

## Fulvic acid – a small but powerful natural substance for agricultural and medical applications

*Asam fulvat – bahan alami kecil tetapi berdayaguna untuk pemanfaatannya di pertanian dan kesehatan*

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### Abstrak

Bahan humat didefinisikan sebagai hasil dekomposisi bahan organik apapun dan mengandung utamanya humin, asam humat, dan fulvat. Dibandingkan dengan humin, asam humat (AH) dan asam fulvat (AF) adalah senyawa yang lebih banyak dipelajari. Namun, AF lebih jarang dikaji dibandingkan dengan AH, karena jumlahnya yang lebih sedikit. Dengan mempertimbangkan bahwa aktivitas biokimianya lebih kuat daripada AH, ulasan ini menyajikan penilaian tentang keefektifan aplikasi AF untuk tanaman dan tanah pertanian serta potensinya sebagai pengatur kekebalan pada manusia. Aplikasi AF secara luas dihadapkan pada dua faktor utama yang menentukan mutu dan efektifitasnya, yaitu jenis bahan baku dan metode ekstraksi. Lignit, suatu batubara bermutu rendah, merupakan bahan baku utama untuk produksi AF di samping gambut rawa, gambut pantai, dan kompos. Ekstraksi AF dilakukan dengan menggunakan beberapa metode, seperti hidrogen peroksida ( $H_2O_2$ ), larutan basa (NaOH/KOH), dan/atau air. Metode modifikasi terbaru menunjukkan bahwa kombinasi  $H_2O_2$  dengan tenaga gelombang mikro mampu menghasilkan ekstraksi AF yang cukup baik meskipun masih sulit untuk diaplikasikan dalam skala industri. Akibat karakteristik yang sangat spesifik, AF menunjukkan potensi yang besar untuk meningkatkan kinerja tanaman dan kesehatan tanah sehingga sangat strategis untuk mendukung pertanian berkelanjutan di masa depan melalui pengurangan cekaman biotik dan abiotik dan pencapaian produksi yang berkelanjutan termasuk untuk tanaman perkebunan serta manfaatnya via pendekatan bioteknologi. Untuk tujuan pengobatan, beberapa kajian telah menunjukkan prospek yang sangat menjanjikan sebagai pengatur kekebalan, pengobatan gangguan penurunan fungsi syaraf, terapi kanker, dan fortifikasi makanan sehat yang dapat sejalan dengan paradigma baru pengobatan yang disebut pendekatan One-Health. Pasar AF masa depan juga diperkirakan tumbuh pesat dengan nilai ekonomi yang menarik. Bagaimanapun juga, akibat data yang tersedia masih terbatas, berbagai

*bidang aplikasi potensial menjadi tantangan terbuka dan dapat memacu kegiatan riset dan upaya pengembangan produk untuk mewujudkan pertanian berkelanjutan dan kesehatan bagi manusia.*

*[Kata kunci: aplikasi pengobatan, senyawa alami, stimulan nabati, pertanian berkelanjutan]*

### Abstract

Humic substances are defined as the result of the decomposition of any organic matter, and they consist mainly of humin, humic, and fulvic acids. Compared to humin, humic acids and fulvic acids (FA) are the most explored compounds. However, FAs are less studied than humic acids because of the usually small residual quantities. Considering that its potential for bioactivity is stronger than that of humic acids, the current review was performed to evaluate the effectiveness of FA application for crops and soils and its potential as an immunomodulator for humans. The wide application of FA is challenged by two main factors affecting the quality and the effectiveness, i.e., the type of raw material and extraction method. Lignite, low-energy coal, is the most common material for FA production besides bog, peat, and compost. Fulvic acid extraction is done through several methods, i.e., hydrogen peroxide ( $H_2O_2$ ), alkaline (NaOH/KOH), and/or water. The latest modified method shows that the combination of  $H_2O_2$  with microwave power can recover a considerable amount of FA. However, it is still difficult to apply this method for industrial scale. Due to highly specified characteristics, FA showed a great potential to improve crop performances and soil health, making it strategic for supporting sustainable agriculture in the future through biotic and abiotic stress alleviations and sustainable yield achievement, including various plantation crops and biotechnological approach. For medicinal purposes, some studies have shown highly promising results, especially as an immunomodulator and in combating neurodegenerative disorders as well as for cancer therapy and health food fortification, which might be in line with the

new paradigm so-called One Health approach. The future market of FA is also estimated to grow in a very attractive economic value. However, as data are still limited, the wide range of potential use should encourage concerted and wide research and product development efforts to achieve sustainable agriculture and human health.

[Keywords: medical application, natural substances, phyto-stimulant, sustainable agriculture]

### Introduction

Any organic material in nature will decompose in time in interaction with water, oxygen, and microbial activities. The main products of these decomposed materials are referred to as humic substances, and they consist of humin, humic, and fulvic acids. The humin fraction consists of insoluble solid particles, whereas humic and fulvic acids are soluble in alkaline and acid solutions. Humic acid (HA) is soluble in solutions with pH > 9 and becomes insoluble at a pH level of 2 and lower (Tan, 2005). On the other hand, FA is soluble at any pH level, ranging from acid, neutral, and alkaline. Compared with HAs, FA is inferior in terms of development potential as a commercial product worldwide. However, many superior functions were reported for many uses, including agronomical, poultry, and health treatment practices. In contrast, the use of HA under control condition was reported positive, but under field conditions were less certain and questioned as a real substance or smoke and mirrors (Lyons & Genc, 2016).

FAs have also been studied for their beneficial effect on soils and crops. Some researchers have published their results showing a great potential of FAs as a plant growth stimulator, such as on rice and radish (Khang, 2011), vegetables (Yang *et al.*, 2014), safflower (Moradi *et al.*, 2017), coffee seedlings (Justi *et al.*, 2019), tobacco (Moradi *et al.*, 2019) and wheat (Sootahar *et al.*, 2020). Also, it was reported by Yang *et al.* (2013) that the application of FAs on soils significantly improved soil-P availability. In poultry, Mao (2019) reported that the application of dietary FAs was capable of modulating the growth performance, meat composition, oxidative status, and immunity of broilers. The acid fraction of FAs was also reported to have potential as a natural feed supplement improving growth and feed efficiency without affecting carcass yield of broiler chickens (Supriyati, 2019).

Shilajit is a well-known natural source of FA found in specific mountain regions of the world, including the Himalaya Mountains and is also referred to as mumie, vegetable asphalt, or mineral pitch. It is a semi-hard brownish-black resin formed through long-term humification of several plant types, mainly bryophytes (Schepetkin *et al.*, 2009). More than three decades ago, Goel *et al.*

(1990) reported that shilajit has been used as a treatment for genitourinary diseases, diabetes, digestive disorders, nervous disease, tuberculosis, chronic bronchitis, asthma, anemia, eczema, bone fractures, and other diseases. Such products are available widely in the online market nowadays. Other evidences were also reported by Vučkits *et al.* (2010) about performance, immune response, and thyroid function in rats.

Continued efforts are being made to find the most efficient options in producing FAs both in terms of raw material and methods of extractions. Peats, lag bogs, composts, and low-quality of coal (brown coal or lignite) have been investigated as alternative sources for humic substances (HS), including FA (Goenadi, 2001; Wali *et al.*, 2019; Cheng *et al.*, 2019; Gong *et al.*, 2020b). A conventional fractionation method for humic substances employs sodium hydroxide (NaOH) also in combination with sodium pyrophosphate (Na<sub>2</sub>P<sub>2</sub>O<sub>7</sub>), with or without pre-treatment with HCl (Bannach-Sczott & Debska, 2008). Many patented technologies are, with some modifications, also based on these approaches. However, all of these focused mostly on HAs production and very limited technology research, if any, is specifically oriented towards the production of FAs. This is understandable as FAs are soluble at all pH levels, including water (pH neutral), and depending on the raw materials, the concentration is much lower than that of the HAs. Considering the techno-economy aspects, the production of FAs should be integrated with the production of HAs, if and only if the use of additional extracting chemicals yields in a significantly high amount of the FAs without any trade-offs to their quality.

This review article discusses the current development of applying FAs for agricultural and medicinal purposes. Understanding the potential functions of FAs will enable the further development and exploitation of the substances to become a high-economic value product. For agricultural uses, the discussion will be limited on the potential of FAs as crop phyto-stimulant in improving fertilizer use efficiency and productivity for both food and plantation crops, biotic and abiotic stress alleviations; biotechnological approach for soil health and crop metabolism improvements, and some reported application in animal feed as immuno-stimulator. Its potential use for medicinal purposes will focus on exploring its immune modulator function against inflammatory disease and some neurodegenerative disorders. Challenges, opportunities, and future market outlook will also be described briefly to indicate the prospects of research and product development for FA's further exploitation beneficial to humankind.

### Raw Materials and Extraction Methods

As indicated before, the raw organic base materials and the extraction methods are the two

most highly important factors regarding an efficient production process of FAs. The followings are brief discussions about each of these factors. For further reading related to HAs, the readers are advised to access, among others, see Santi *et al.* (2000), Goenadi (2001), Tan (2005), and Goenadi (2006).

*Raw materials*

Production technology efficiency will be determined by the concentration of FAs in the raw base materials in decomposition. In general, decomposed organic matter contains HAs originating from lignin humification resulting in humic and fulvic acids and humin formation. Old data from HumiTech (Mema, 2005) in Table 1 show that leonardite, a soft waxy, black or brown, shiny, vitreous mineraloid that is easily soluble in alkaline solutions as an oxidation product of lignite, was considered as the highest HS-containing material compared to the others. These data show that the concentration of FAs fraction was in general below that of the HAs fraction. As mentioned before, the natural product with high FAs currently available in the market is shilajit with HS contents, including FAs about 60-80% (Carrasco-Gallardo *et al.*, 2012). However, Sasikala & Deeptha (2018) carried out a study on Oriens Shilajit only found 0.96% (w/w) FAs. A commercial company from China presumably using lignite as raw materials claims to provide fulvic acid fertilizer grade product containing 50-60% (w/w) FAs (Khumic, 2020). On the other hand, Sootahar *et al.* (2019) indicated that different sources of FAs contain different amounts of

chemicals (C, H, N, and S), as shown in Table 2. The data show that chemical compositions of the FAs from mineral-derived liquid (MDLF) are somewhat similar to that of plant-derived liquid (PDLF), and both tend to be higher than of plant-derived solid (PDSF). These differences were assumed responsible to some extent for the effectiveness of the FAs in changing soil properties.

