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Review Article

POTENTIAL OF HUMIC SUBSTANCES - A REVIEW

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Organic farming has attained significant attention due to the impact of continuous use of chemical fertilizers and synthetic pesticides on deterioration of soil health, environmental pollution, and contamination of food chain. The demand for organic products has also created new export opportunities for the developing world. Application of organic manure improves soil physical properties, nutrient availability, soil carbon storage and microbial activity. At the same time, it reduces pollution of soil, water and air leading to a healthy food chain. By using these organic manures, the health of living things is improved because the food products produced are free of harmful chemicals. Humus is the final outcome of the decomposition of the organic matter found in soil, which comes from plant and animals. Humic substances constitute approximately 40–60% of the soil organic matter (Paul *et al*., 2001). Humic substances are more stable organic matter compounds, which make up a significant portion of the total soil organic C and N (Lal, 1994). They can improve soil buffering capacity, increase moisture retention, and supply plants with available nutrients. Moreover, these compounds can also bind metals, alleviating both heavy metal toxicity and metal deficiency in soils (McCarthy, 2001).

Based on their solubility and resistance to deterioration, humic compounds are divided into three primary fractions.

(1) Humic acids (HA or HAs) - acid insoluble (2) Fulvic acids (FA or FAs) that are soluble in both acid and alkali (3) Humin – Insoluble

Humic substances contain different functional groups including phenols, carboxylic acids, quinones, enolics, and ethers (Amir *et al*., 2010). The presence of these functional groups is related to their various agronomic effects, including growth enhancement, water- and nutrient-holding capacity, and disease suppression. Humic substances affect agronomic functions through their interaction with soil, plants, and microorganisms. These effects are mainly attributed to the carboxylic and phenolic groups, quinones, and other functional groups of humic substances (Mladkova *et al*.,2006). The main phytochemicals, which are responsible for antioxidant properties are phenolic compounds. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. Antioxidants are quenchers of free radicals, and play an important role in preventing diseases. Humic acids have been considered to have antioxidant action in radical-chain oxidation (Efimova *et al.*, 2012). Physicochemical characterization and antioxidant activity of humic acids extracted from peat of diverse origins were investigated by (Zykova *et al*., 2018). The medicinal activities of humic acids (HAs) extracted from nine distinct types of peat are diverse. In both radical scavenging and electrochemical experiments, all HA fractions showed antioxidant activity. The influence of humic substances on cancer cells were reported by Aydin *et al.* (2017) and among the humic substances, the positive effects of fulvic acid on several cancer cell lines were significant. Various studies are available on the immense benefits of humic substances in different areas and its isolation from different sources of organic matter. The antioxidant activity of humic substances opens wide application in food industry too. Humic acid may cause the peroxymonosulfate (PMS) to create sulphate radicals, which aid in the breakdown of methylene blue is quite interesting (Pang *et al*., 2018).

Isolation and characterization of humic substances from soil

The humic substances constitute largest fraction of soil organic matter and its key role in soil productivity is quite interesting. Its supreme nature on increasing soil fertility is significant. Humic compounds have been studied scientifically for more than 200 years (MacCarthy,1990). Among the several procedures reported by various studies, alkali reagent was the earliest, efficient and widely used method (Senesi *et al*., 1994; Stevenson, 1982).The studies of Shurygina *et al.* (1971) on soil humus components using differential thermal analysis (dta) and thermogravimetry (tg) indicated two architectures in their molecules: aromatic and aliphatic. Humic acids have an aromatic structure, whereas fulvic acids have an aliphatic structure. Among the two techniques of humic and fulvic acid synthesis, the NAGOYA method (Nagoya University) recovered more alkali extractable humus, whereas the IHSS (International Humic Substances Society) approach only recovered 80 and 26 per cent of the maximal yield by extracting with 0.1 N NaOH from two soils studied (Waksman,1936; Kononova,1966; Schnitzer and Khan,1972; Stevenson,1982; Kumada,1987) have also documented a variety of humus sample preparation procedures.Aguilera *et al.* (1997) studied organic matter in volcanic soils in chile

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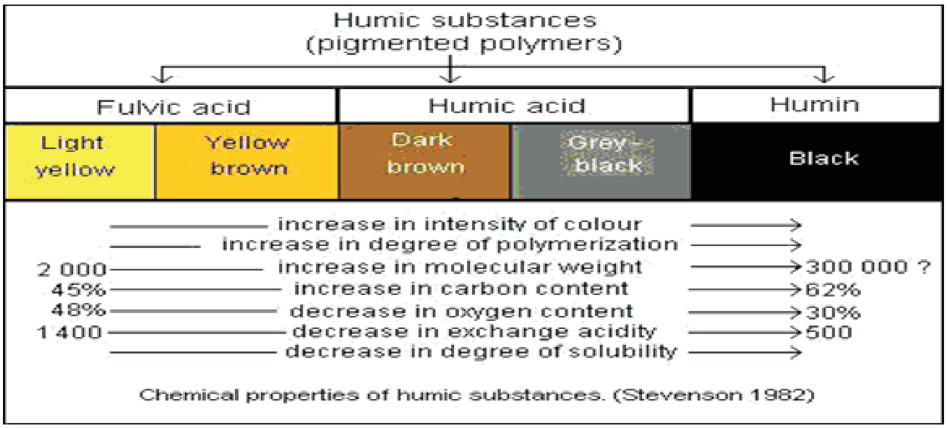

