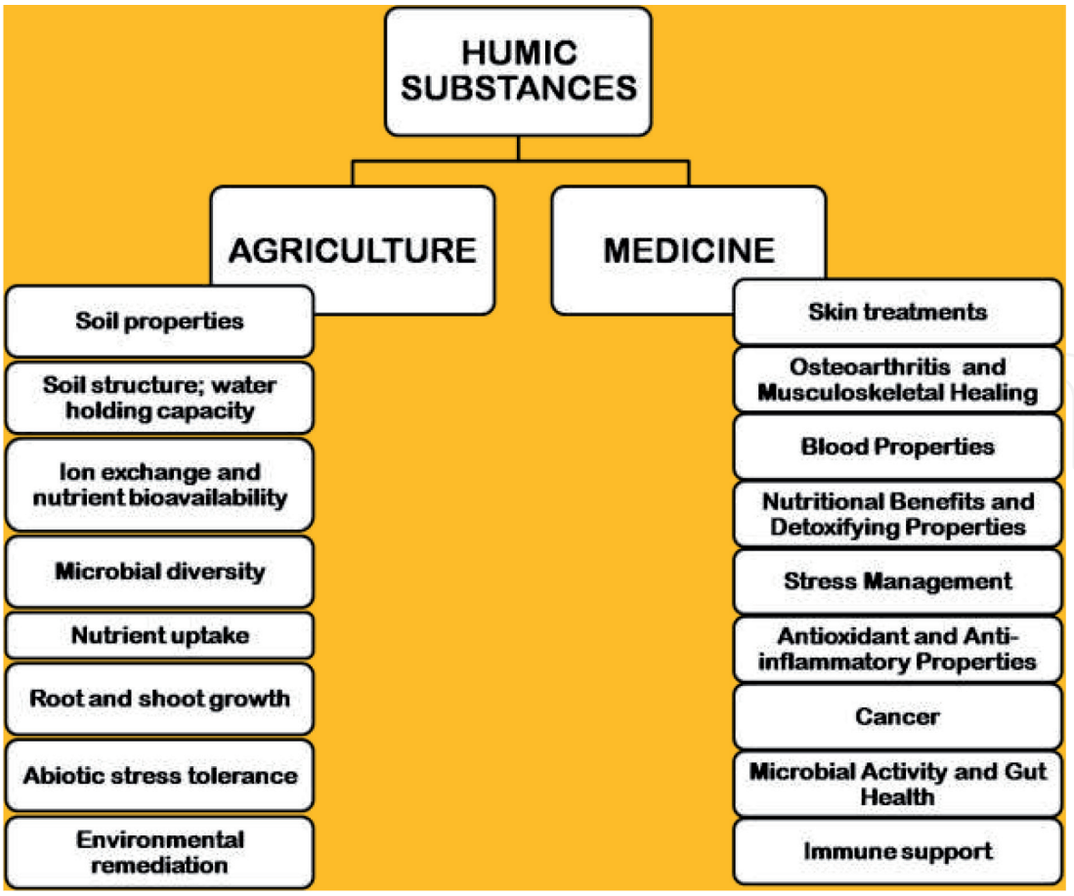

Figure 1.
Applications of Humic Substances in various fields of agriculture and medicine.

positive effect on the microbial communities in the soil, hormone like impact on plant growth. Thus, proving their vitality for farmers' economic status. HSs have also been applied in the treatment of various human diseases and maintaining overall vigour and growth of the body. Being rich in minerals which are easily assimilated in our body, their consumption in the form of dietary supplements and addition in cosmetic products is highly recommended. **Figure 1** tabulates various fields of agriculture and medicine where humic substances have shown their applications.

2. Role of humic substances in agriculture

2.1 Impact on soil properties

Humic substances play an important role with their effect on the quality and productivity of the soil. They influence the soil structure and various physico-chemical properties and are involved in the majority of soil surface phenomena. Humic substances, increase soil aggregation, water retention, infiltration rate, and water-holding capacity [1]. Research shows that aggregation in soils is improved with humic substances over a wide range of texture grades and mineral suites [2]. Polymerization reaction between the hydroxyl group and carboxyl group of humic acid in the fertilizer with the calcium in the soil, lessens the soil bulk density, improves the porosity, provides good permeability; thus, improving the soil structure [3]. Significant differences have been observed between the field capacity, permanent wilting point, and available water capacity of untreated and humic acid treated soils and emphasized that humic substances can improve structure of

degraded soils even [4]. The combination of colloid in humic acid and the calcium in the soil forms flocculent gels that impacts the soil loosening, increases air permeability and water storage capacity and develops soil aggregate structure [5]. Humic acids have potential to improve soil structure in short term as aggregate stability increased with humic acid application while soil modulus of rupture decreased [6]. The application of humic acids in arid white sandy and red sticky soil can provide the opportunity for use of reserved cultivated land resources, as these substances decrease the bulk density band increase the porosity of soil [7]. Positive effect have been observed of humic acid application on physico-chemical characteristics of soil embankment with pH value in range of 7–8, cation exchange to 60% saturated and soil bulk density in the range of 1.1–0.97 g/cm^3, thus making embankment favorable for fish or shrimp [8].