Other potential raw materials that have been reported in the literature are lignin and carbohydrate. Aro & Fatehi (2017) stated that lignin is the largest reservoir of aromatic compounds on earth and can be used in many industrial applications. According to these two researchers, the lignin materials originated from pulp and kraft industries, occur in the form of lignosulfonate and sulfonated lignin. Humic substances, including FA, are believed because lignin humification took place in nature due to humifying fungi activities. Therefore, it is worthwhile to develop a control mechanism employing such fungi for producing FA. Jeong *et al.* (2018) demonstrated an alternative utilization of technical lignin originated from the Kraft processing of woody biomass transformed into humic-like plant fertilizers through what they called “one-pot Fenton oxidations”, i.e. an artificially induced fungus reaction, mimicking a fungus-driven nonspecific lignin oxidation process in nature. The variants of lignin resulting from this reaction, and manufactured using a few different ratios of FeSO<sub>4</sub> to H<sub>2</sub>O<sub>2</sub>, have been shown to

Table 1. Different organic materials containing various concentrations of HSs (Mema, 2005)  
 Table 1. Beberapa bahan organik yang mengandung berbagai konsentrasi AH (Mema, 2005)

Natural sources (Bahan alami)	Humic/Fulvic acid (%) Asam humat/fulvat (%)
Leonardite	25 - 90
Composts	5 - 25
Peats	5 - 20
Peat Moss	5 - 20
Lignite	5 - 15
Manure	1 - 3
Soft Coal	2 - 5
Hard Coal	0 - 1

Tabel 2. Composition of elements (%) found in several-origin FAs (Sootahar *et al.*, 2019)  
 Table 2. Komposisi unsur yang ditemukan di dalam AF asal beberapa bahan berbeda (Sootahar *et al.*, 2019)

Type of FA origin (Asal bahan)	C	H	N	S
PDSF <sup>*)</sup>	5.39	25.31	5.75	8.47
MDLF	10.29	52.48	9.74	14.84
PDLF	10.78	50.61	11.56	16.96

\*) PDSF: Plant-derived solid, MDLF: Mineral-derived liquid, and PDLF: Plant-derived liquid.  
 \*) PDSF: padatan asal tanaman, MDLF: cairan asal mineral, dan PDLF: cairan asal tanaman.

successfully accelerate the seed germination of *Arabidopsis thaliana* and to increase NaCl-induced abiotic stress tolerance of this plant. Moreover, the stimulation effect on the growth of this plant by these HS products was similar or higher to that induced by commercial HAs. As a similar effort, FA had also been developed from less-harmful natural resources like a carbohydrate. This substance composes organic acids found in nature and known for having anti-inflammatory, antimicrobial and antioxidant properties. However, heavy metals embedded in the molecular structure of FAs extracted from numerous environmental sources have limited them for safe application as medicines. A commercial company has made a new invention to produce FA synthetically from sucrose, identified as Carbohydrate-Derived Fulvic Acid (CHD-FA), which then becomes a major international breakthrough in production FA with no heavy metals. This CHD-FA produced by employing a non-catalytic wet oxidation process complied with standardized product specifications for molecular consistency and safety (Jordaan, 2019).

*Extraction methods*

Since the first book of Orlov on Humus Acids of Soils published in Russian in 1974 and translated into an English version in 1985 by the United States Department of Agriculture and

National Sciences Foundation, researchers worldwide show great interest in the development of methods to extract HS, including its fractionation, see Tan (2005). In general, all methods for FA extraction are in one package with the HA employing the alkaline solution as extracting agent, pH adjustment, and centrifugation. Gong *et al.* (2020a) as shown in Table 3, have outlined different extraction methods of FA. Based on techno-economical aspects, some methods were considered inefficient because of the time required. On the other hand, methods that are reliably efficient in time consumption are difficult to industrialize using available technology. As an illustration, a common systematic extraction, reported by Bannach-Szcott & Debska (2008), is shown in Figure 1. This figure shows that the FA extraction is in one line of HA extraction when conducted using an alkaline solution (0.5M NaOH). The HA and FA are then separated through precipitation of the former with lowering the pH with 6 M HCl addition. It should be noted that the whole process takes a considerable time, *i.e.*, 3x24 hours, for obtaining FA and HA. Besides, as this process employs high amounts of sodium (Na<sup>+</sup>) or potassium (K<sup>+</sup>) ions in the process, it is hard to get FA with high purity by using this technique and the use of a large volume of alkali and acid, it is considered as a non-environmentally friendly method.

Table 3. Different characteristics of selected FA extraction methods (Gong *et al.*, 2020a)

Tabel 3. Perbedaan karakteristik dari beberapa metode ekstraksi AF (Gong *et al.*, 2020a)

Method (Metode)	Principle (Prinsip)	Equipment (Peralatan)	Time (Waktu)	Advantages (Keunggulan)	Disadvantages (Kelemahan)
Precipitation using alkali-soluble acid (Romaris-Hortas <i>et al.</i> , 2007 & Khanna <i>et al.</i> , 2008)	Different in solubility	Ordinary extractor	5 – 6 hours	Easily operated	Time-consuming Low yield High impurity
Chemical degradation (Yao <i>et al.</i> , 2010 & Isoda <i>et al.</i> , 1998)	Acid solubility increased by oxidation	Ordinary extractor	36–72 hours	High yielding	Time-consuming Expensive
Ultrasound-assisted (Raposo <i>et al.</i> , 2016)	Applying ultrasound energy	Ultrasonic water bath	1 hour	Fast	- Efficiency is lower than the microwave method - Narrow range of ultrasonic action
Microwave power (Javed <i>et al.</i> , 2013)	Microwave energy	Microwave extractor	10 – 20 mins	Very fast	Laboratory-scale only

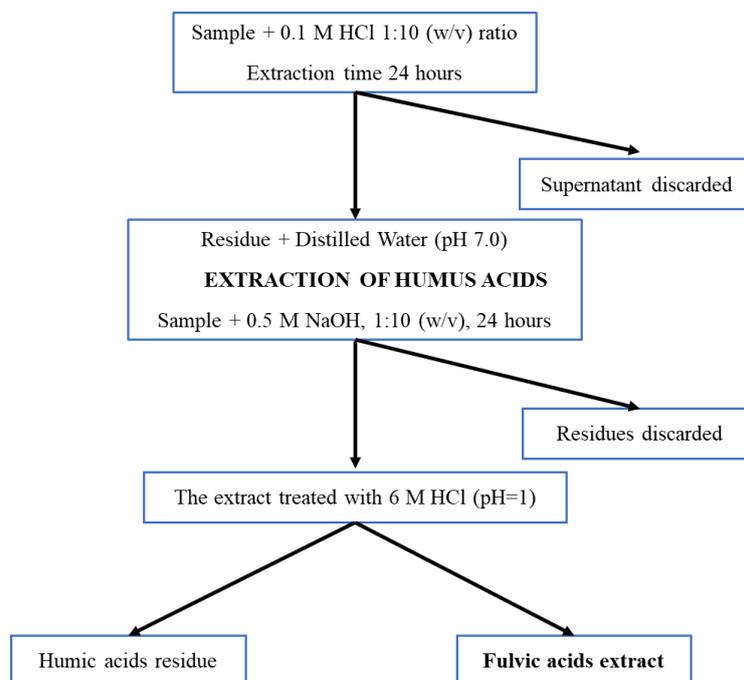


Figure 1. Scheme of humic substance extraction procedure (modified from Bannach-Sczott & Debska, 2008)

Gambar 1. Skema prosedur ekstraksi bahan humat (dimodifikasi dari Bannach-Sczott & Debska, 2008)

Lately, Gong *et al.* (2020a) described a novel method for FA extraction from low-quality lignite by using hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) in a microwave apparatus that was later modified by Zhang *et al.* (2020). By using oxidizing of the material approach, the researchers have been able to develop a fast and relatively simple extraction procedure to obtain less-cation-contaminated FA. Their data collected from a microwave reactor, shown schematically in Figure 2, indicate that the best yield of FA from lignite was obtained under the following conditions: oxygen-coal ratio 11, the concentration of  $\text{H}_2\text{O}_2$  21%, microwave power 400W, and time of extraction 10 minutes yielding approximately 28.0 – 29.5% (w/w) FA. On the other hand, Zhang *et al.* (2020) reported a higher FA yield, i.e., 60.9%, by introducing acetic acid glacial and higher microwave power (700 W). However, as indicated in Table 3, this microwave technique would still be difficult in a commercial application. Therefore, this will stimulate further exploration of the more efficient extraction techniques in terms of low cost and good quality of FA. In addition, extraction of FA from weathered coal by Zhang *et al.* (2017) by using hydrothermal catalytic oxidation employing catalyst of nano-CuO and an  $\text{H}_2\text{O}_2$  green oxidant. Total yields of both HA and FA reached the maximum of 73.23% and 8.98%, respectively. On the other hand, Swiech *et al.* (2017) stated that HA molecules

could be broken up into two soluble and two insoluble fractions by successive dissolution in deionized water at near-neutral pH.