Figure 1. Chemical nature of humic substances (Stevenson,1982)

and a standard alkaline extraction approach (Schnitzer and Khan,1972) to separate pure humic acids (HA) and fulvic acids (FA) in Chilean volcanic soil. The presence of humines, as well as humic and fulvic acids, suggests that Chilean volcanic soil SOM is highly humified, according to the findings.

Quality of humic compounds in acid forest soils was determined based on the ratio of absorbances of pyrophosphate soil extract at wavelengths of 400 and 600 nm (A 400/A 600) (Mladkova *et al.,* 2006). Hayes (2006) reported how urea in aqueous base can enhance the isolation of soil humic components, and suggested mechanisms involving the breaking of hydrogen bonds binding the humic substances (HS) to the humin matrix, or allowing conformational changes enabling the liberation of the molecules. Hayes *et al*. (2008) found that a fractionation of HS is achieved when exhaustive extractions are carried out at increasing pH values. Isolation and characterization of humic compounds and humin from grey brown podzolic and gley grassland soils was examined by Hayes *et al.* (2010). The exhaustive extraction of the classical humic components of soil organic matter (SOM), including the isolation of humin residues from a well-drained Grey Brown Podzol (GBP) and of the humin materials from a poorly drained Gley soil after the humic acid (HA) and fulvic acid (FA) had been isolated during exhaustive extractions in aqueous sodium hydroxide (NaOH) solutions adjusted to different pH values. To illustrate differences in the compositions of the different isolates, nuclear magnetic resonance (NMR)-spectroscopy (C13) techniques were used. Helal *et al.* (2011) investigated the characterization of several humic materials using various analytical technique and found that low-cost, simple analytical procedures were suitable for describing humic materials, with excellent agreement between the results. The E3/E5 ratio was suggested as a similar indication to the E4/E6 ratio. Lamar *et al*. (2014) investigated a new standardised method for measuring humic and fulvic acids in raw humate ores, as well as solid and liquid products made from them. The procedures for preparing humic acid (HA) and fulvic acid (FA) were adapted to the International Humic Substances Society's requirements, which included alkaline extraction followed by acidification to separate HA from the fulvic fraction. After that, the FA was separated from the fulvic fraction by adsorption on a non-ionic macro porous acrylic ester resin with an acid pH. It varied from earlier methods by using gravimetric analysis to measure HA and FA concentrations on an ash-free basis. According to Sardashti and Alidoost (2015), the parameters of humic acid recovered from northern Iranian forest soil correspond better with Stevenson's model than the other models. Raposo (2016) determined humic compounds in sediments using focused ultrasound extraction and ultraviolet visible spectroscopy. The study revealed that ultrasonic solid liquid extraction (FUSLE) followed by separation of humic substances (HSs) in acidic media and determination by UV–Vis was an excellent analytical approach for speeding up HSs extraction, particularly humic acid (HA) and fulvic acid (FA) (FA). Using sophisticated solid-state NMR, Xu *et al.* (2017) revealed significant structural changes in HSs and distinct quantitative differences in aliphatic of fulvic acids (FAs) in two different soils.

The particle and structural characterization of fulvic acids from agricultural soils was studied by Ukalska-Jaruga *et al.* (2018).The results showedmajority of FAs are tiny molecules that associate or form molecular aggregates in liquids. Because of their chemical composition and aggregation behavior. FA in a solution with a comparable ionic strength might have a positive or negative charge, which influences their characteristics in the soil. Zavarzina *et al.* (2019) compared the characteristics of humic acids recovered from soils by 0.1M Na OH in the presence and absence of oxygen. Between HAs extracted by alkaline extraction in the presence and absence of oxygen, no statistically significant variations in quantitative yield, molecular weight distribution, or absorption spectra in the visible, UV, and IR regions were detected for both soils studied. At the same time, larger O: C ratios, higher concentrations of quinone and carboxyl groups, and significantly higher content of free radicals were observed in the HAs isolated from the Retisol soil in the presence of oxygen.

Isolation and characterization of humic substances from compost

Compost produced from different feed stocks are commonly used to elevate the organic carbon level and associated properties of soil (Stevenson, 1994).

Long term and labile carbon, cation exchange, water and nutrient holding benefits,beneficial soil biota, humic and fulvic soil conditioning compounds, immediate and slow release nutrients are the main benefits of compost application to soil.

