The Pharma Innovation Journal 2023; SP-12(7): 177-184

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(7): 177-184

www.thepharmajournal.com
Received: 18-04-2023
Accepted: 22-05-2023

**Gowthamchand NJ**
Department of Soil Science, University of Agricultural Sciences, Dharwad, Karnataka, India

**Sujaina M**
Department of Soil Science, University of Agricultural Sciences, Dharwad, Karnataka, India

**Corresponding Author:**
**Gowthamchand NJ**
Department of Soil Science, University of Agricultural Sciences, Dharwad, Karnataka, India

# A review on humic substances: Their significance in agriculture as stimulations of plant physiological growth and development

**Gowthamchand NJ and Sujaina M**

**Abstract**
Currently, agricultural production management approaches are greater emphasis on environmental sustainability. Humic substances (HSs) are the most abundant component of soil organic matter, formed through the breakdown of organic material through a series of biochemical processes. HSs with distinctive physiochemical and biochemical features, such as humic acid, fulvic acid, and humin, play an important role in crop physiological growth and development. The stimulatory effects of HSs on plant growth might be indirect (Through increased fertiliser efficiency or reduced soil compaction) or direct (through improvement of total plant biomass). The effect of HS on plant development is dependent on the source, concentration, and molecular weight of the humic fraction. The physiological effects of humic substances on plant development and metabolism are investigated. This study provides a summary of the research results on humic compounds diverse regulatory functions and their influence on plant physiological growth and development.

**Keywords:** Humic acid, fulvic acid, humin, plant nutrition, plant metabolism

## Introduction

Humic sustances (HSs) are naturally occurring, biogenic, heterogeneous organic molecules that are yellow to black in colour, have a large molecular weight, and are resistant (MacCarthy *et al.* 1990) [38]. HSs are more common organic fractions in earth, which may be found in soil, water, lake sediments, peat, brown coal, and shales. HSs account for around 25% of all organic carbon on the planet. These substances are a type of naturally occurring complex molecular structures that originate from the breakdown of plant and animal residues and cannot be categorised as proteins, polysaccharides, or polynucleotides (Nardi *et al.* 2002) [50]. The future of natural organic matter, particularly HSs, has piqued the curiosity of scientists from a variety of fields in recent decades. HSs research is complicated among the various organic substances that occur naturally because these organic compounds are bound by or associated with soil mineral fractions and require physical and/or chemical separation from the inorganic components via an extraction procedure prior to physico-chemical analysis. Many fractions of humic compounds have been separated and given specific names over the years. However, only three of them (Humic acid, fulvic acid, and humin) have stood the test of time as being generally beneficial. These fractions, like humic substances as a whole, are operationally defined (Aiken *et al.* 1986) [4]: Humic acid-A humic compound that is not soluble in water under acidic circumstances (pH 2) but is soluble at higher pH values. Fulvic acid is the portion of humic compounds that is soluble in water at all pH levels. Humin-A humic compound that is not soluble in water at any pH level.

Since the 1980s, the use of HS in crop production, which is comprised of humic (HA) and fulvic acid (FA), has been acknowledged as a long-run product (Calvo *et al.* 2014) [12]. The use of humic compounds in agriculture as fertiliser and soil conditioner is becoming increasingly significant for long-term crop development. Ihsanullah and Bakhashawin (2013) found that these humic compounds had a significant influence on soil structure and plant development. HSs are essential for soil fertility and plant nourishment (Omaima *et al.* 2014) [54]. Plants growing in soils rich in humin, humic acids (HAs), and fulvic acids (FAs) are less susceptible to stress, generate larger yields, and the nutritional content of produced crops and feeds is superior. The chemical makeup (e.g., functional groups), hydrophobicity, and flexible conformational shape of HS have all been linked to its role as a biological activator for plant development (Muscolo *et al.* 2013; Canellas and Olivares, 2017) [44, 14].

The favourable benefits of HS on plant development can be attributed to either indirect (Through increased fertiliser efficiency or reduced soil compaction) or direct (Through increased total plant biomass) effects.

One of the most significant effects of HS on plant development is the stimulation of nutrient absorption and the lengthening of lateral root growth, which is known as the "auxin-like effect," and is associated with incresed ATPase activity in the plasma membrane (Maggioni *et al.* 1987; Nardi *et al.* 1991; Pinton *et al.* 1992; Canellas *et al.* 2002; Quaggiotti *et al.* 2004; Zandonadi *et al.* 2007) [39, 49, 55, 16, 57, 73]. The basic mechanisms include ATPase induction providing a wider electrochemical gradient and speeding food uptake rate, which may also be validated by overexpression of transporter genes (Jindo *et al.* 2016; Zanin *et al.* 2018; Nunes *et al.* 2019) [30, 77, 53].With HSs, the availability of micronutrients such as iron (Fe) can be enhanced not just through chelation but also by improving the root's ability to absorb nutrients from the soil solution (Aguirre *et al.* 2009; Zanin *et al.* 2019) [3, 76]. This impact has been linked mostly to HS's complexing characteristics, which improve the availability of micronutrients from sparingly soluble hydroxides (Stevenson, 1991) [60]. Although only to a limited extent, HS, particularly those with a low molecular mass, are taken up by plants and, as a result, may actively influence plant metabolism (Vaughan and Malcom, 1985; Muscolo *et al.* 1993) [85, 43]. By functioning as hormone-like compounds, their actions appear to be primarily exerted on cell membrane activities, boosting nutrient absorption (Visser, 1986; Varanini and Pinton, 1995) [69, 67], or plant growth and development (Vaughan and Malcom, 1985; Nardi *et al.* 1996) [68, 48]. The use of humic compounds increased plant height, lateral shoot number per plant, leaf number per plant, stem diameter, leaf area, dry weight, and total leaf chlorophyll content, resulting in increased crop growth and development (Mustafa and El-Shazly, 2013; Eisa *et al*. 2016) [45, 16].