#### Patented technology

Interest in a proven extraction method of FA from various organic materials is also shown in the form of patent documents. Michael Charles Karr holds a patent in Europe since 2004 for the technology of FA extraction employing ultrafiltration and digestion techniques (Patent # EU EP1797190A1) (Karr, 2010). The first step is to solubilize some FA molecules by mixing humus material, such as oxidized lignite, with water. An ultrafiltration apparatus could be used for the first filtration to separate some solubilized FA molecules from the humin and HA molecules. For the separation of other FA molecules from water and other impurities, the second filtration is performed using the same equipment or a nanofiltration apparatus. Some digestion step needs to be done when there is a possibility of microbial contact with the mixture to get unoxidized organic matter all oxidized. Phosphate is added to remove Fe and Al by precipitation so the purity of the end product could be improved. In 2014, Van Dyke and co-workers patented their invention in the USA regarding technology to produce FA from compost materials (Patent # US20120279266A1) (USPTO, 2014). The method

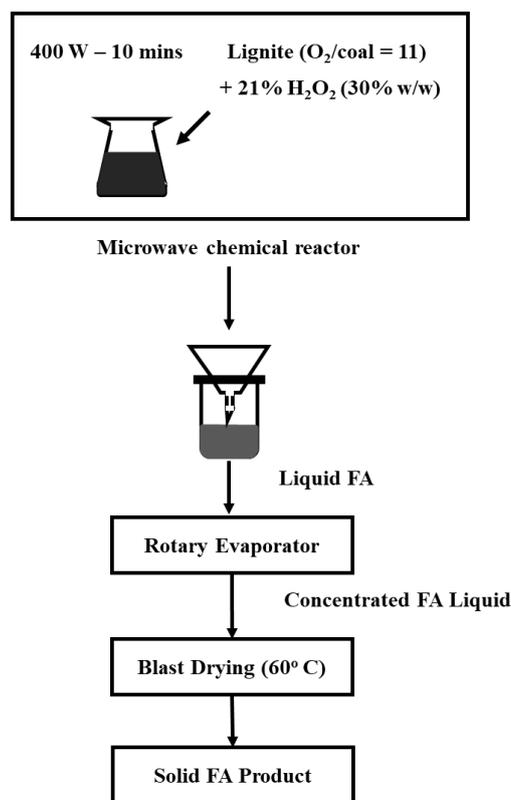


Figure 2. Schematic illustration of a microwave reactor (redrawn & modified from Gong *et al.*, 2020a)  
 Gambar 2. Gambaran skematis dari sebuah reaktor gelombang mikro (Digambar-ulang dari Gong *et al.*, 2020a)

presented in this patent document includes providing an extraction procedure to separate a liquid containing HA and FA from a mixture of compost with the crop materials that have been heated previously and added with water. In practice, the organic compost material could include straw and manure, which have been fermented a few days or a week aerobically. The crop materials could be any type, including fruits and fungi or mushrooms.

At least there are three patent documents found in China dating from 2017. The first about technology for extracting FA from lignite (Patent # CN104629062A) (Gong *et al.*, 2017), the second was related to diatomite as an FA source (Patent # CN104974356A) (Feng *et al.*, 2017), and the third was related to a novel method for extracting FAs from industrially produced waste edible fungi residues by fermentation (Patent # CN 105331669A) (Li & Li, 2017). The first invention by Gong Guanqun and colleagues disclosed a method for extracting FA from the lignite. The method comprises the following steps: (1) weathering the lignite crushed to 80-200 mesh in the sunlight (the weathering illumination temperature is 15-30° C and the weathering effective illumination time is 140-210 hours); (2) adding the hydrogen peroxide with the mass

concentration of 30% in the lignite sample processed in step 1 according to the mass ratio of the lignite to the hydrogen peroxide of (1:1.5)-(1:2.5), and performing microwave oxidation degradation on the mixtures at 40-60° C for 10-30 minutes; and (3) adding distilled water in the oxidation-degraded mixtures, wherein the mass ratio of the distilled water to the lignite sample is 5:1; followed by centrifugal separation, filtering the supernatant, evaporating by a rotary evaporator, performing vacuum drying to obtain the FA solid. The method has been claimed to be green and environmentally friendly, economical and effective, and high in productivity and extraction efficiency of the fulvic acid, simple in process, low in cost, and little in environmental pollution.

In contrast, the second invention by Feng Quanli and co-workers (Feng *et al.*, 2017) describes an extraction method of FA from diatomite, which belongs to the technical field of comprehensive utilization of mineral resources. The FA substances were extracted from crushed diatomite in a microwave environment, with sulfuric acid as a solvent, and separation was carried out to obtain a main product- active diatomite and by-products - the FA substances. It was claimed as simple and easily practicable

because it greatly mitigates environmental problems caused by calcining, washing, and refining diatomite and effectively improves the extraction rate of the FA substances without influencing the activity and structure of diatomite.

The last of the three inventions from China issued in 2017 is by Li Jin and Li Deshun. They concern a novel method for extracting FAs from industrially-produced waste edible fungi residues by fermentation. It includes the steps of (1) preparing a primary seed solution; (2) preparing a secondary seed solution; (3) inoculating the secondary seed solution, *Bacillus subtilis*, *Candida tropicalis*, and *Trichoderma pseudokoningii* into a 1000-2000L of the fermentation tank, respectively and fermenting for 10-15 days to obtain a fermented seed solution; (4) treating *Pleurotus ostreatus* residues; (5) inoculating the fermented seed solution into the *P. ostreatus* residues to be inoculated, and fermenting at the temperature 20-40° C aerobically for 4-5 days to obtain fermented fungi residues; (6) extracting the fulvic acids; and (7) performing spray drying to obtain a flavescent fulvic acid powder. The fulvic acid content can be above 35% after fermentation, and the yield of crude fulvic acids can be up to about 30% (dry matter percentage) through extraction.

#### Chemical Characterization

In addition to the high yield of extraction, it is considered important to characterize the quality of the FA obtained from given extraction procedures and/or type of organic materials. For many decades, much literature focuses on the characterization of FAs employing many available tools and equipment. Most include the elemental analysis, functional group identification, and micromorphological evidence of the material. As an illustration, the latest report by Gong and his co-workers (Gong *et al.*, 2020a) provides the common analyses performed to evaluate the quality of FA obtained from a certain extraction procedure, i.e., H<sub>2</sub>O<sub>2</sub> oxidation under microwave field (Figure 2), using lignite as extracted material. In short, these researchers performed elemental analysis by using a series of analyses employing fluorescence spectrophotometer, photoelectron spectroscopy,

synchronous thermal analyzer, and Fourier transform infrared (FTIR) spectrometer.

Their elemental analysis results are presented in Table 4. It is clearly shown that C and H contents in lignite were significantly higher than in extracted FA, while the opposite is observed for the O content. An interesting observation was that the values of C/H and C/O ratios in FA were less than 1. For this phenomenon, they believed that under microwave influence, the peroxide used has destroyed weak covalent bonds (i.e., R–O–R, R–OH, α-H, and so forth). Meanwhile, they found in the lignite sample, cleavage of fatty side chains and other substituents, along with many oxygen-containing hydrogen-rich groups were introduced into the molecular structure. As a result, small oxygen- and hydrogen-rich molecules were formed, such as C=O, –OH, and –COOH, from fragments of the broken alkyl chains. Low aromaticity and short molecular chains of FA were obtained and contained many functional groups such as –COOH and C–O. These functional groups containing O in FA were confirmed to be C–O > –COO > C=O, as shown by fluorescence spectroscopy.

Compared with other conventional FA extraction methods, especially with the use of alkali and acid, this process clearly showed many advantages: a high yield recovery, environmentally safe reagents, and a more simple process. This study also revealed that a –COOH and C–O containing low molecular weight substance dominated FA extracted from coal materials. Hence, this method could be used as an effective technique for utilizing low-quality coal and provides a strong basic for an in-depth understanding of the molecule of FA extracted from coal. Gong and other co-workers (Gong *et al.*, 2020b) also reported the molecular structure of FA obtained from the extraction of Halunbuir lignite in Inner Mongolia by using H<sub>2</sub>O<sub>2</sub> and microwave-assisted technique (Gong *et al.*, 2020a). In contrast, Zhang *et al.* (2020) employed a similar technique in low-rank lignite extraction but with the addition of glacial acetic acid. The optimal conditions were obtained as follows: reaction time of 13 min, microwave power of 700 W, acid/coal ratio of 1.32, H<sub>2</sub>O<sub>2</sub> concentration of 22.5%, and oxygen/coal ratio of 9, and under these optimized conditions, they achieved 60.97% average yield of FA.

Table 4. Analysis results of the element in lignite and extracted FA (Gong *et al.*, 2020b)

Tabel 4. Hasil analisis unsur di dalam lignit dan AF ekstraksi (Gong *et al.*, 2020b)

Samples (Contoh)	Elemental content (% w/w) (Kandungan unsur, % b/b)					Molar ratio (Nisbah molar)	
	C	H	N	S	O <sup>*</sup>	C/H	C/O
Lignite sample	76.20	4.25	1.70	0.39	17.46	1.49	5.82
Extracted FA	40.22	3.81	1.82	0.22	53.93	0.88	0.99

\* ) obtained by the different from 100% (diperoleh dari pengurangan)

## Potential Use of FA in Sustainable Agriculture

### Effects of FA on growth and yield of crops

It has been reported that FA has only a few hundred Daltons (lower than that of HA), is soluble in a wide range of pH values, and poses a good chemical activity due to its molecular structure containing many functional groups such as -OH and -COOH groups (Gong *et al.*, 2020b & c). Due to its highly specified properties, FA has been used in various practices, such as agriculture. Some researchers have shown that the morphological characteristics of crops, as well as their seeds and straw yields were significantly improved by FA application, and its ability to chelate metals can regulate metal absorption by crops capable of influencing the growth and metabolism of plants (Abdel-Baky *et al.*, 2019; Ali *et al.*, 2015 & Priya *et al.*, 2014). Suh *et al.* (2014) reported that a foliar spray application of FA improved marketable yield and, at the same time, reduced greatly blossom-end rot in all FA-treated plants, and no incidence was observed in the 1.6 g·L<sup>-1</sup> treatment. Their study demonstrated that foliar application of FA at about 0.8 g·L<sup>-1</sup> could be used to promote plant growth and increase marketable yield in tomato production. Based on their earlier study, Anjum *et al.* (2011) found that exogenous FA application significantly ameliorated the negative impacts of drought by maintaining the chlorophyll contents and gas exchange. These pieces of evidence were assumed due to increased levels of antioxidant enzyme activities such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)) and proline. Furthermore, these phenomena influenced the growth and allometry, and grain yield of the crop. It was also shown that under well-watered conditions FA application, improved the crop performances. This confirms that FA application enhanced the crop performance under both drought and well-watered conditions.

It seems obvious from the above evidence that FA is highly beneficial to growth promotion and yield improvement, although the mechanism is somewhat unclear still. Capstaff *et al.* (2020) explored the beneficial effects of using FA by applying two different sources of FA, i.e., FA from Holland and the USA, on three *Medicago sativa* cultivars. They found that FA application did not significantly stimulate the growth of grass species. However, *Rhizobium* grew better after direct application to bacteria, and in addition, the root nodulation was stimulated in *M. sativa* trials. Moreover, from their RNA transcriptional analysis of FA-treated plants, it was shown that many important early nodulation-signalling genes were up-regulated after only three days. The experiments in plate, glasshouse, and field environments showed yield increases, providing substantial evidence for the use of FA to benefit *M. sativa* forage production. Based on their analyses, the effects of FA application on these forage

cultivars indicate a bio-stimulant effect rather than a nutritional one.