The agricultural value of composts is increased when they are added to soil with more humified organic matter because the impacts of this organic matter in the soil persist longer. Therefore, a deeper comprehension of the humification process during composting would aid in producing a final product with a high level of organic matter stabilization and with a higher agricultural value (Sanchez-Monedero *et al*., 2002).Garcia *et al.* (1992) adopted pyrolysis-gas chromatography to investigate the chemical and structural properties of organic wastes and their humic acids during composting. Temperature was found to have profound influence on the yield of humic substances. The study on the effects of heat on the alkali extraction of humic substances from peat revealed that at the two higher temperatures (50^{o}C, and 80 °C) and with the most concentrated alkali solution (0.25M KOH), the maximum extraction yields were obtained (Cegarra *et al.,*1994). Francioso *et al.* (1998) adopted fourier transform infrared (FT-IR), surface-enhanced Raman spectroscopy (SERS), and nuclear magnetic resonance (NMR) spectroscopy to characterise unfractionated and fractionated fulvic acids isolated from an Irish peat. The application of these spectroscopic techniques yielded valuable structural information about the aromaticity, carboxylate, and carbohydrate group contents in each fraction. Sanchez-Monedero *et al.* (2002) explored the chemical and structural evolution of humic acids in seven different organic waste mixtures of various origins during composting. As per the experimental data, the composting process produces humic acids that are chemically and structurally similar to higher humified soil humic acids (mostly elemental and functional concentrations). The main effects of the composting process on humic acids are the formation of a higher molecular weight structure with more aromatic characteristics, higher oxygen and nitrogen concentrations, and a greater number of functional groups, all of which are consistent with widely held humification theories in soil.According to Ahmed *et al.* (2005) HA from compost can be isolated in 24 hours or less, rather than the current average time of 48 hours, allowing for the production of potassium-humate (K-fertiliser) from composted pineapple leaves rather than open burning, which has negative environmental consequences.

The 13C solid state NMR assessment of decomposition pattern during co composting of sewage sludge and green wastes was evaluated by Albrecht *et al.,* (2008). Chemical and spectroscopic characteristics revealed that HA and lignin breakdown were important in the compost's development. The 13C-NMR spectra indicated that aromatic and phenolic C increased during composting, while polysaccharides and other aliphatic structures decomposed.

Characterisation of structural properties of fulvic acids isolated from sewage sludge during composting was carried out by using thermochemolysis–gas chromatography–mass spectrometry (Amir *et al.,* 2006). Asing *et al.* (2009) characterised the humic acid derived from coals and composts as well as the optimization of the extraction procedure. Humic acid (HA) was recovered from Mukah, Sarawak subbituminous coals, compared to commercial humic acid (HA) product (leonardite), humified peat, and several types of compost. The presence of major HA structural elemental groups such as H bonded OH (3400 cm–1 peak), C=O of carbonyl (1710 cm–1 peak) functional groups, aliphatic components CH2 and CH3 (2930, 1420 and 1370 cm–1 peak), and C=C of aromatic ring (1620 cm–1 peak) functional groups could be seen in the Fourier Transformed Infrared (FTIR) spectra of extracted HA from coals, leonardite and chrysanthemum compost. Fialho *et al.* (2010) also utilized physicochemical and spectroscopic approaches to characterise organic matter from composting of various residues. The extraction of both pectin and lignin in the humic acid (HA)-like fraction was done by the 13C NMR and FTIR data. Fourti *et al.* (2010) studied changes in the humic substances during the co-composting of municipal solid wastes and sewage sludge. The humin and fulvic acid fractions in both the windrows were decreasing from the beginning of composting process, and were turning to stabilise. At the end of the composting process, the humic acid fraction was more dominant in the windrow without sludge than in the windrow with sludge. Palanivell *et al.* (2012) reported that extraction from rice straw compost could be done within 21.6 hours, and purification with distilled water (done in 1 hour) was successful in removing contaminants. More over K-humate, HA from rice straw compost could be extracted in less than 24 hours, and purified in a short amount of time without affecting their chemical or spectral properties. The characterisation of humic acids and fulvic acids produced from sewage sludge was examined by Li *et al.* (2013) through elemental analysis, functional analysis, infrared spectroscopy, and ultrafiltration fractionation were used to compare sludge humic acids with fulvic acids. Results revealed that humic acid made up the majority of sludge humic compounds, with fulvic acid accounting for only one eighth of humic acid. Enev *et al.* (2014) examined the spectrum characteristics of selected humic compounds of various origins using ultraviolet-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), "steady-state" fluorescence spectroscopy, and 13C nuclear magnetic resonance (13C NMR). Soil (Gleyic Luvisol), compost, and South-Moravian lignite from the mine Mr at Mikulcice were used to isolate sodium humates.