Humic acid can improve the chemical properties of the soil. The application of humic substances influences the cation exchange capacity of the soil. Exogenous application of humic acid increased exchangeable Na, Ca, Mg and K while decreased soil pH and EC [9]. Similar positive effects on soil EC, organic matter and total nitrogen were reported with increasing dose of humic acid [6]. Humic substances buffer the soil pH and release carbon dioxide. The biological and physio-chemical properties of organic substances may play role in nitrogen mineralization from organic sources during incubation [10]. Long term effect of humic acid application on soil nutrient status was confirmed through a three-year experiment on peanut in which humic acid treatment increased not only soil total nitrogen, phosphorus, potassium content and available NPK contents but also the organic matter content [11]. Increase in organic matter content of the soil can be useful for the improvement of agricultural soils [12]. In saline-alkali soils, humic acid adsorbs soluble salts in the soil, obstructs unfavorable cations, and decreases the salt concentration and pH of the soil. These substances have potential to counterbalance the acidity and salinity of the soil and act as a natural chelator for metal ions under alkaline conditions. It exchanges H^+ ions with Na in the soil thereby declining Na^+ content and increasing H^+ levels, consequently soil pH and SAR is reduced [9]. Humic substances decrease the soil Na, EC and pH likely due to high supplies of Ca, Mg and K which hold the cation-exchange sites on soil particles, restrict the Na adsorption and thus enhance Na leaching losses during precipitation events [13].

Soil microorganisms are important components involved in the processes viz., decomposition of soil organic matter, humus formation and soil nutrient cycling [14, 15]. Humic substances affect the structure of microbial community and impact the soil fertility [16]. The activity of soil enzymes represents the metabolism in the soil, which in turn reflects the nutrient uptake and growth of plants. Humic acid treatment increased the activities of soil enzymes- urease, sucrose and phosphates in a three year experiment on peanut [11]. Furthermore, microbial diversity of the soil was also altered with the application of humic acid; promotion of bacteria while the effect was reverse with fungi. Humic acids facilitate the growth of microbes by providing a source of carbon and food for micro-organisms. They also provide a source for colonization of microorganisms because of their large size; thus, providing continuous supply of nutrients for establishment of beneficial microbial communities in soil [17]. Humic acids at a concentration of 0.1 g/l stimulated the growth of the soil bacteria, probably, modulating the cell metabolism [18]. Application of humic substances has significant impact on carbon and nitrogen transformation processes occurring in the soil. This effect may be attributed to the functional groups of humic substances, which can be readily oxidized and subsequently act as electron donors for bacterial respiration, thus affecting the CO_2 and CH_4 production [19]. Microbial processes affected by humic acids can help to reduce greenhouse gas emissions [20].

2.2 Impact on plant growth

Humic substances are fairly stable products of organic matter decomposition and, thus, accumulate in the environmental systems. Due to their advantageous effects on different soil properties, their role in plant growth has been recognized. For several decades, commercial humic products have been available and applied to increase crop growth and economic yield [21]. Commercially available humic substance-based products can be applied both to the soil (root area) as well as foliar sprays. The impact of soil application has been studied extensively but for foliar application mechanism needs to be explored. In foliar application, humic substances do not come in contact with rhizosphere where important effect of humic substances on nutrient availability takes place [22]. Two hypothesis have been proposed to explain the effect of humic substances on plant growth- (a) Nutritional hypothesis, which proposes that the humic substances affect the plants due to their indirect effect on soil properties and the mineral nutrients available in the soil and (b) Hormone-like hypothesis, which proposes their direct impact on plant metabolism through roots [23]. The impact of humic acids on the plants depends on the concentration used, their genesis, molecular size and number and distribution of hydrophilic and hydrophobic sites. Humic substances mainly act upon proteins involved in nutrient transport, nitrogen assimilation, cell division and development; plasma membrane H^+-ATPases, hormone pathways [24].