### Role of FA in soil properties improvement

Sustainable agriculture always focuses on managing soil quality issues to some extent. Any usage of agricultural inputs as well as agronomical practices should not create trade-offs in terms of soil properties deterioration. Goenadi (2017) has outlined highly significant soil characteristics related to soil quality or soil health based on the guidance set by the Natural Resources Conservation Service – United States Department of Agriculture (NRCS-USDA). The indicative soil characteristics determining the best candidate for soil quality improvement include dissolved organic matter, texture, bulk density, soil respiration, infiltration rate, and worm population. Chlorophyll content and root density in topsoil are also closely related to those soil properties mentioned. In relation to FA application, some studies also indicated its significantly positive effect on soil properties improvement. However, the volume of work is not as big as the work on humic acids.

Sootahar *et al.* (2019) researched in the Northeast Plain of China to evaluate the effect of FA application on the properties of Albic Black soil. Using three different FA origins (see Table 2), they showed that among the treatments, soil organic C and light fraction C were greater by 29% to 21% and 38% to 21%, respectively, compared to that of the control. Besides, in PDLF and PDSF treatments, they reported that available N and P contents were found significantly greater, respectively. However, lower contents of the following were reported, i.e., K-available and Mg-extractable, as well as organic-inorganic degree complexes and organic-inorganic composites in all FA treatments compared to the control. The results also show that FA application significantly affected exchangeable Ca, organic-inorganic composites of SOM, and organic-inorganic compounds, but no significant effect on exchangeable Mg. In 2020, Sootahar *et al.* (2020) reported another work related to the application of FA on three different soil textures (sandy loam, silty clay, and clay loam) and found that the content of heavy fraction C was 10-60% higher, and the light fraction C increased by 30-60% in all treated soils compared to those of controls. However, different soils were observed wherein treated soils, the available N content significantly improved (30%-70%) as well as the available K content by 20%-45%. In Aridisols and Vertisols, soil P content significantly increased by 80-90% but decreased significantly by 60-70% in Mollisols. On the other hand, they found that in both Aridisols and Vertisols P organic-inorganic compounds were greater and lower in Mollisols. Relative to the other two soils, however, organic-inorganic composites decreased in Vertisols.

### *Role of FA in abiotic stress alleviation*

Another challenge in achieving sustainability is how successfully we combat abiotic stress, such as prolonged droughts and salinization, as observed in China. This is caused by the freshwater shortage and the intrusion of saltwater into the groundwater. In order to ameliorate such a coastal saline soil type, biochar originated from wheat straw, and FA has been considered a potential natural soil ameliorant. Sun *et al.* (2020) studied the effects of several dosages of biochar and FA applications to Aquic Halaquepts on alleviating salinity and its impacts on yield performance of maize-barley rotation crops. They found that soil characteristics of the salt-affected soil were improved due to combined amendment of biochar and FA, and as a result, the yields of maize-barley used in this study were improved. This phenomenon is believed that this could be attributed to the multiple benefits resulting from reducing salt effect, as well as improvement in water retention, nutrient supply, and crop growth. Amelioration with biochar at 15 t ha<sup>-1</sup> biochar and 1.5 t ha<sup>-1</sup> FA resulted in the largest changes in total grain yields of the four experimental seasons compared to the control. As crop yields generally have a strong relation with soil fertility and microbial activities, further investigation was required to evaluate the impact of biochar and FA on soil nutrition and microbial community. Although many agronomic factors such as weed and pest controls and timing of field operations may affect yield, the trial should be possible to identify various ways in which biochar and FA could have affected yields. In conclusion, biochar derived from wheat straw in combination with FA could reduce the secondary salinization risk in soil and help to improve crop yields.

### *Effects of FA on plant disease control*

Under unfavorable climate conditions, biotic stress would also be amplified especially related to the diseases that potentially lowers the yield of the crops. A series of greenhouse experiments carried out by Kamel *et al.* (2014) showed the efficacy of FA in controlling downy and mildew on cucumber cultivar DP-164 during the seasons 2012-2013 compared with commercial fungicides. The application of three concentration levels of FA was also evaluated to determine their effects on plant growth, chlorophyll content, and yield of the plants. It was proven that FA application significantly reduced both diseases' severity. The application of FA was shown more effective in reducing the severity of downy mildew compared to that of the recommended fungicide. The inhibition effect of FA on powdery mildew diseases increased gradually with increased FA concentration, and the disease severity reduced equally to or greater than that of commercial fungicides. Foliar application of FA combined with commercial fungicides was shown to improve plant morphological characters, but all FA

concentrations resulted in non-significantly different yields. They also found that FA application increased plant exudates and directly promoted microbial activity, as indicated by increasing dehydrogenase and nitrogenase activity. This study confirmed that improvement of plant growth and yield quantity and quality as well as controlling powdery and downy mildews of cucumber plants could be achieved by foliar application of FA. Similar evidences were reported by El-Sawy & Afifi (2014) in controlling powdery and downy mildew in cucumber plants. On the other hand, Wu *et al.* (2016) indicated that the soil HAs and FAs were found to show inhibition activities against phytopathogenic fungi for the first time and continue to decline with time upon extensive cultivation.

### **Potential Application of FA in Plantation Crops and Biotechnology**

#### *Improvement of plantation crop performances by FA*

As a natural product, FA has a high potential for its application in plantation crops, including improving plantation crop performances. Sun *et al.* (2020) carried out a study on a tea plantation. They found that the tea plant's drought tolerance was improved with FA application by the following mechanisms: (i) the ascorbate metabolism enhancement, (ii) the glutathione metabolism improvement, and (iii) flavonoids biosynthesis promotion that significantly improved the antioxidant defence of tea plants during drought stress. These pieces of evidence were valuable to mitigating drought effects on tea plantations and provided meaningful understanding related to the molecular mechanism of FA to strengthen the strategies for minimizing crop damage due to drought stress. The reason is that their study can be considered a breakthrough by revealing genomic observation as a result of FA application on the crop. By applying FA on tea plants at different periods of drought stress, the effects of FA addition on genes and metabolites were evaluated by using transcriptomics and metabolomics profiles. In total, 30,702 genes and 892 metabolites were observed. It was evidenced that compared with controlled groups, 604 differentially expressed metabolite genes (DEGs) were found in FA-treated tea plants at four days, and this number improved to 3331 at eight days under drought stress. In addition, it was found that 54 and 125 differentially expressed metabolites (DEMs) at those two-time points, respectively. Bioinformatics analysis showed that DEGs and DEMs participated in diverse biological processes such as ascorbate metabolism (*GME*, *AO*, *ALDH*, and L-ascorbate), glutathione metabolism (*GST*, *G6PDH*, glutathione reduced form, and *CYS-GYL*), and flavonoids biosynthesis (*C4H*, *CHS*, *F3'5'H*, *F3H*, kaempferol, quercetin, and myricetin). Moreover, co-expression analysis

results showed that the interactions of identified DEGs and DEMs are diversely involved in the above-mentioned processes, indicating that FA may regulate of these processes during drought stress.

Another study was reported by Silva *et al.* (2020) regarding the effect of FA application on mango in Sao Francisco Valley in the semi-arid zone of Brazil in combination with the application of paclobutrazol (PBZ), a plant growth regulator commonly used in mango production systems in semi-arid conditions to inhibit gibberellin biosynthesis and promote a better flowering uniformity. They found that the use of FA, free amino acids, or both affected PBZ absorption by local variety mango. The absorption of PBZ molecules by the plant was improved, whereas the application of FA significantly inhibited vegetative growth. At the end of the productive cycle, there is a lower soil PBZ residue when applied together with fulvic acid. Therefore, they recommended this treatment for mango crop management in semi-arid conditions. Considering that the generative pattern of mango is more or less similar to cocoa and coffee, the application of those findings could apply to the crops in tropical regions. As indicated earlier, the application of FA improved the growth performances of coffee seedlings (Justi *et al.*, 2019) and also of tobacco (Moradi *et al.*, 2019), whereas in oil palm, the use of FA isolated from palm oil mill effluent as urea coating reduced 50% ammonia volatilization more effective than HA (Rosliza *et al.*, 2009). Moreover, El-Boray *et al.* (2012) reported that the combination of FA application with selected micronutrients (Fe, Zn, and Mn) and a consortium of microbes (*Serratia sp.* + *Bacillus polymyxa* + *Pseudomonas fluorescens* + *Trichoderma viride* + *Trichoderma harzianum*) improved significantly the yield of Zaghoul date palm in Egypt significantly. Based on the reported evidence, it is believed that the application of FA will offer similar benefits to other plantation crops in Indonesia, such as sugarcane, cocoa, and rubber.

#### Biotechnological application potential of FA

FA has also enjoyed some applications related to its unique biological character and function in biotechnological-related works. A study carried out by Liu *et al.* (2019) in PR China has been reported in relation to the biotechnologically produced FA (BFA). They prepared BFA from four different processes involving liquor's grains as the main raw material originated from fermented straw or woods enriched with soybean meal and wheat bran, the use of fermentation engineering and enzyme engineering core technology, combined with excellent probiotic strain, the probiotics-composite enzyme coupled biological transformation method, auxiliary physical and chemical processing. The fermenting microbes used were *Lactobacillus*, *Bacillus*, yeast, and the

combination of the three. Produced BFAs were then fed to the Sprague-Dawley rats mixed with normal feed at 1.5% concentration for four weeks. It proves that the BFA has a positive effect on improving the health indicators and nutritional status, blood physiology and biochemistry, weight gain of rats, which can effectively promote the growth of animals and raise feed reward. Other Chinese co-workers (Li *et al.*, 2020) have reported using FA produced from the liquid waste of molasses fermentation as a raw material for high-value products of poly- $\gamma$ -glutamic acid ( $\gamma$ -PGA). This compound has a wide application in food, medicine, cosmetic, and agriculture nowadays as it is proven as a natural anionic polymer of D/L-glutamic acids linked together via amide bonds between the  $\alpha$ -amino group and the  $\gamma$ -carboxylic acid group, resulting in numerous properties, such as holding water, biodegradability, and non-toxicity. However, its production cost using the conventional method via fermentation was expensive due to low yield and the use of glutamate and other high-cost components as substrate. Therefore, some low-cost feedstocks like FA powder are urgently needed to overcome the economic and sustainable obstacles to produce  $\gamma$ -PGA biotechnologically. Using a microbial strain of *Bacillus velezensis* GJ11, they found that FA power could partially substitute the high-cost substrates such as sodium glutamate and citrate sodium for producing  $\gamma$ -PGA. With FA powder in the fermentation medium, the amount of sodium glutamate and citrate sodium used for producing  $\gamma$ -PGA were both decreased by around one-third. This result exhibited that FA as a waste produced by the fermentation process of molasses offers a great opportunity as cheap and abundant raw material to be bio-converted into high value-added  $\gamma$ -PGA. In other words, this invention opens the possibility of a circular economy in agricultural-based product development.