The International Humic Substances Society proposed a traditional process for extracting sodium humates (SH) (IHSS). The presence of O-containing functional groups (carbonyl in aldehydes and ketones, carboxyl in carboxylic acids, ester and ether groups) was more in compost followed by >soil > lignohumate > lignite. The use of non-destructive analytical methods like UV-VIS, FTIR, 13C NMR, and fluorescence spectroscopy aided in determining the major properties of various humic compounds. Humic compounds during co-composting of food waste, sawdust, and Chinese medicinal herbal residues were examined by Zhou *et al.* (2014). Moghadam *et al.* (2015) found that sodium hydroxide was the most effective extractant, while urea extracted the least quantity of fulvic acid from vermi compost. Highest functional groups of total acidity and phenolic OH were found in fulvic acid extracted with urea. In EDTA solution, the most carboxyl functional groups and spectrophotometric ratios were found. Haddad *et al.* (2015) tracked evolution of different humic fractions (humin, total humic substances, humic and fulvic acids) generated during composting to determine quality of compost along with organic carbon content, humic spectroscopic UV-visible ratio (E4/E6), humification parameter estimation, and humification fraction number. Al-Faiyz (2017) characterised humic acids from composted agricultural Saudi waste of various origins by adopting, CPMAS 13C NMR method (A). All the samples had prominent peaks for a carboxyl group carbonyl. The carbonyl carbon, on the other hand, had no absorption in any of them. The analysed samples' composition and functional groups did not exactly match those of any previously described HA models, but there was some overlap between the Dragunov's model and the others. Zhang *et al.* (2017) investigated the characterization of pH-fractionated humic acids produced from Chinese weathered coal. A new sequential dissolution process for humic acid (HAs) generated from Chinese weathered coal was described in the study. By changing the pH (3-10) of the extraction solution, this approach was used to separate HAs into seven fractions. Elemental analysis, ultraviolet–visible (UV-Vis), Fourier transform infrared (FTIR), and solid-state 13C-nuclear magnetic resonance (NMR) spectroscopies were used to assess the compositional and structural properties of the HA fractions. Ch'ng *et al.* (2018) carried out the extraction of humic acid from rice straw compost by determining the extraction period and extractant ratio. The study determined the shortest extraction time and extractant ratio for humic acid extraction from rice straw compost. Huculak-Mczka *et al.* (2018) evaluated the possibility of employing lignite-derived humic acids in the manufacturing of commercial fertilisers. The qualities of humic acids were dependent on the source of origin as well as the method of extraction. Humic acids extracted with NaOH and KOH solutions were less condensed than those extracted with $Na_4P_2O_7$ solutions. Humic acids extracted from lignite using $Na_4P_2O_7$ solutions were having low transformation degree and a higher number of carboxyl groups. Guo *et al.* (2019) discussed the humic compounds produced during organic waste composting, including their formation mechanisms, structural features, and agronomic activities. Zara *et al.* (2017) evaluated humic acid extraction and characterisation from Pakistani lignite coals. The study revealed optimal concentration of KOH for humic acid extraction as 3.5 per cent. The quantitative assay of extracted humic acid was done using gravimetric and UV spectrophotometer techniques. The presence of carboxylic, phenolic, alcoholic, and amine functional groups were noted in FTIR analysis of extracted humic acid samples. Three humic acids' 13C NMR spectra revealed a wide range of aliphatic and aromatic chemicals, including carboxylates, carbohydrates, ethylene, phenols, and amines.