2.2.1 Nutrient uptake from soil

Because of their structure and chemical composition, humic acids make nutrients more available in soil that might not otherwise be available. Negatively charged humic substances readily attract the cations and bind with them; thus affecting the cation exchange capacity of the soil. Humic substances form complexes with metallic ions and enhance the availability of micronutrients like Zn, Mn, Cu, and Fe and macronutrients like P, and specifically in the soils deficient in these nutrients [25]. Liquid fulvic acids polarize the soil and increase the uptake of nutrients [26]. So, humic substances form systems for ion exchange and complexation of metals, thus exerting beneficial effects on nutrient uptake, their availability and transport. Many researchers reported positive effect of both soil and foliar application of humic acids on uptake of different macronutrients (N, Mg, Ca, K and P) and micronutrients (Cu, Zn, Mn, Fe) in various crops viz., lettuce [27], maize [28, 29], wheat [30], peppers and cucumbers [16].

Application of either dry or liquid humic substances to soils improves the fertilizer efficiency. Humic substances diminish the leaching of nitrogen compounds into subsoil water and reduce the toxicity. They bind to these nutrients in a molecular form which decreases their solubility in water; thus, increasing soil exchangeable NH^{4+} and available NO^{3-} while retaining more nitrogen in the soil. These binding processes also prevent volatilization of N into the atmosphere. This role of humic substances is related to their effects on plant cell membranes, and associated biochemical and molecular processes initiated at post-transcriptional level in roots and shoots [31]. Humic substances might increase the uptake efficiency of nutrients, because of their ability to alter the activity of the root membrane H^+-ATPase, which is a recognized as a marker of biostimulant action and contributes to regulate the rhizosphere pH, thereby affecting nutrient availability [32].

2.2.2 Root and shoot growth

Humic compounds have been reported to improve plant growth in terms of parameters like plant height and weight. These effects vary with the concentration

and origin of the humic substance and the plant species. Applications of humic and fulvic acids at lower concentrations is advantageous for root proliferation. However, use of these compounds at high concentrations might decrease root and shoot growth. The positive effects of humic substances on root and shoot growth can be ascribed to their hormone like activities as different hormones have been identified in the humus structure [33]. The potential capacity of humic substances to induce root development in plants with auxin-like activity was first postulated by an independent research group working on mobilization of soil organic matter by root exudates [34].

Humic acids prevent the oxidation and breakdown of hormones, thus, auxins remain active for longer time. Application of humic acid, increase the root and thus, the plant is better able to take up water and nutrients. Auxins induce activity of plasma membrane H^+-ATPase root cells, which couple ATP hydrolysis to H^+ transport across cell membranes. The apoplast is acidified, the cell walls are loosened and resulting in cell elongation, thereby increasing the root growth. The stimulation of H^+-ATPase affects nutrient uptake, by enhancing the electrochemical proton gradient that drives ion transport across cell membranes. Humic acid-like auxins also induce H^+-ATPase synthesis and it activity [35]. Humic substances induce the synthesis of plasma membrane and increase the root growth with similar mechanism. Humic acid treated maize seedlings recorded higher root fresh weight than control plants along with increased number, diameter and length of roots [36]. Similar effects of root elongation, lateral root formation and increased activity of H^+-ATPase were observed in maize seedlings treated with humic substances obtained from earthworm compost which showed the presence of exchangeable auxin groups in the structural analysis [37]. Mechanism of effect of humic substances on root structure is still not clear. HA is also hypothesized to affect root architecture in cucumber seedlings and enhance nitric oxide, ethylene and auxin levels though the latter not being responsible for the former [38]. He suggested the participation of some other factors in improvement of root architecture with humic acid application. Also, research has shown that foliar application of humic substances did not result in increased activity of H^+-ATPase and various hormones like ABA as observed with application to the root; while both treatments increased the jasmonic acid content in root which can be related to adaptation to mild transient stress caused by humic substance application [22].

Humic substances also affect shoot growth, and it has been shown to affect multiple plant processes like photosynthesis, respiration, protein metabolism and activities of enzymes, uptake of water and nutrients. The mechanisms for these effects have been related to changes in hormones, cell membrane permeability, components of ETC, hydroxyproline:proline ratio, free radical activity within the humic structure, and reactive oxygen species in plants [21]. Although it is still not fully understood, humic acids result in better shoot growth. Application of humic acidsimproves the health and vigor of the plant. Apart from auxin, humic substances also exhibit gibberellins and cytokinin like activities [39]. These activities stimulate plant metabolism and in turn shoot growth. It has also been speculated that effect of humic substances on H^+-ATPase might affect the root-shoot distribution of various hormones like cytokinins, polyamines etc., thus improving the shoot growth [40]. Foliar applications of humic acid resulted in higher leaf and stem dry matter contents than the control in tomato [41]. Hormone like activity of humic acids also increased the number of harvested flowers per plant and extended the vase life of harvested flowers in gerbera [42]. Humic acid treatment showed highly significant effect on dry biomass, root/shoot ratio and photosynthesis parameters like stomatal conductance, transpiration rate, photosynthetic rate in potato along with 23–37% increase in tuber yield [43].