Effects of FA on soil enzymes and microbe have also become the interests of some researchers. Zhang *et al.* (2020) studied the FA dynamics in soil amended with different composts and their effect on the soil microbes. They proved that the formation of FA in soils was influenced by compost amendment. By using structural equation models (SEMs), they demonstrated that transformation of FA components was taken place directly by microbes or indirectly through changes in total organic carbon (TOC) and total nitrogen (TN) contents, C/N ratio, HS levels, and the ratio of HA to FA (HA/FA). These mechanisms believed to regulate microbial community structure, and as a result, would be helpful to improve the bioavailability of compost products and implement sustainable utilization of the soils. Among many agricultural inputs, such as soil amelioration, fertilizer application, and plant growth regulator, the foliar application of FA as the most bioactive humate molecule improved K levels in leaves of tobacco plant grown on

Vertisols soil in India (Vadlamudi *et al.*, 2014). Although the mechanism is still plausible, they postulated that FA acts like the plant hormone auxin in tobacco, influencing the expression of key genes encoding transporters and enzymes involved in K uptake and starch metabolism. Li *et al.* (2016) reported the effect of FA application on microbial denitrification. The study revealed that the presence of FA was not only improved the efficiency of total N (TN) removal (99.9% vs. 74.8%) significantly but also reduced nitrite accumulation (0.2 vs. 43.8 mg L<sup>-1</sup>) and N<sub>2</sub>O emission (0.003 vs. 0.240 mg N/mg TN removed) compared to the control. It was also shown that FA addition increased C source metabolism via glycolysis and tricarboxylic acid (TCA) cycle pathways to produce more available nicotinamide adenine dinucleotide (NADH).

To examine the effectiveness of HS on enzyme activity, Li *et al.* (2013) compared HA with FA in influencing the activity and stability of lysozyme and urease. These two molecules are negatively charged, whereas the two enzymes studied have pH-dependent charges, i.e. lysozyme is net positive at pH values < 10.4, and urease is net positive < pH 5.2 or net negative above pH 5.2. They found that the enzyme activities were reduced when the HS has an opposite charge to the enzymes. As the hydrophobicity of HA is higher than that of FA, the enzyme activity suppression was stronger for HA. However, when urease and HS were negatively charged it was found that no complexes were formed, and the presence of HA or FA increased the activity and stability of the enzyme. Yu *et al.* (2015) identified the endophytic microbial consortia on the stevia leaves at different growth stages, and FA application reported an interesting study. They found that Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes were the dominant phyla on stevia leaves, and the stevia growth stages strongly controlled the endophytic microbial community. *Agrobacterium* and *Erwinia* genera were dominant during the early growth stage until flowering, whereas *Sphingomonas* and *Methylobacterium* dominated in mature leaves at harvesting. These latter two microbes were found to be positively correlated with stevioside content and *UGT74G1* gene expression. Fulvic acid application increased the variation of endophytic microbes along the growth stages and improved the stevioside content of stevia leaves. These findings could be used as a strategic approach to improve stevia growth and steviol glycosides accumulation by applying key endophytic microbial and FA via foliar application.

### Potential Use of FA in Medicinal Application

#### *Application of FA as antimicrobial and anti-inflammatory treatments*

Antonio Celestino Fernandes, Elizabeth Medlen & Stephen Leivers were granted a patent

(Patent # US 20110207687A1) in 2013 regarding an invention of a combination of FA and one or more antibiotics from the classes of penicillin and aminoglycosides for use in the treatment of various diseases and conditions. The antibiotic was oxacillin, gentamicin, or both oxacillin and gentamicin intended for various diseases, including bacterial infection, particularly bacteria which are antibiotic-resistant ones. Van Rensburg (2015) stated that humic substances, humic and fulvic acids effectively suppress delayed-type hypersensitivity, raw paw edema, a graft-versus-host reaction, and contact hypersensitivity in rats. Further, Van Rensburg (2016) described the wide-spectrum antimicrobial activity of FA, thus very potential for eradicating those microbes with highly resistant to antibiotics. To overcome the high cost of converting bituminous coal to humic substances, including FA, a pharmaceutical company developed a so-called carbohydrate-derived FA (CHD FA). They carried out a preclinical study and found that the cutaneous immune response in mice can be suppressed by FA addition and confirmed that a dosage of 40ml of a 3.8% solution taken twice daily for three days was considered safe. This study also showed strong evidence on the improvement of anti-allergenic when applied to most allergic patients. It was observed that the wheel and flare reactions were successfully decreased in the treatment group. In conclusion, they believed that the product is safe and effective as an anti-inflammatory agent at oral dosages of up to 40 mL taken twice daily for one week. Efficacy and safety tests of the product as an a-topical treatment for eczema were also conducted. It was found that a significant improvement in the symptoms was observed in the treated group, meaning that the product was well tolerated and effective as a topical treatment for eczema. Other researchers have also tried to develop other methods in producing synthetic FA, such as from hematoxylin (Litvin *et al.*, 2015).

Other CHD FA had been reported by Boste *et al.* (2018), showing its tolerability, safety, and effect on disease marker of pre-ART HIV-1 given a wellness drink containing FA. They concluded that the CHD FA wellness drink was well tolerated in an ART-naive study population and did not negatively affect the disease-specific parameters and hence did not adversely affect the natural progression of the HIV-1 disease or patients' general health. Sabi *et al.* (2012) also shown the positive effect of CHD FA to evaluate the safety and anti-inflammatory and wound-healing characteristics of CHD FA in rats. They concluded that CHD FA was a safe compound with anti-inflammatory and wound-healing properties for patients induced by carrageenan. However, this material still merits further evaluation in the treatment of patients suffering from similar conditions.

In 2013, another study reported the positive effect of CHD FA by studying the biological

properties of CHD FA as a potential novel therapy for the management of oral biofilm infections (Sherry *et al.*, 2013). They found that CHD FA possessed broad-spectrum antibacterial activity, with a supplementary function of down-regulating inflammation. These properties offer an attractive spectrum of function from a naturally derived compound, which could be used as an alternative topical treatment strategy for oral biofilm diseases. However, Sherry *et al.* (2013) advised that further studies *in vitro* and *in vivo* are required to determine the precise mechanism by which CHD FA modulates the host immune response. Winkler & Ghost (2018) conducted a thorough review regarding FA's therapeutic potential in chronic inflammatory diseases and diabetes. Proinflammatory markers were reported to decrease and activate the immune system to kill bacteria due to FA treatment. The application of FA was shown that oxidative stress was reduced, and apoptosis in hepatic cancer lines was even induced while also influencing the microbiome and possibly gut function improvement. They believed that FA has a yin-yang effect when it comes to these physiological states. Although the supporting literature currently is minimal, if considered in combination, the potential for FA to be a candidate in preventing inflammation like in diabetes could be encouraging. Besides, as our current approach to these kinds of diseases is lacking in this direction, this is highly promising. However, it is important to note that some conflicting evidence exists in FA research results, which is presumably due to variance in dosage, parent material, and isolation procedure. In addition, there is no consensus on the structure of FA, standard isolation, or parent material. Thus, it is of iceberg concern to reconcile these factors and establish the proper dosages for different ages of patients and various sources of FA. It is believed that by doing so, a conclusive statement regarding FA function and its related beneficial effect to cure immune-related diseases could be achieved.

#### *Potential use of FA for the healing of neurodegenerative diseases*

It has been indicated that FA application also promoted the healing of Parkinson's disease (PD) and Alzheimer's disease (AD), a very important neurodegenerative disorder. Both diseases had been known to share the so-called Lewy bodies (LB) in PD and neurofibrillary tangles in AD, which are the accumulation of characteristic protein aggregates. Primarily component of LBs is misfolded  $\alpha$ -synuclein (aSyn), whereas tau protein is a primary component of neurofibrillary tangles. Dominguez-Mejide *et al.* (2020) reported their study in which K18 tau aggregation *in vitro* was inhibited by FA application. Very interestingly, FA seemed to disaggregate previously formed tau aggregates in cells and was consistent with

findings using heparin-induced tau aggregation while, apparently not being able to disaggregate previously formed aSyn aggregates, as shown by proteinase K (PK) digestion and homogeneous time-resolved fluorescence (HTRF)-based aggregation assay, implying differences in their mechanism of aggregation inhibition depending on the target proteins. Another interesting fact was that FA could also lead to an increase in neurite outgrowth and when used in combination with B complex vitamins may stabilize cognitive function in AD patients. They believed that the absence of changes in the percentage of positive cells observed using flow cytometry, in contrast with what they observed for aSyn, might be due to the different subcellular distributions of the proteins. In cells, tau is mostly bound to microtubules, with free C-terminal tails that can interact with other tau molecules. Additionally, monomers of the longest isoform of tau might fold into a paperclip shape, with N- and C-termini  $\sim 2.3$  nm away from each other. They believed that this distance is small enough to enable the reconstitution of the venus-based BiFC assay. Hence, this effect may also explain the differences for tau and aSyn they found. A few years earlier, Verma *et al.* (2012) believed that AD is directly related to the aggregation of A $\beta$  peptides. These peptides can self-assemble from monomers to higher oligomeric or fibrillar structures in a highly ordered and efficient manner. Their study revealed from the 14ns molecular dynamics simulation that fulvic acid interrupted the dimer formation of A $\beta$ (17-42) peptide while in its absence A $\beta$ (17-42) dimer formation occurred at  $\sim 12$ ns. In addition, FA disrupted the preformed A $\beta$ (17-42) trimer in a very short time interval (12ns). These results may provide an insight into the drug design against A $\beta$ (17-42) peptide aggregation using as lead molecule against A $\beta$ (17-42) mediated cytotoxicity and neurodegeneration.