Influence of humic substances on plant growth

The positive benefits of humic substances on plant development can be attributed to either direct (improving the efficacy of fertilizers) or indirect (improving the effects on total plant biomass). Typically, root growth is more noticeable than shoot growth (Vaughan and Malcom, 1985). Humic substances are usually recognized as being advantageous for plant uptake of Fe (Chen and Aviad, 1990). The complexing properties of humic substances leads to increasing the availability of micronutrients from sparaingly soluble sodium hydroxide (Stevenson, 1991).Aso and Takenaga (1968) examined the effect of nitro humic acid on the iron-manganese interaction in soybean plants by comparing it to EDTA as a chelating agent. The foliar application of fulvic acid (FA) resulted in increased levels of chlorophyll in the leaves and more root uptake.Drought-stricken plants produced 97 per cent of the yield of watered controls after being sprayed with FA (Xudan, 1986). Physiological effects of humic substances (HS) on several aspects of plant growth and metabolism were studied by Nardi *et al.* (2002). The effect of HS on plant growth was shown to be dependent on the source, concentration, and molecular weight of the humic component. The main candidate for determining the favourable effects of HS on plant growth was a low molecular weight components fraction. HS appeared to have an effect on both respiration and photosynthesis, as well as a hormone-like function. Nikbakht *et al.* (2008) explored the role of humic acid on gerbera (*Gerbera jamesonii* L.) cv. 'Malibu' growth, macro- and micronutrient content, and postharvest life. Humic acid concentrations of 0, 100, 500, and 1000 mg l^{-1} were added to the nutritional solution. HA considerably increased the macro- and micronutrient content of leaves and scapes, including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn). However, large doses of HA reduced the concentration of some nutrients.Katkat *et al.* (2009) investigated the effects of foliar and soil application of humic compounds on plant development and nutrient element uptake in wheat (*Triticum durum* Salihi) cultivated at varying salt concentrations. The results showed that humus in the soil boosted wheat N uptake, while humic acid applied to the leaves increased P, K, Mg, Na, Cu, and Zn uptake. The review by Trevisan *et al.* (2010) provides an overview of the existing information on the molecular structures and biological activities of humic compounds, with a focus on their biological functions and with special emphasis on their hormone-like activities. Silva-Matos *et al.* (2012) found that a foliar spray of humic compounds @.22.5 ml m^{-2} improved the aerial part and root system of water melon seedlings. Palanivell *et al.* (2013) investigated the effects of compost and crude humic compounds derived from selected wastes on growth and nutrient uptake by *Zea mays* (L.) The study examined and compared the nutrients content of rice straw (RS), rice husk (RH), saw dust (SD), and empty fruit bunch (EFB) composts and crude humic substances, as well as their effects on *Zea mays* (L.) growth, dry matter production, and nutrient uptake, as well as selected soil chemical properties. When compared to RS, RH, and EFB, sawdust compost humic acid (HA) had higher carbon, carboxylic, K, and Ca concentrations. The pH, total K, Ca, Mg, and Na levels of RS compost's crude fulvic acid (FA) were the highest. The ash, N, P, and CEC content of crude humin from RS compost was greater. Canellas and Olivares (2014) evaluated physiological responses to humic compounds as plant growth promoters. Changes in root architecture and development dynamics, which resulted in increased root size, branching, and/or greater density of root hair with bigger surface area, have been extensively acknowledged as a plant growth booster. Gomes Junior *et al.* (2019)studied the influence of humic acids from vermicompost on nutrient absorption in mangosteen seedlings. Humic acids induced curvilinear increments in N, P, K, Ca, Mg, and S contents from shoots and roots, as well as quadratic increased in Zn and Mn uptake by shoots, all at quadratic rates.

Deotale *et al.* (2019) investigated the effects of humic acid (HA) through vermicompost wash (VCW) with NAA on sesame. The results showed that foliar application of 50 ppm NAA + 300-400 ppm HA significantly enhanced nutrient uptake, chlorophyll content, seed yield, oil content, and other yield contributing parameters, leading to improved overall productivity. The responses of humic acid and NAA on morpho- physiological and yield attributes of pigeonpea have been investigated Padghan *et al.,* (2018). Specifically, this study evaluated the efficacy of foliar sprays of humic acid derived from vermicompost wash, in combination with NAA, on the growth, physiology, and yield of pigeonpea cultivar PKV-Tara. Guddhe *et al.* (2019) examined the effects of naphthaleneacetic acid (NAA) and foliar application of humic acid via vermicompost wash on the morpho-physiological characterstics and yield of sesame (*Sesamum indicum*). The results showed significant enhancements in plant height, number of branches, leaf area, dry weight, relative growth rate (RGR), net assimilation rate (NAR), seed yield (ha^{-1}), and harvest index. Ghodpage *et al.* (2021) examined the effects of integrated nutrient management practices on soil phosphorus and humic substances in a Vertisol under wheat cultivation. Neware *et al.* (2017) investigated the effects of varying concentrations of humic acid and naphthaleneacetic acid (NAA) on the morpho-physiological parameters and yield of linseed (*Linum usitatissimum*). Deotale *et al.* (2020) evaluated the impact of foliar application of humic acid and NAA on chemical, biochemical parameters, yield, and yield attributing characters in safflower, revealing significant improvements in various parameters.

Antioxidant activity of humic substances

The main phytochemicals which are responsible for antioxidant properties are phenolic compounds. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. Antioxidants are also quenchers of free radicals, and increasing the intake of dietary antioxidants may help to maintain an adequate antioxidant status and, play an important role in preventing diseases.Humic acids have antioxidant action in radical-chain oxidation processes, according to Efimova *et al*.(2012).Smirnova *et al.* (2012) investigated the antioxidant and pro-oxidant activity of ascorbic and humic acids in radical-chain oxidation processes. They investigated the role of ascorbic and humic acids in radical-chain oxidation of model hydrocarbons. Ascorbic and humic acids were found to be inhibitors of cumene's liquid-phase radical-chain oxidation. Humic acids have antioxidant action in radical-chain oxidation processes, according to Efimova *et al.* (2012) using model hydrocarbons, the gas-volumetric approach was used to investigate the behaviour of humic acids in radical-chain oxidation processes (cumene and ethylbenzene). The rate of hydrocarbon oxidation was determined by the amount of humic acid present. Humic acids might be considered potential natural antioxidants for biological and technological (antioxidants for liquid fuels and oils) uses due to their strong inhibitory characteristics. Aminifard *et al.* (2012 a) studied the effect of fulvic acid (FA) on antioxidant components and pepper fruit quality in the field. The results showed that fulvic acid treatment altered fruit antioxidant activity, total phenolic, carbohydrate, capsaicin, and carotenoids content, but not total flavonoid or ascorbic acid content. Aminifard *et al.* (2012 b) explored the role of humic acid on the antioxidant activities and fruit quality of the hot pepper (*Capsicum annuum* L.). In an open-field study, they investigated the influence of humic acid (HA) on antioxidant components and fruit quality of the hot pepper (*Capsicum annum* var. Red chilli). Antioxidant activity, total flavonoid, capsaicin, lycopene, and -carotene were all influenced by humic acid (HA) treatments.