2.2.3 Abiotic stress tolerance

The effect of humic substances in the alleviation of abiotic stress effects in plants is ascribed to the elevation in enzymatic and non-enzymatic antioxidants, formation of compatible solutes and changes in ionic balance. In various abiotic stresses, reactive oxygen species (ROS) are one of the significant causes responsible for cellular damage. The oxidative damage due to ROS is prevented by induction of enzymatic antioxidants such as superoxide dismutase, peroxidases, catalase and ascorbate peroxidase and non-enzymatic antioxidants like ascorbate, tocopherol and phenolics and production of osmo-protectants like proline, sugars. These stress combating mechanisms are set off by humic substances also. Response of humic acid treatment has been evaluated on tolerant and sensitive melon varieties under drought and reported increased shoot weight, leaf area, chlorophyll content and activities of antioxidant enzymes with humic acid application under stress [44]. Humic acids enhance the stress tolerance by reducing the evaporation of water with reducing effect on the stomatal opening; helping the plant and soil to retain more moisture [7]. The oxidative stress is prevented by humic substances through their positive effect on peroxidase activity and proline levels; thus reducing ROS levels and maintaining cellular homeostasis [45].

Humic acid treatment is also known to improve photosynthetic rate under different watering conditions through increase in rate of gas exchange and electron transport flux in rapeseed [46]. Similar effects of stimulatory effect of humic substances on proline contents, grain yield, total dry weight and harvest index was reported in chickpea under stress as well as control conditions [47]. The exploration of impact of chemical priming with humic acids on maize seedlings at transcriptional level, revealed that signaling pathways of various hormones like ABA, gibberellins and auxins and stress-responsive genes were positively modulated and humic acid priming alleviated drought, heavy metal and salinity stress effectively [48]. Application of humic acid effectively imparted tolerance to maize plants against salinity stress in terms of increased plant biomass, chlorophyll content, mineral elements and antioxidant enzymes activities [49]. An increase in permeability of root cell membranes might be responsible for enhance nutrient uptake in tomato plants grown under saline medium added with humic acid [50].

Humic substances may act through proliferation of root which is important for plant adaptation under adverse conditions like salinity for exploration of deeper layers. In addition to biochemical responses, the effects of humic substances have been studied at molecular level also. Salt-induced inhibition of HKT1 (sodium influx transporter high-affinity K^+ transporter 1) gene was overcome by application of humic acid in *Arabidopsis* through post-transcriptional control of the *HKT1* transporter gene under saline conditions [51]. Likewise, humic substances confer heat tolerance by transcriptional activation of heat shock proteins in *Arabidopsis* [52]. Thus, humic substances tend to be the potential sources to sustain crop production under arid, semi-arid and saline environments.

2.3 Environmental remediation

Humic acid-based fertilizers increase crop yield and stimulate plant enzymes and hormones in an eco-friendly manner. High adsorption capacity and high ion exchange capacity enable them to be the key candidates for environmental remediation. In environmental applications, the significant roles of humic substances are removal of toxic metals, anthropogenic organic chemicals and other chemicals like herbicides, fungicides, insecticides, etc., and pharmaceutical products from soil and water. Humic substances have the ability to adsorb, form complexes, and exert redox effects. These interact with different types of chemical groups like metal ions,

hydroxides, oxides, minerals, organic matter, and toxic pollutants in the environment. In addition, these substances also participate in the biogeochemical cycles like carbon and nitrogen cycle, thus, modulating the man-made pollution in the soil. Furthermore, the detoxification organic and inorganic inhibitors of biological processes and biodegradation of toxic organic substances, speculates the efficiency of humic substances. Humic substances also hinder the uptake and availability of heavy metals *viz,* Cu, Fe, Zn etc., in soil by immobilization of these metals.

Apart from soil, humic substances in aqueous systems, affect aquatic ecosystems along with the organisms. In rivers, streams and lakes, about 50% of the dissolved organic materials are humic acids, which affect their pH; the physical and chemical properties of water are altered and they act as natural neutralizing components by stimulating biotransformation of xenobiotics. Humic acids can be deployed in water treatment and water purification technologies for removal of pollutants [53].