#### *Other potential uses of FA in disease and health cares*

The studies on FAs properties with plants and plant cells have shown a positive effect of this substance on animal organisms. Kishor *et al.* (2012) suggested that a humic substance indicated some anticarcinogenic properties, consisting of 60–80% FAs. Due to their heavy metal chelating properties, binding of proteins delivering anticancer drugs, and inhibition of cancer cell proliferation, these properties may be beneficial in cancer therapy (Aydin *et al.*, 2017). Moreover, products consisting of natural ingredients enjoy a growing interest from consumers nowadays, especially without preservatives, and have beneficial effects on humans. Besides economic considerations, a great impact on the market of

food products, including beverages, is determined by the taste and the health awareness of consumers on the importance of food to their health. In fact, in 2004, the Ministry of Health, Labour, and Welfare of Japan has designated FA as a food (Motojima *et al.*, 2009). A study carried out by Swat *et al.* (2019) disclosed the quality of FA-based food products bought from online stores in 2015 and 2018 in Poland. The concentrated FA used in the products was originated from the Great Salt Lake in Utah (North America) and England, whereas FA in ready-to-drink beverages was obtained mainly from an aquatic source and/or soils of North America and South Africa. The concentrations of Ca, K, Mg, Na, Cu, Fe, Mn, and Zn, and antioxidant capacities of FA concentrates and ready-to-drink beverages available on the global market were determined by using microwave plasma-atomic emission spectrometry and antioxidant capacities and ferric reducing ability of plasma (FRAP) values were evaluated. From 14 products' daily portion studied, eight of them have various levels of Fe (45–135% of recommended daily allowance (RDA)). The data obtained also showed that some product was also suitable as a source for Mg (about 40% of RDA), and another Mn (about 70% of RDA). Overall, it is indicative that a good source of antioxidant polyphenolic compounds and some minerals could be obtained from dietary supplements or food products with FAs.

### **The Future Challenge, Opportunity, and Outlook**

#### *Challenges and opportunities*

Based on the brief illustrations described above, there is much evidence showing the power of FA as a natural compound with a wide-spectrum application for supporting sustainable agricultural practices and its high potential in an application for medicinal purposes and healthy foods. However, as a natural product, FA needs to be guaranteed for its beneficial functions and quality. As outlined before, the type of materials and/or extraction procedures would have much influence on the quality and as consequence the beneficial effects of its uses. For consumers and manufacturers, the large-scale production technology should be in place to provide an efficient process, and finally a competitive price of products. It is time to develop a cheap and simple method in FA large-scale production. Besides, many works await further efforts in understanding the mechanisms of FA as a plant growth stimulator and as a disease curing agent.

It has been widely exhibited that the application of FA for various plantation crops and biotechnological approaches is highly promising. Oil palm, sugarcane, tea, coffee, cocoa, tobacco, and other perennial horticultural crops will potentially enjoy the benefits of using FA for yield and quality improvements. In biotechnological

aspects, FA opens the possibility of improving crop metabolism alone and/or in combination with selected beneficial microbes. Therefore, the development of new material as an efficient source for FA production, like from carbohydrate and plantation organic wastes (empty fruit bunches composts, POME, and molasse wastes), will open broader possibilities to avoid natural raw material shortage. Wide ranges of FA application in agriculture, including as phyto-stimulant, and ameliorating soil agent have been shown convincingly to be part of the solutions in practicing sustainable agriculture to achieve sustainable development goals connected with reduced environmental pollution, healthy foods, and farmer welfare improvement targets. Many pieces of evidence have also been reported regarding the powerful effect of FA in medical treatments. Although available research is still limited, there is an urgent need to extend FA research activities to prevent chronic inflammatory diseases, including diabetes, neurodegenerative disorders (AD & PD), cancer, and digestive diseases.

The opportunity of FA application in medicinal cares is also possibly prospective along with the so-called One Health concept as a new paradigm. According to the Center for Disease Control and Prevention, One Health concept is an approach viewing that the people's health is closely related to the animal's health and the environment shared (CDC, 2018). It is not new, but it has become more important in recent years. The reason is that many factors have changed interactions between people, animals, plants, and our environments, such as human population increase, climatic and land-use change, and high mobility of people, animal, and animal products. Gebreyes *et al.* (2014) stated that zoonotic infectious diseases had been an important concern to humankind for more than 10,000 years. Until almost a decade ago, approximately 75% of newly emerging infectious diseases (EIDs) are zoonoses that result from various anthropogenic, genetic, ecologic, socioeconomic, and climatic factors. These interrelated driving forces make it difficult to predict, and to prevent zoonotic EIDs. In 2017 World Health Organization (WHO, 2017) described One Health as an approach to designing and implementing programs, policies, legislation, and research in which multiple sectors communicate and work together to achieve better public health outcomes. The areas of work in which a One Health approach is particularly relevant to include food safety, the control of zoonoses (diseases that can spread between animals and humans, such as flu, rabies, and Rift Valley Fever), and combatting antibiotic resistance (when bacteria change after being exposed to antibiotics and become more difficult to treat). Based on these assumptions, FA will enjoy further beneficial applications supporting the One Health concept in the future, particularly as disease curing agents for plants, animals, and humans.

### Current market outlook of FA

Transparency Market Research (2020) has conducted a global industry analysis for 2015-2019 and an opportunity assessment for 2020-2030. The key prospect of FA is related to its function as a nutrient booster and it involves FA in binding the minerals and trace elements and transporting these in the animal or plant system. Further justification is that the healthy plant needs proper nutrition and FA improves the nutrient absorption by the plant from the soil. Farmers worldwide are looking for effective products to increase the productivity of crops that are propelling the demand for fulvic acid in global markets. By improving the structure of the soil, it increases the water holding capacity and buffering property. It is believed that FA also helps the plant to endure environmental stress (both biotic and abiotic), root respiration, and formation. Moreover, the consumption of dietary supplements had increased as the consumers need to boost the immune system, propelling the global FA.

This review study also considers opportunity in relation to the current common situation worldwide as caused by the covid-19 pandemic. This pandemic has been posing major challenges to humans, industries, and countries worldwide. Various adverse effects have been shown on all the global market sectors, but the governments keep also monitoring the production and distribution of essentials. All agrochemicals, especially fertilizers and other crop protection products are made available for the farmers to ensure productivity for the upcoming crop season. At the same time, this pandemic made consumers realize the benefits of nutrition and healthy food, thus the demand for organic and natural produces is strengthening. As the pharmaceuticals and nutraceutical industries are still operating in most of world's region, the global FA market may expect to witness an upsurge. The growers are working on farms and want to keep their crops safe for the consumers. On the other hand, the manufacturers are facing challenges in supplying the products across borders since the borders are sealed due to this pandemic. Based on these pieces of evidence, then it is believed that the future market of FA all over the world is prospective.

Another market report was published by MarketWatch in January 2021 (<https://www.marketwatch.com>), providing the following forecast. MarketWatch sees that in the coming years in North America, Europe and Asia will be facing an increasing demand for FA. Global production and consumption developed steadily in the past few years from 2012-2017. It is projected that the global FA market size in terms of production will grow to 190,763 MT by 2022. At the same time, China and North America are remarkable in the global FA industry because of FA's market share and technology status. In the future, production and consumption are estimated

to continue developing with a stable growth rate. To meet the great and increasing demand, more and more manufacturers will go into this industry. According to a new study, the worldwide market for FA is expected to grow at a CAGR of roughly 3.6% over the next five years, will reach 250 million USD in 2024, from 210 million USD in 2019. However, concerns for an excessive rate of the product together with lack of knowledge approximately the right usage of FA 2021 amongst users are a number of the elements predicted to behave as a barrier to market growth.

### Concluding Remarks

Based on the information gathered and collated in this review, it is clear that a natural compound as part of humic substances (HS) so-called FA exhibits a wide range of application in both agriculture and medicine. The exploitations of FA are shown to be based on its unique physical and chemical properties, which are different compared to HA, particularly in molecular size. Although the composition of FA is considerably lower than the other two fractions of HS, it seems that this fraction has more powerful effects on improving nutrient uptake by crops and facilitating crucial metabolism in human disease healing. In addition, since the FA structure, isolation standard procedure or raw material have not been agreed yet, then it is a huge concern to conform these factors and determine standard dosage for any uses and different FA origin. This will help make a more conclusive statement regarding FA function and its benefit on both soils and crop productivity especially related to plantation commodity and human health, particularly on immunity improvements become a reality. Moreover, further research is opened in developing a small volume – high-value product from FA in medical application and pharmaceutical grade FA, including healthy foods and feeds, which will certainly have a high economic value and saving lives. Potential use of FA to boost immunity against widespread diseases such as covid-19 will also be in the front-row of interest by using less and less dependent on non-renewable natural resources.

### References

- Abdel-Baky YR, HF Abouzena, AA Amin & MR Elsh (2019). Improve quality and productivity of some faba bean cultivars with foliar application of fulvic acid. *Bull. Natl. Res. Ctr.* 43(1), 1-11. DOI. 10.1186/s42269-018-0040-3.
- Ali S, SA Bhawarna, M Rizwan, M Farid, S Kanwal, Q Ali, M Ibrahim, RA Gill & MD Khan (2015). Fulvic acid mediates chromium (Cr) tolerance in wheat (*Triticum aestivum* L.) through lowering of Cr uptake and improved antioxidant defense system. *Environ. Sci. Pollut. Res.* 22(14), 10601-10609. DOI: 10.1007/s11356-015-4271-7.