The antioxidant effects of humic compounds were examined by Aeschbacher *et al.* (2012). The electron donating capacities (EDCs) of a representative number of terrestrial and aquatic HS and natural organic matter (NOM) samples were measured using mediated electrochemical oxidation over a wide range of pH values and applied redox potentials (Eh) using 2,22 -azino-bis (3-ethylbenzthiazoline-6- sulfonic acid) as an electron transfer mediator over a wide range of pH values and applied redox potentials (Eh). The electrochemical oxidation of three model humic acids (HAs) was mostly irreversible, and their EDCs increased as

Eh and pH increased. The EDCs of the HS corresponded well with their titrated phenol concentrations at a particular pH and Eh, implying that phenolic moieties are important electron donating groups in HS. Aminifard *et al.* (2013) investigated the impact of compost on antioxidant components and fruit quality in sweet pepper (*Capsicum annuum* L.). In an open field experiment, researchers looked at the influence of compost (CO) on antioxidant components and fruit quality in sweet pepper (*Capsicum annum* L.). Pepper fruit antioxidant components were positively impacted by compost treatments (antioxidant activity, total phenolic and carbohydrate content). However, there was no discernible difference in total flavonoid concentration between the compost and control treatments.

Organic manures enhanced the phenolic content, antioxidant capacity, and soluble solids in pepper, according to Moreno-Resendez *et al.* (2016). The study looked at the effects of three organic manures (vermicompost (VC), simple compost (SC), and compost with gypsum (CG)) mixed with river sand at four different levels as fertilisers (RS). The phenolic content of fruits rose when VC-RS, SC-RS, and SC-RS were combined in 1:2, 1:1, and 1:2 ratios, respectively.

Shang *et al.* (2017). investigated the relative impact of humic and fulvic acid on ROS production, dissolution, and toxicity of sulphide nanoparticles. Natural organic matter (NOM) such as humic acid (HA) or fulvic acid (FA) was found to act as an antioxidant, reducing the production of reactive oxygen species (ROS) (O2•, •OH, and 1O2) by tungsten disulphide (WS2) and molybdenum disulphide (MoS2) nanoparticles. Physicochemical characterisation and antioxidant activity of humic acids extracted from peat of diverse origins were investigated by Zykova *et al.* (2018). The medicinal activities of humic acids (HAs) extracted from nine distinct types of peat are diverse. Basic (HAb) and pyrophosphate (HAp) extractions were used to isolate HA fractions. In comparison to HAb fractions, HAp fractions contained a larger number of aromatic structures. Furthermore, phenolic OH groups were found in much larger concentrations in HAp fractions (3.6 0.5 mmol g^{-1}) than in HAb (3.1 0.5 mmol g^{-1}). In both radical scavenging and electrochemical experiments, all HA fractions showed antioxidant activity.Vuolo *et al.* (2019) investigated the structure, categorization, and antioxidant properties of phenolic compounds. The hydroxyl groups on aromatic rings describe phenolic substances, which have a wide structural diversity. Simple phenols, phenolic acids, flavonoids, xanthones, stilbenes, and lignans are categorised and categorised based on the number of phenol rings and the structural components that bind rings to one another. They primarily scavenge reactive oxygen, nitrogen, and chlorine species, but they can also chelate metal ions, and they play a role in both the beginning and propagation of the oxidative process. Bayat *et al.* (2021) compared the effects of humic and fulvic acids as bio stimulants on yarrow (*Achillea millefolium* L.) growth, antioxidant activity, and nutritional content. Along with the control, the experimental treatments included HA and FA at doses of 5, 10, 15, and 20 kg ha^{-1}. The administration of both HA and FA increased the growth metrics, photosynthetic pigments, and antioxidants of yarrow in field and greenhouse trials. Singla and Pradhan (2019) investigated the antioxidant activity of various common weeds found in Punjab plains agriculture regions. The goal of the study was to look at the phenols, flavonoids, and antioxidant activity of common weeds found in the Punjab plains' agriculture areas. A UV-Vis spectrophotometer was used to measure total phenolic, flavonoid, and DPPH radical scavenging activity, as well as total antioxidant capacity. A total of eight weed species were examined. All weed species with significant phenol and flavonoid content had substantial antioxidant potential in terms of DPPH radical scavenging activity and total antioxidant capacity, indicating that these weeds could be a future supply of natural antioxidants for the pharmaceutical industry, according to the study.