3. Role of humic substances in medicine

3.1 History of the use of HSs in medicine

HSs have found special place in traditional systems of medicine for roughly 3000 years in many countries, particularly India and China. Transcripts from 'Ayurveda' and 'Siddha' systems of medicine strongly refer to the use of humic substances in treatment of various ailments. Chinese Materia Medica pharmacological compendium, the medical text of the 15th century Ming Dynasty, reports the use of HSs in traditional medicine. Owing to their numerous benefits, HSs were referred to as "Wujinsan", meaning "golden medicine" in China.

The pharmacological properties of humic substances and products derived and extracted from them, have been systematically reviewed [54]. A positive approach of these substances has been documented through well-designed studies for treatment of various rheumatological and other musculoskeletal diseases. Shilajit, a traditional exudate from Himalayas in India occurs in the form of naturally occurring mineral substance. Shilajit has proven stimulatory, rejuvenatory, revitalizing, anti-ageing and anti-inflammatory properties. In addition, topical application of Shilajit has proven its antiseptic and analgesic properties [55]. Shilajit typically constitutes humic substances in which fulvic acid act is the most bioactive component (15–20%) that is known to have immunomodulatory and psychoactive behavior. Peat extracts, derived from soil – abundant in HS, have been used extensively in ancient Rome during the 19th century as mud baths for its healing effects in gynecological and rheumatic diseases [56]. Peat was also given orally to treat cardiac, gastric, intestinal or hepatic conditions.

Owing to their numerous benefits, HA and FVA have recently gained popularity in 'natural' food market. They can potentially be used as novel, natural, valuable food supplements or additives. Already some are available in the form of ready-to-drink beverages and OTC pills, but still this market needs detailed exploration in terms of combinations and variety.

3.2 Skin treatment

Direct application of mud pack on skin or as bath therapy has shown successful results in treating painful body and skin ailments. The anti-inflammatory and antioxidative properties of FvA and HA extracts present in mud help combating the symptoms and treating the condition effectively. The extracts are found safe when applied in amounts as high as 10 percent weight-by-volume. Fulvic acid or humic extracts can be applied in the form of masks, bandages, bath therapy or mud

packs as in balneotherapy done in various spas round the world. These extracts are selectively absorbed via skin and have a stimulatory response on the spontaneous contractile activity of smooth muscle tissue [57].

Hospital studies have shown effective use of mud in baths or packs in curing various skin diseases, eczema, dermatitis and psoriasis including ulcers, common cold or flu, osteoarthritis and rheumatoid arthritis and other bone, joint and muscle disorders. Patients exhibit healing effects and are significantly relieved of pain and inflammation in a few sessions only. Peat is referred to as a magical, anti-ageing substance. Detailed microbial and chemical analysis of Dead Sea mineral mud, Israel used in certain dermatological and cosmetic preparations revealed that the antibacterial properties of Dead Sea mud are probably owing to some chemical and/or physical phenomena and not of the bacteria present therein [58]. Sterilization of the mud by gamma irradiation could not deter its effects. FvA and HA mineral baths have shown 90% success rate in curing ulcers; Surprisingly these baths were successful in curing internal ulcers as well [59].

3.3 Osteoarthritis and musculoskeletal healing

High temperature peat or mud bath provides depth warming, when given at specific consistency. Besides, other conditions such as temperature, ionic strength and pH of the mud or peat also influence the benefits reaped during the application. This treatment improves blood circulation and regeneration processes in the patient's body. Studies have found significant effect of mud bath therapy on osteoarthritis, a rheumatic condition associated with the progressive destruction of cartilage. Peat therapy improves the level of chondrocytes, inflammation markers-interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α).

A trial study explained the benefits of Potassium humate treatment reducing the levels of inflammation in osteoarthritis patients over a period of 6 weeks. The levels of C-reactive protein (associated with inflammation) were significantly reduced in the blood of patients [60]. Studies on mice indicate effectiveness of Shilajit in treating induced-RA [61]. Pro-inflammatory cytokines were found to be suppressed. Its anti-arthritic mechanism of action was established through the suppression of pro-inflammatory cytokines, which suggests FVA as a potential therapeutic candidate for rheumatoid arthritis.

3.4 Blood properties

Presence of humate increases the oxygen carrying capacity of red blood cells and improves blood circulation rate. Feelings of euphoria have been reported in people taking humate in experimental studies, even during the first few days [62]. They have also shown improved rate of healing of injuries. This might be linked to the extra content of oxygen in the body. Fulvic acids have shown improved rate of absorption of iron, making higher concentrations available to bone marrow stem cells for blood formation.

Feeding animals with humic acid-rich diet has shown improvement in blood parameters viz. increased levels of total protein, globulin and glucose levels, reduced blood urea nitrogen, cholesterol, non-esterified free fatty acids and ketone body concentrations in goat fed on HA [63].