- Anjum SA, L Wang, M Farooq, L Xue & S Ali (2011). Fulvic acid application improves the maize performance under well-watered and drought conditions. *J. Agron. Crop Sci.* 197(6), 409-417.
- Aro T & P Fatehi (2017). Production and application of liginosulfonates and sulfonated ligin. *Chem. Sus.* 10(9),1861-1877. <https://doi.org/10.1002/cssc.201700082>.
- Aydin SK, S Dalgic, M Karaman, OF Kirlangic & H Yildirim (2017). Effects of fulvic acid on different cancer cell lines. In: *Multidisciplinary Digital Publishing Institute Proceedings* 1(10), 1031.
- Bannach-Szott M & B Debska (2008). Content of phenolic compounds in fulvic and humic acid fractions of forest soils. *Polish J. of Environ. Stud.* 17(4), 463-472.
- Boste ME, IS Gilada, JR Snyman & JPL Labuschagne (2018). Carbohydrate-derived fulvic acid wellness drink: its tolerability, safety and effect on disease markers in pre-ART HIV-1 positive subjects. *South Afric. Fam. Pract.* 60(3), 91-96.
- Capstaff NM, F Morrison, J Cheema, P Brett, L Hill, JC Muñoz-García, YZ Khimiyak, C Domoney & AJ Miller (2020). Fulvic acid increases forage legume growth inducing preferential up-regulation of nodulation and signalling-related genes. *J. Experiment. Bot.* 71(18), 5689–5704. <https://doi.org/10.1093/jxb/eraa283>.
- Carrasco-Gallardo C, L Guzman & RB Maccioni (2012). Shilajit: A natural phytocomplex with potential precognitive activity. *Internat. J. Alzheimers Dis.* doi: 10.1155/2012/674142.
- CDC (2018). One Health Basics. <https://www.cdc.gov/onehealth/basics/index.html>.
- Cheng G, Z Niu, C Zhang, X Zhang & X Li (2019). Extraction of humic acid from lignites by KOH-hydrothermal method. *Appl. Sci.* 9(7), 1356. <https://doi.org/10.3399/app9071356>.
- Dominguez-Meijide A, E Vasili, A König, M Cima-Omori, AI de Opakua, A Leonov, S Ryazanov, M Zweckstetter, C Griesinger & TF Outeiro (2020). Effects of pharmacological modulators of  $\alpha$ -synuclein and tau aggregation and internalization. *Sci. Rep.* 10, 12827. <https://doi.org/10.1038/s41598-020-69744-y>.
- El-Boray MS, M Mostafa, AA El-wahab & A Elattar (2012). Effect of fulvic acid, some micro-elements and micro-organisms on yield and its components, content fruits of macro- and micro- elements content of Zaghoul date palm. *Journal of Plant Production* 3(8), 2277-2285. DOI: 10.21608/jpp.2012.84971.
- Feng Q, C Sun, J Cao & H Liu (2017). *Method for extracting fulvic acid from diatomite*. CNIPA. Patent # CN104974356A. (<https://patents.google.com>)
- Fernandes AC, E Medlen & S Leivers (2013). *Fulvic acid and antibiotic combination*. United States. Patent # US 20110207687A1. (<https://patents.google.com>)
- Gao Y, J He, Z He, Z Li, B Zhao, Y Mu, J Lee & Z Chu (2017). Effects of fulvic acid on growth performance and intestinal health of juvenile loach *Paramisgurnus dabryanus* (Sauvage). *Fish Selfish Immunol.* 62, 47-56.
- Gebreyes WA, J Dupouy-Camet, M Newport, CJ B de Oliveira, L Schlesinger, YM Saif, S Kariuki, L Saif, WJA Saville, T Wittum, A Hoet, S Quessy, R Kazwala, B Tekola, T Shryock, MS Bisesi, P Patchanee, S Boonmar & LJ King (2014). The global one health paradigm: challenges and opportunities for tackling infectious diseases at the human, animal, and environment interface in low-resource settings. *PLoS Neglected Tropical Diseases* 8(11), e3257. DOI: 10.1371/journal.pntd.0003257
- Goel RK, RS Banerjee & SB Acharya (1990). Antiulcerogenic and anti-inflammatory studies with Shilajit. *Ethnopharmacol.* 29, 95-103.
- Goenadi DH (2001). The potential use of humic acids. *J. Tanah Link.* 2(2), 23-31.
- Goenadi DH (2006). *Developing Technology for Biodecomposition of Fresh Solid Wates of Plantation Crops under Tropical Conditions*. IPB Press. Bogor. 229pp.
- Goenadi DH (2017). *Penilaian Mutu Tanah Secara Cepat – Sebagai Dasar Solusi Ameliorasinya*. PT RPN Press, Bogor, Indonesia. 160pp, *In Indonesian*.
- Gong G, L Xu, Y Zhang, W Liu, M Wang, Y Zhao, X Yuan & Y Li (2020a). Extraction of fulvic acid from lignite and characterization of its functional groups. *ACS Omega* 5(43), 27953-27961.
- Gong G, X Yuan, Y Zhang, Y Li, W Liu, M Wang, Y Zhao & L Xu (2020b). Characterization of coal-based fulvic acid and the construction of a fulvic acid molecular model. *RSC Adv.* 10 (9), 5468-5477.
- Gong G, Y Zhang, Y Zhao, G Xie, H Zheng, Y Li & K Shi (2017). *Method for extracting fulvic acid from lignite*. CNIPA. Patent # CN104629062A. (<https://patents.google.com>)
- Gong G, Y Zhao, Y Zhang, B Deng, W Liu, M Wang, X Yuan & L Xu (2020c). Establishment of a molecular structure model for classified products of coal-based fulvic

- acid. *J. Fuel* 267, 117210. <https://doi.org/10.1016/j.fuel.2020.117210>.
- Javed S, K Kohli & M Ali (2013). Microwave-assisted extraction of fulvic acid from a solid dosage form: a statistical approach. *J. Pharm. Innov.* 8, 175–186. 10.1007/s12247-013-9157-y.
- Jeong, HJ, J Cha, JH Choi, K Jang, J Lim, W Kim, D Seo & J Jeon (2018). One-pot transformation of technical lignins into humic-like plant stimulants through fenton-based advanced oxidation: accelerating natural fungus-driven humification. *ACS Omega* 3(7), 7441–7453.
- Jordaan IT (2019). Synthesis, characterisation and properties of fulvic acid, derived from a carbohydrate. Ph.D. Diss. NWU Lib. <https://orcid.org/0000-0003-4221-582X>.
- Justi M, E Morais & C Silva (2019). Fulvic acid in foliar spray is more effective than humic acid via soil in improving coffee seedlings growth. *Arch. Agron. Soil Sci.*, 1-15. DOI: 10.1080/03650340.2019.1584396.
- Kamel SM, MMI Afifi, FS El-shoraky & MM El-Sawy (2014). Fulvic acid: a tool for controlling powdery and downy mildews in cucumber plants. *Internat. J. Plant Pathol.* 3(2),101-108. DOI: 10.33687/phytopath.003.02.0866.
- Karr MC (2010). *Methods for extracting fulvic acid molecules*. European Patent Office. Patent # EP1797190A1. (<https://patents.google.com>)
- Khang VT (2011). Fulvic foliar fertilizer impact on growth of rice and radish at first stage. *Omonrice* 18, 144-148.
- Khanna R, M Witt, MK Anwer, SP Agarwal & BP Koch (2008). Spectroscopic characterization of fulvic acids extracted from the rock exudate Shilajit. *Org. Geochem.* 39, 1719–1724. 10.1016/j.orggeochem.2008.08.009.
- Khumic (2020). The largest humate products in China. <https://www.khumic.com>. Downloaded November 10, 2020.
- Kishor P, S Bimala & T Nagendra (2012). Shilajit: Humic matter panacea for cancer. *Int. J. Toxicol. Pharmacol. Res.* 4, 17–25.
- Li J & D Li (2017). *Novel method for extracting fulvic acid from industrially-produced waste edible fungi residues by fermentation*. CNIPA. Patent # CN105331669A. (<https://patents.google.com>)
- Li M, Y Su, Y Chen, R Wan, X Zheng & K Liu (2016). The effects of fulvic acid on microbial denitrification: promotion of NADH generation, electron transfer, and consumption. *Appl. Microbiol. Biotechnol.* 100(12), 5607-5618. DOI 10.1007/s00253-016-7383-1.
- Li Y, J Wang, N Liu, L Ke, X Zhao & G Qi (2020). Microbial synthesis of poly- $\gamma$ -glutamic acid ( $\gamma$ -PGA) with fulvic acid powder, the waste from yeast molasses fermentation. *Biotechnol Biofuels* 13, 180. <https://doi.org/10.1186/s13068-020-01818-5>.
- Li Y, W Tan, LK Koopal, MX Wang, F Liu & W Norde (2013). Influence of soil humic and fulvic acid on the activity and stability of lysozyme and urease. *Environ. Sci. Technol.* 47(10), 5050–5056.
- Litvin VA, BF Minaev & GV Baryshnikov (2015). Synthesis and properties of synthetic fulvic acid derived from hematoxylin. *J. Mol. Struct.* 1086, 25-33.
- Liu Y, J Mao, X Wei, M Yi, X Zhang, K Zheng, X Chen, G Wang & B Chen (2019). Effects of biotechnologically produced fulvic acid on nutritional status and health indicators of sprague-dawley rats. *Pakistan J. Zool.* 51(3). 961. DOI: <http://dx.doi.org/10.17582/journal.pjz/2019.51.3.961.970>.
- Lyons GH & Y Genc (2016). Commercial humates in agriculture: real substance or smoke and mirrors? *Agon.* 6(4),1-8. DOI: 10.3390/agronomy6040050.
- Mao Y (2019). Modulation of the growth performance, meat composition, oxidative status, and immunity of broilers by dietary fulvic acids. *Poultry Sci.* 98(10), 4509-4513.
- Market Watch (2021). Fulvic Acids as Biostimulant Market Analysis, Growth, Size, Share, Trends, Forecast, Supply Demand and Sales to 2026. <https://marketwatch.com>. Downloaded January 30, 2021.
- Mema V (2005). Identification of extraction methods for the production of humic acids from black liquor. MS thesis. Dept. Proc. Eng., the Univ. Stellenbosch. 106p. <http://scholar.sun.ac.za>.
- Moradi P, B Pasari & F Fayyas (2017). The effect of fulvic acid application on seed and oil yield of safflower cultivars. *J. Centr. European Agric.* 18(3), 584-597. DOI:10.5513/JCEA01/18.3.1933. Corpus ID: 39540112.
- Moradi S, P Babak & R Talebi (2019). Study of the effects of mycorrhiza, fulvic acid, seaweed extract and urea on physiological traits and leaf yield of tobacco (Burley 21). *European J. Environ. Sci.* 9(1), 33-40.
- Motojima H, P Yamada, J Han, M Ozaki, H Shigemori & H Isoda (2009). Properties of fulvic acid extracted from excess sludge and its inhibiting effect on beta-hexosaminidase