Application of antioxidants in food industry

Antioxidants are substances found in fresh fruits and vegetables, and the research studies into their involvement in the prevention of degenerative diseases is constantly being published. Some fruits and vegetables lose antioxidants through post-harvest management, such as mild processing and storage. In this context, postharvest treatments are required to protect the fresh produce's quality and antioxidant potential. The study on the effect of antioxidants on fresh-cut apples, either alone or in combination with edible coatings revealed that adding the antioxidant to the coating reduced browning (Perez-Gago *et al*., 2006). The exogenous application of methyl jasmonate vapour to strawberry increased the production of the most relevant aroma-active compounds in general (De la Pena Moreno *et al.*, 2010). According to Villa-Rodriguez *et al.* (2013) postharvest treatments and technology solutions for maintaining fruit and vegetable antioxidant capacity were effective and promising. Khoddami *et al.* (2013) highlights the use of several approaches in the measurement of phenolic chemicals in plant-based products, as well as recent technology advancements in phenolic quantification.

Antioxidant-containing films and edible coatings (Eca *et al.*, 2014) revealed that incorporating natural antioxidants into films and edible coatings could change their structure, boosting their usefulness and usability in meals like fresh-cut fruits. Because of the potential for toxicity, synthetic antioxidants have been avoided in meals. To boost their bioactive characteristics, a variety of natural antioxidants (such as essential oils and plant extracts, as well as pure molecules like ascorbic acid and -tocopherol) included into edible films and coatings. Films and coatings with additional antioxidants assisted to preserve or enhance the sensory characteristics of foods while also enhancing the shelf life of the food goods (Eca *et al.*, 2014). Oroian and Escriche (2015) reviewed the studies on characterization, natural sources, extraction, and analysis of antioxidants. Vitamins, carotenoids, and polyphenols are the three main types of antioxidants considered for the studies. A variety of analytical approaches employing a variety of instrumental techniques had been developed for the extraction, separation, identification, and quantification of these chemicals, Quantification of antioxidants was carried out using in *vivo*, in *vitro*, electrochemical, chemiluminescent, electron spin resonance, chromatography, capillary electrophoresis, nuclear magnetic resonance, near infrared spectroscopy, and mass spectrometry methods.

Antioxidant and antiradical effects of phenolic compounds and flavonoids such as - malvin, oenin, ID-8, silychristin, callistephin, pelargonin, 3,4-dihydroxy-5-methoxybenzoic acid, 2,4,6-trihydroxybenzaldehyde, and arachidonoyl dopamine were all examined for their total antioxidant, metal chelating, Fe^{3+} and Cu^{2+} reduction, and free radical scavenging capabilities. The antioxidant activities of these compounds were compared to those of reference antioxidants such as BHA, BHT, -tocopherol, and trolox at various doses (10–30 g ml^{-1}) (Huyut *et al.*, 2017). The findings of Lopez-Palestina *et al.* (2018) suggested that gelatin coverings containing tomato oily extracts were particularly effective in safeguarding garambullo (*Myrtillocactus geometrizans*) fruits stored at 5 degrees Celsius. The most efficient coatings for retaining bioactive components and quality of garambullo fruits were gelatin-based coatings with tomato oily extract (TOE)-3 per cent. Garambullo fruits, which are a great source of antioxidants in the human diet, are preserved by gelatin-based coatings containing tomato oily extract.

Application of nano in food industry

Studies have shown that using phenolic compounds and plant-based essential oils as coating materials can prolong food shelf life, inhibit microbial growth, and keep nutrients from being lost (Salmieri and Lacroix, 2006). These substances are a natural substitute for preventing pathogenic and spoilage organisms that may arise in food on account of their potent antibacterial and antifungal qualities. In recent years a bit of works focused on natural nano coating instead of artificial one.

The response of mango (var. Alphonso and Banganapalli) to pre-harvest sprays of a nano-emulsion of hexanal (Enhanced Freshness Formulation) in extending fruit retention and shelf-life was investigated in the field. Pre-harvest EFF sprays dramatically decreased post-harvest illnesses such as anthracnose, stem end rot, and other infestations, reducing post-harvest losses while boosting net fruit yield (Anusuya *et al.,* 2016).