3.5 Nutritional benefits and detoxifying properties

Humic extracts, especially fulvic acids, are unique in their chelating ability. When taken orally, they provide a natural chelation therapy as an alternative

medicine for removing toxins from the body. They attach to the toxins, including heavy metals, pesticides, radioactive particles, and environmental carcinogens, and hence detoxify the body - the liver and the digestive tract. The toxins are neutralized such that their cellular absorption gets difficult inside the body and get dragged out as waste products, via urine, skin, lungs or in stools. Their ability to bind toxins has led to the commercial use of humic acid in industry, animal husbandry and human nutrition. Humic acid was shown to be effective in removing botulinum neurotoxins in sublethal chronic botulism in cattle [64], removal of lead in hen thereby alleviating the toxic effects of lead poisoning on their thyroid gland [65], reduce the accumulation of lead and cadmium in fish and improving their growth rate [66].

Since they are derived from naturally occurring organic substances, humic acids particularly FvA are good source of minerals. Humic acids act as dilator, increasing the cell membrane permeability which facilitates the transfer of minerals from blood to the bone and cells. Humic substances or their extracts are medically proven to cause rehabilitation of muscles, bones and nerves and in dealing with geriatric disorders viz. arthritis, diabetes, allergic symptoms and dementia [54]. Owing to their low molecular weight and water solubility in both acidic and basic substances, FvAs are readily absorbed through semi-permeable membranes, and function actively in association with living cells. Fulvic acid acts as a chelator; it readily complexes with essential mineral ions and rare earth elements. This makes it an excellent mineral-carrier in the body. At the same time, FAs capture and remove toxic metals from the body, eliciting the process of detoxification. Within 3–4 days of FvA usage, both animal and human subjects have shown considerable results. Treatment with humic extracts have shown noticeable results in treating patients with normally incurable epidemic hemorrhagic fever. The extracts exhibited instant results in arresting bleeding, restoring circulation rates, removal of clots, beside displaying anti-viral, and immune modulatory properties.

3.6 Stress management

Humates block or reduce the production of stress causing hormones, improving the animal behavior. Humate-fed animals are less aggressive, least affected by the outside disturbances like heavy crowd or traffic or during confinement in closed arenas. Humate supplementation in diet ameliorates the adverse effects of increased caging density in hen [67, 68] suggesting the role of trace elements, having antioxidative properties, present in humates. This effect has been noted in farm animals and even fish [69].

3.7 Antioxidant and anti-inflammatory properties

Antioxidants are substances that protect cells from the damage caused by free radicals, by neutralizing their electrical charge thereby preventing the oxidative damage to the cell. Humic substances, including peat and sodium humates, are known to exhibit excellent antioxidative and anti-inflammatory properties. Fulvic acids act as good free radical scavengers, neutralizing dangerous free radicals like superoxide (O_2^-), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), peroxynitrite ($ONOO^-$), and singlet oxygen ($^1O2$) radicals. An increase in the level of antioxidant enzymes such as glutathione, superoxide dismutase and catalase is generally taken as a biomarker for antioxidant properties of HAs. Inside the cell, FvA can uncouple the electron transport chain in liver mitochondria, which is associated with lowering ROS production.

The ages-old FvA-rich compound, Shilajit is known to reduce hyperglycaemia in diabetic animal models and increase SOD activity in pancreatic beta cells. They have the ability to significantly increase superoxide dismutase (SOD) activity that helps preventing and combating free radical damage to pancreatic B islet cells. This property, as shown in several hospital studies, stops the advancement and progression of diabetes, plus assists in the treatment. With fulvic acid treatment, diabetes patients became more energetic and the tingling, painful feeling and numbness experienced in the nerve endings disappears or gets reduced [54].

Fulvic acid (FvA) belong to the category of natural health products (NHP) known to cure diseases associated with chronic inflammation such as diabetes, cardiovascular diseases, and colitis. They also help in relieving inflammatory states of the cervix, especially cervical erosion (generally known as cervicitis), swelling from joint inflammation as in Rheumatoid Arthritis (RH). HSs have been shown to bond to the collagen fibres to aid in repair of damaged tendons and bone.

The neuroprotective effect of HAs on cerebral ischemia rat model [70] and gerbil Hippocampus [71], therapeutic effects in renal ischemia reperfusion injury in rats [72], protective effects in iron-induced hepatotoxicity and cardiotoxicity in rats [73] have been associated with their antioxidant and free radical scavenging effects. Administration of fulvic acid to rats for 4 weeks [74] and fish for 60 days [75] showed significant decrease in lipid peroxidation and increase in the expression of antioxidant enzymes glutathione, superoxide dismutase and catalase.