- release. *Biosci. Biotechnol. Biochem.* 73(10), 2210-2216.
- Priya BNV, K Mahavishnan, DS Gurumurthy, H Bindumadhaya, P Ambika & NK Sharma (2014). Fulvic acid (FA) for enhanced nutrient uptake and growth insights from biochemical and genomic studies. *J. Crop Improv.* 28(6),740-757.
- Raposo JC, U Villanueva, M Olivares & JM Madariaga (2016). Determination of humic substances in sediments by focused ultrasound extraction and ultraviolet visible spectroscopy. *Microchem. J.* 128, 26–33. 10.1016/j.microc.2016.04.004.
- Romarís-Hortas HV, PA Moreda & P Bermejo-Barrera (2007). Application of microwave energy to speed up the alkaline extraction of humic and fulvic acids from marine sediments. *Anal. Chim. Acta* 602, 202–210. 10.1016/j.aca.2007.09.022.
- Rosliza S, OH Ahmed, NMA Majid & M Jalloh (2009). Reduction of ammonia volatilization through mixing urea with humic and fulvic acids isolated from palm oil mill effluent sludge. *Am. J. Environ. Sci.* 5(3), 382-386.
- Sabi R, P Vrey & C van-Rensburg (2012). Carbohydrate-derived fulvic acid (CHD-FA) inhibits Carrageenan-induced inflammation and enhances wound healing: efficacy and toxicity study in rats. *Drug Dev. Res.* 73(1), 18-23. 10.1002/ddr.20445.
- Santi LP, DH Goenadi, H Widiastuti, N Mardiana & Isroi (2000). Extraction and characterization of humic acid from plantation solid organic waste composts. *Menara Perkebunan*, 68(2), 29-36.
- Sasikala S & K Deeptha (2018). Titrimetric estimation of fulvic acid substances in Oriens Shilajit as a part of herbal nutraceutical standardization. *Internat. J. Multidisc. Res. Dev.* 5(5), 198-199.
- Schepetkin IA, G Xie, MA Jutila & MT Quinn (2009). Complement-fixing activity of fulvic acid from Shilajit and other natural resources. *Phytother Res.* 23(3), 373-384.
- Sherry L, E Millhouse, DF Lappin, C Murray, S Culshaw, CJ Nile & G Ramage (2013). Investigating the biological properties of carbohydrate derived fulvic acid (CHD-FA) as a potential novel therapy for the management of oral biofilm infections. *BMC Oral Health*, 13(47). <https://doi.org/10.1186/1472-6831-13-47>.
- Silva LS, PTS Silva, PTS Silva & IHL Cavalcante (2020). Impact of fulvic acid and free amino acids on paclobutrazol absorption by 'Keitt' mango. *Rev. Ambient. Agua* 15(4). <http://dx.doi.org/10.4136/ambi-agua.2519>.
- Sootahar MK, X Zeng, S Su, Y Wang, L Bai, Y Zhang, T Li & X Zhang (2019). The effect of fulvic acids derived from different materials on changing properties of albic black soils in the Northeast plain of China. *Molecules* 24(8), 1535. doi:10.3390/molecules24081535.
- Sootahar MK, X Zeng, Y Wang, S Su, P Sootahar, L Bai, MK Sootahar, Y Zhang, A Mustafa & N Ye (2020). The short-term effects of mineral- and plant-derived fulvic acids on some selected soil properties: improvement in the growth, yield, and mineral nutritional status of wheat (*Triticum aestivum* L.) under soils of contrasting textures. *J. Plants* 9(2), 205. <https://doi.org/10.3390/plants9020205>.
- Suh HY, KS Yoo & SG Suh (2014). Effect of foliar application of fulvic acid on plant growth and fruit quality of tomato (*Lycopersicon esculentum* L.). *Horticul. Environm. Biotechnol.* 55(6), 455-461.
- Sun J, C Qiu, Y Ding, Y Wang, L Sun, K Fan, Z Gai, G Dong, J Wang, X Li, L Song & Z Ding (2020). Fulvic acid ameliorates drought stress-induced damage in tea plants by regulating the ascorbate metabolism and flavonoids biosynthesis. *BMC Genomics* 21(1), 411. <https://doi.org/10.1186/s12864-020-06815-4>.
- Sun Y, J Yang, R Yao, X Chen & X Wang (2020). Biochar and fulvic acid amendments mitigate negative effects of coastal saline soil and improve crop yields in a three-year field trial. *Sci Rep* 10(1), 1-12. <https://doi.org/10.1038/s41598-020-65730-6>.
- Supriyati S (2019). The effect of fulvic acid acid fraction supplementation on broiler chicken performance. In: *Proc. 8<sup>th</sup> Internat. Sem. Trop. Anim. Prod (ISTAP)*. Yogyakarta, 23-25 September 2019 p, 2, 643-647.
- Swat M, I Rybicka & A Gliszczynska-Swigło (2019). Characterization of fulvic acid beverages by mineral profile and antioxidant capacity. *Foods* 8(12), 605. <https://doi.org/10.3390/foods8120605>.
- Swiech WM, I Hamerton, H Zeng, DJ Watson, E Mason, E Spencer & SE Taylor (2017). Water-based fractionation of a commercial humic acid. Solid-state and colloidal characterization of the solubility fractions. *J. Colloid Interfaces Sci.* 508, 28–38.
- Tan KH (2005). *Soil sampling, Preparation, and Analysis*. 2<sup>nd</sup> Ed. CRC Press, Taylor & Francis. Boca Rato, FL. USA. 623pp.
- Transparency Market Research (2020). Fulvic Acids Market – Global Industry Analysis 2015-

- 2019 and Opportunity Assessment 2020-2030. <https://transparencymarketresearch.com>. Downloaded November 10, 2020.
- USPTO (2014). Method for Producing Fulvic Acid. <https://patents.google.com>.
- Vadlamudi PBN, K Mahavishnan, DS Gurumurthy, H Bindumadhava, AP Upadhyay & NK Sharma (2014). Fulvic acid (FA) for enhanced nutrient uptake and growth: insights from biochemical and genomic studies. *J. Crop Improv.* 28(6), 740-757. <https://doi.org/10.1080/15427528.2014.923084>.
- Van Rensburg CEJ (2015). The anti-inflammatory properties of humic substances: a mini review. *J. Phytotherapy Res.* 29(6), 791-795. <https://doi.org/10.1002/ptr.5319>
- Van Rensburg CEJ (2016). Medicinal applications of fulvic acid. *3<sup>rd</sup> World Congress on Pharmacology*. Birmingham, 08-10 August 2016 p.
- Verma S, A Singh & A Mishra (2012). The effect of fulvic acid on pre- and postaggregation state of A $\beta$ (17-42): molecular dynamics simulation studies. *Biochim. Biophys. Acta.* 1834(1), 24-33. DOI: [10.1016/j.bbapap.2012.08.016](https://doi.org/10.1016/j.bbapap.2012.08.016) PMID: 22940640.
- Vucskits AV, I Hullar, A Bersenyl & E Andrasofszky (2010). Effect of fulvic and humic acids on performance, immune response and thyroid function in rats. *J. Anim. Physiol. Anim. Nutr.* 94(6), 721-8. DOI: [10.1111/j.1439-0396.2010.01023.x](https://doi.org/10.1111/j.1439-0396.2010.01023.x).
- Wali A, IB Salah, Z Mostapha, CB Abderrahim, K Youssef & M, Ksibi (2019). A novel humic acid extraction procedure from Tunisian lignite. *Euro-Mediterranean J. for Environ. Int.* 4(1), 1-9. DOI: [10.1007/s41207-019-0115-z](https://doi.org/10.1007/s41207-019-0115-z).
- WHO (2017). One Health. <https://www.who.int/news-room/q-a-detail/one-health>.
- Winkler J & S Ghosh (2018). Therapeutic potential of fulvic acid in chronic inflammatory diseases and diabetes. *J. Diabetes Res.*, 1-10. Doi: [10.1155/2018/539/5391014](https://doi.org/10.1155/2018/539/5391014).
- Yang S, L Cong, X Wang, Z Zhang & Z Zhang (2014). Fulvic acid displaces manure to improve soil in vegetable greenhouse. *Acta Agric. Scandinavica, Sect. B-Soil & Plant Sci.* 64(5), 454-461.
- Yang S, Z Zhang, L Cong, X Wang & S Shi (2013). Effect of fulvic acid on the phosphorus availability in acid soil. *J. Soil Sci. Plant Nutr.* 13(3), 526-533.
- Yao ZS, X Wei, J Lu, F Liu, Y Huang, J Xu, F Chen, Y Huang, Y Li, Y Lu & Z Zong (2010). Oxidation of shenfu coal with RuO<sub>4</sub> and NaOCl. *Energy Fuels* 24(3), 1801-1808. [10.1021/ef9012505](https://doi.org/10.1021/ef9012505).
- Yu X, J Yang, E Wang, B Li & H Yuan (2015). Effects of growth stage and fulvic acid on the diversity and dynamics of endophytic bacterial community in *Stevia rebaudiana* Bertoni leaves. *Front. Microbiol.* 6, 867. doi: [10.3389/fmicb.2015.00867](https://doi.org/10.3389/fmicb.2015.00867).
- Zhang X, Y Zhao, H Meng, L Li, H Cui, Z Wei, T Yang & Q Dang (2020). Revealing the inner dynamics of fulvic acid from different compost-amended soils through microbial and chemical analyses. *J. Agric. Food Chem.* 68(12), 3722-3728.
- Zhang XY, WJ Zhen & MG Sun (2017). Green preparation process of fulvic acid from Qitai weathered coal based on hydrothermal method. *Mod. Chem. Ind.* 37, 116-119.
- Zhang Y, G Gong, H Zheng, X Yuan & L Xu (2020). Synergistic extraction and characterization of fulvic acid by microwave and hydrogen peroxide-glacial acetic acid to oxidize low-rank lignite. *ACS Omega* 5(12), 6389-6394.