Pesticide degradation

Pesticides are classes of synthetic chemicals that are hazardous and non-biodegradable in the environment. They also linger in the environment after application and are susceptible to various chemical processes in the ecosystem, such as hydrolysis, oxidation, and photolysis (Ormad *et al.,* 1997). Among the chemical remediation methods, the advanced oxidation process (AOP), which is based on peroxymonosulfate (PMS) or persulfate, is a highly effective method for a wide range of pollutants, including dyes, phenol, and polychlorinated biphenyls (Andreozzi, 1999). On the other hand, adding artificial chemicals or metals to the system could make it harder to operate or increase the risk of secondary pollution. For large-scale operation, the use of heat and radioactivation is complex and challenging. In recent years, natural materials such as humic substances were tried to remove the pollutants and pesticides from the earth surface.

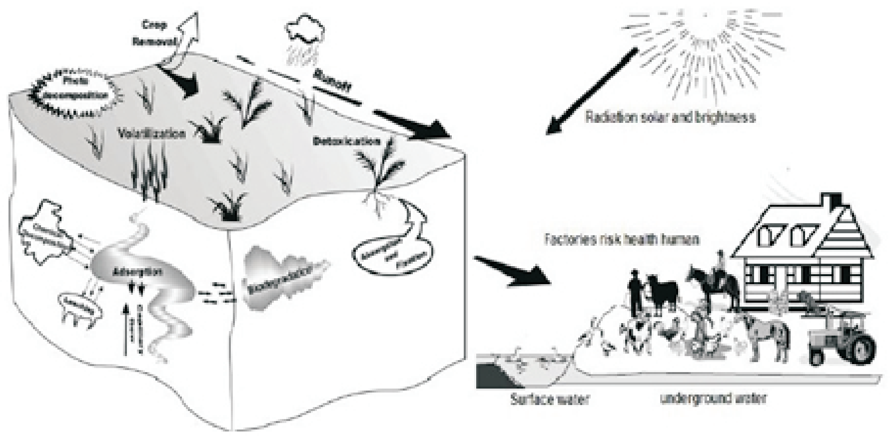

Figure 2. Pesticide contaminants in the environment (Strandberg *et al.*, 1998)

Based on photocatalyzed reactions, (Cardeal *et al.,*2011) examined the process of carbofuran degradation. The progress of the pesticide degradation was tracked using HPLC and GC procedures, both of which were connected to mass spectrometer (MS) detectors. Because of its superior selectivity, specificity, and capability for identification, the GC/MS approach was the only way to identify carbofuran intermediate products. Miguel *et al.* (2012) studied the influence of hydrogen peroxide on photocatalytic pesticide breakdown in natural water and concluded that photocatalytic treatments could decompose parathionmethyl, chlorpyrifos, endosulphan, 3,4-dichloroaniline, 4-isopropylaniline, and dicofol very effectively whereas poorest degradation was observed in HCHs, endosulphan-sulphate, heptachlors epoxide, and 4,4dichlorobenzophenone.Pang *et al.*(2018) tested humic acid, a naturally occurring organic compound, on the breakdown of methylene blue using peroxymonosulfate (PMS). Results revealed that PMS could successfully decomposed 50 mg l^{-1} methylene blue (>95%) at a concentration greater than 1.0 mM.

Influence of humic substances on cancer cells

Though the biological significance of fulvic acid was recognized long back, scientific understandings about its biological properties are still under way.

The effects of fulvic acid on several cancer cell lines were investigated by Aydin *et al.* (2017). They looked at how fulvic acid affected various cancer cells. Hep3B, HT29, and PC3 cells were treated with fulvic acid at various doses during 48 and 72 hours, and cell proliferation was measured using the MTT method. In PC3 cells, changes in the mRNA levels of apoptotic genes were also investigated. MTT assay revealed that fulvic acid decreased the growth of all of the cell lines employed in this investigation. Hep3B cells were shown to be the most sensitive for 48-hour application, with an IC50 value of 1.58–2.43 g l^{-1}. Furthermore, as compared to the non-treated control group, fulvic-acid dramatically increased the expression of apoptotic genes at the mRNA level. Aykac *et al.* (2018) examined the cytotoxic effects of humic acid on human breast cancer cells. Using the MTT method, the study looked at the cytotoxic effects of humic acid (HA) at doses of 5, 10, 20, 50, and 100 g ml^{-1} in human breast adenocarcinoma MCF-7 cells for 24 and 48 hours. The results showed that HA produced at a concentration of 100 g ml^{-1} has a cytotoxic impact in human breast cancer MCF-7 cells after 24 and 48 hours of incubation. It was also noted that 50 g ml^{-1} HA was the efficacious dose on MCF-7 cells after 24 and 48 hours of incubation.

Future potential

The antioxidant activity exhibited by humic substances presents a promising avenue for innovation in the food sector, with potential benefits for food quality, safety, and nutritional value. This property presents great opportunities for applications in two critical domains: the emerging nano food industry and cancer research, particularly effects of fulvic and humic acids on several cancer cell lines. Moreover, the capacity of humic substances to modulate pesticide degradation pathways presents a significant opportunity for the development of novel, environmentally friendly approaches to pesticide management and soil remediation.

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