3.8 Cancer research

Conventional anti-cancer therapies like chemotherapy, radiotherapy, immunotherapy and surgical approaches for cancer treatment often result in recurrence of the symptoms. During the procedures of cancer chemotherapy and radiation therapy, large amounts of free radicals are generated which damage the normal cells around the tumor cell. Hence, there has always been search for development of cancer management approaches using non-toxic and natural products.

The naturally occurring humic substances, consisting of 60–80% fulvic acids, have known anticarcinogenic properties. Naturally-occurring humic substances have proven antioxidant, anti-inflammatory, antiviral, antimutagenic, heavy metal chelating, apoptotic and photo-protective properties. These properties make them useful agents for cancer therapy and prevention. Another plus point is that when taken orally, HSs have no reported side effects and can be administered as a nutritional and rejuvenating tonic in daily routine of the patient. Numerous hospital studies have shown that humic substances, especially fulvic acids, have the power to protect against cancer and related cancer-causing viruses. Humic extracts destroy cancer cells as well as decrease cell proliferation and angiogenesis and thus, curb growth of the cancer. Special humic substance therapies have shown promising results in reversing deadly cancers and tumors.

Molecular structure of humic substances gives them excellent free radical scavenging and antioxidant properties. This antioxidant activity increases with increasing concentration of the humic extracts present in the compounds used in cancer therapy. Naturally-occurring humic substances stimulate the production of cytokines, including interferon-gamma, interferon-alpha, interferon-beta, and tumor necrosis factor-alpha.

The effect of fulvic acid on three cancer cells lines - Hep3B, HT29 and PC3 at different concentrations of FvA using MTT assay [76]. The assay revealed that FvA inhibited the proliferation of cancer cellsas well as upregulated the mRNA levels of apoptotic genes in all the three cell lines. The cytotoxic effect ofHA with Arsenic trioxide on human cervical cancer cells [77], FvA on hepatic cell lines [78] and HA

on human breast adenocarcinoma cells [79] have been well documented. Even low concentrations of Mumie–a natural component found in high mountains such as the Himalayas and rich in humic compounds – could destroy human cervical cancer cells [80].

3.9 Microbial activity and gut health

Sufficient evidences link imbalance in the gut microbiome with inflammation and bowel diseases. Humic substances are known to improve the gut flora, increase the expression and activity of gut enzymes that aid in nutrient absorption and settle certain gut disorders. In other words, humic substances promote microbial activity in gut and thus, enhance nutrient absorption.

When used as feed additives, humic substances may increase rumen microbial population or shift microbiota in the gut to suit animal requirements. Increased microbial activity, in turn, improves fermentation and digestibility of nutrients in the gut rumen. HSs act as natural antibiotics, resulting in increased growth performance in farm animals and early-weaned baby animals. HSs boost the animal gut by promoting good microbes and inhibiting bad microbes when fed at high concentrations in the diet. Several studies have shown that specific species of microbes such as *Turicibacter, Clostridium, Campylobacter, Dehalobacterium, Desulfuvibrio,* and *Paludibacter* are promoted while *Prevotella, Blautia, Faecalibacterium, Lactobacillus*, and *Coprococcus* are inhibited in the cecum and colon of the animals when fed on humate supplemented diet [81, 82]. Similarly, a high relative abundance of *Serratia*, *Acinetobacter*, *Aeromonas* and *Edwardsiella* and low abundance of *Lactobacillus* was obtained in the intestine of fish fed on FvA rich diet for 60 days. The activity of digestive enzymes like lysozyme, proteases, and acid/alkaline phosphatases was also increased [75].

Humic substances particularly FvA increases the absorption of metal ions and other nutrients. There are significant proofs to show their influence on absorption of drugs in the body. FvA has also been shown to mediate drug delivery in animal models. Studies have shown that FvA enhances the uptake of iron, making it more bioavailable to bone marrow stem cells for formation of blood as well as supplying hormone stimulating micronutrients. In Broiler chicken, supplementation of diet with HA improved the utilization of nutrients and growth performance by improving the overall gut health [83, 84].

3.10 Immune support

Use of humic substances exert a beneficial effect on the animal immune system, including humans. Humates possess ability to bind sugars and assemble them to form complex saccharides within the body like glycoproteins that function as modulators of intercellular interaction. Glycoproteins bind to T cells and Killer cells, keeping them in balance and thus, regulating the immune system. Excessive T cells may lead to development of auto-immune diseases while excess killer cells can attack bone and joints causing arthritis.

Administration of HSs, especially fulvic acids, both topically and orally can bolster the immune system. They act as powerful immunomodulators, may show potent antioxidant and anti-inflammatory properties that can be used to enhance immunity and overall growth of the organism. This has been observed in growing swine [85], juvenile Loach [75] and broiler chicken [86]. Medical studies have indicated that HSs prove more effective than several currently prescribed immune regulatory drugs. Studies on mice have reported strong humoral immune stimulation by HA and FvA supplemented diets [87].

In addition, HSs have proved effective in alleviating the adverse effects of lethal doses of irradiation and reducing the incidence of infections, increasing the life span of the animal. This was directly correlated with the increasing capability of immune system to recognize its own dead cells, following administration with HSs supplemented diet or topical application of HSs. The overall health of animals improves with food and water supplemented with HSs [88].

3.11 Anti-viral properties

Anti-viral properties of soil-derived humate materials have traditionally been known to humans and have been studied experimentally since decades. Exploration into the mechanism by which humic substances inhibit viral cytopathicity revealed that these substances attach to the viral envelope protein, thereby coating its receptor sites. This further disables the virus from being able to bind and infect host cells, thus combating viral replication. Their potential range of applications cove both naked and enveloped DNA viruses.

Viral pathogens for which HSs have been found effective include Coxsackie virus A9, herpes simplex virus type 1 and 2, human immunodeficiency virus (HIV), influenza type A and B, cytomegalovirus (CMV) and Vaccinia virus as well as other respiratory tract infections. Excellent multimodal anti-HIV properties of humic substances extracted from mud [89], Shilajit [90] and Lignin-rich solid waste resulting from the processing of vegetable feedstock [91] have been demonstrated. Researches are also underway to explore the potential of HSs against the novel coronavirus COVID-19 [92].

3.12 Overall growth rate

Humic substances, with their miraculous anti-inflammatory, anti-oxidative, immunomodulatory, detoxifying, gut-promoting and nutritional properties helps in improving the overall growth and development of animal body. In animal husbandry, use of humic substances as feed additives promotes the animal growth rate, improves feed efficiency, prevents intestinal diseases and boosts overall immunity, thereby improving the economics and ecology of animal production.

Administration of HA improved the final body weight, daily weight gain and feed conversion ratio in broilers [88], increased the milk output and its quality in goats, and also growth rate of their kids with no adverse effects [63]. Even their young ones show higher survival values and better ability to overcome the effect of weaning at early stages when given diet supplemented with HSs [82].

3.13 Safety aspects

Humic substances are soil-borne materials, so humans and animals are naturally exposed to them. Industry has safely used these products for industrial exposure to heavy metals in both humans and in animals. Various studies have been conducted on both animals and humans over several years to test the effectiveness and safety of fulvic acids on human diseases [54, 93]. Most of those studies suggested that they are safe for oral or topical use, though recommended further exploration to confirm their safety to be used as food additives. No significant changes in body weights, hematological variables, indices of thyroid function, and microscopic organ histology were obtained when adult rats were orally administrated with HA [94]. No mutagenic or genotoxic effects of acute toxic and subtoxic doses of FvA in female rats [95].

However, some studies also hint upon the harmful effects HSs may be associated with. HA can induce oxidative DNA damage and genotoxicity in human

lymphocytes [96]. Some studies also hint upon goitrogenic aspects of HSs [97–99] but no confirmatory studies have been made so far.

4. Conclusion

Humic substances are a group of organic macromolecules formed from dead tissues of plant and animals transformed over the years by physical, chemical and microbiological processes. They exist ubiquitously in soil, sediments and various terrestrial and aquatic environments. The entire group of HSs can be divided into three components based on their solubility: fulvic acids, humic acids, and humin fragments. Specific properties of humic substances and their products qualifies their uses in both agriculture and medicine. Exploration of compounds present in HAs and their potential in agricultural, pharmaceutical and biomedical industry is of utmost importance.

In the era of depleting natural resources, increasing food demands and degrading environment, global warming and erratic weather conditions, we cannot afford more devastation of environment by utilization of chemical fertilizers, pesticides, insecticides etc. Humic substances pose to be potential candidates for improving soil structure and nutrient status along with beneficial effects on plant growth and yield; thus, giving a pavement towards sustainable agriculture.

Primarily used to enhance growth and vigour of the organisms, HSs are increasingly being used to treat several body ailments. Their potential as anti-ageing, anti-oxidative, anti-inflammatory, anti-cancer, immunostimulatory and growth-promoting agents is largely being explored. This will help bringing these organic molecules into the niche market where they can largely replace chemical substances. So, explore and reveal the power of 'dirt'!

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