As agriculture grows more advanced and intensive agricultural systems evolve, the use of pesticides, decreased organic matter content, and poor physical conditions all have a negative impact on the availability of micronutrients. There need to understand the significance of humic compounds and their utility as fertiliser elements has never been greater than now. Those who are worried about soils' potential to support sustainable agricultural production. Most soil scientists and agronomists agree that humic compounds are the most significant component of a healthy fertile soil for crop development. The primary goal of this research is to discuss the importance of HSs on plant growth and development in boosting crop yield in modern agriculture.

## Review of literature

Humic substances have an indirect and direct effect on plant development. Positive relationships between soil humus levels, plant yields, and product quality have been reported in several scientific journals. Indirect impacts include those that offer energy to beneficial organisms in the soil, influence the soil's water holding capacity, influence the soil's structure, release of plant nutrients from soft minerals, enhanced availability of trace minerals, and overall improved soil fertility. Changes in plant metabolism that occur as a result of the absorption of organic macromolecules such as humic acids and fulvic acids are examples of direct impacts. Taking into account the benefits of HSs and their potential impact on plant growth and development is reviewed in the following headings.

### Influence of HSs on seed germination

Humic compounds and their structural features can have a major impact on seed germination and plant development in the early stages. The absorption of humic compounds into seeds promotes seed germination and seedling growth (Ayuso *et al.* 1996) [8]. According to Adetumbi *et al.* (2019) [2], priming rice seed with humic material can improve seedling growth and vigour in both highland and lowland rice cultivation. Fulvic acid had a substantial effect in promoting wheat seed germination. The effect of HSs on seed development due to regulating the amylase activity, which is related to respiration (Qin *et al.* 2016) [56]. Braziene *et al*. (2021) [11] made the following observation: The application of fulvic acids for seed dressing consistently increased the eventual germination percentage while shortening the mean germination time in spring wheat, spring barley, and sugar beet. When planting treated seeds in soil, pre-sowing treatment with humic preparations has a notable stimulating impact on germination (Shoba *et al.* 2019) [58]. Nandhini *et al* (2018) [46] revealed that soaking of seeds with humic acid records maximum seed germination, optimum shoot and root length of maize. Application rates of humic acids (HAs) or fulvic acids (FAs), required for improved seed germination, range from 20 to 100 mg/liter of seed. Humic compounds must be present within the cells of seeds in order for better germination to occur. As the humic substance enter the seed cells, respiration rate increases, and cell division processes are accelerated. These same respiratory processes enhance root meristem development and activate other growing points within the seedlings. In well-regulated tests, humic compounds have been shown to increase mitotic activity during cell division. The application of these humic compounds to seeds (seed treatment) or within the seed furrow improves seed germination and seedling development dramatically.

### Influence of HSs on root growth and development

Humic substances have a strong impact on plant root development. When humic acids (HAs) and/or fulvic acids (FAs) are added to soil, root initiation and growth are improved. Humic acid, according to Mora *et al.* (2012) [42], increased the quantity of secondary roots as well as root growth in cucumber. Humic substance application to roots enhances plasma membrane H+-ATPase activity, shoot mineral nutrient concentration, and ABA concentrations in roots (Nunes *et al.* 2019) [53]. Plants treated with HA had an increase in the quantity, diameter, and length of maize roots. This total improvement might be attributed to the activation of PM H+-ATPase, which is critical in ion absorption and the development of an electrochemical gradient, both of which are required in the growth process under acidic circumstances (De Hita *et al.* 2020) [21]. This enzyme combines ATP hydrolysis with H+ transport across the cell membrane, acidifying the apoplasts, loosening the cell walls, and lengthening cells, resulting in increased root growth (Hager *et al.* 1991) [28]. Plant root development is promoted to a larger extent in most experimental experiments than above ground plant components. The sort of humic material used had a substantial impact on the percentage of increase. In repeated trials, treated root weights ranged from 20 to 50 percent higher than non-treated root weights. When the smaller molecular components of fulvic acid (FA) are present at concentrations ranging from 10 to 100 mg/liter of solution, root stimulation occurs. Fulvic acids (FAs) boost growth even more when combined with humic acids (HAs) and other essential plant nutrients. Humic acids (HAs) and fulvic acids (FAs) should be used at low doses to promote plant development.

**Humic Substances and their role in plant nutrition**

The presence of HS in soil encourages root and shoot development by improving mineral nutrition under the soil surface. The activity of these compounds can be measured in terms of plant yield and active growth (Zandonadi *et al.* 2016) [75]. HS regulate plant growth and mineral absorption through complimentary and possibly different activities. These impacts are categorized as either direct or indirect (Vaughan and Malcom, 1985; Zandonadi *et al.* 2013) [68, 74]. The activities of HS are primarily determined by their structural properties, functional groups, and tendency to interact with inorganic and organic ions and molecules found in the soil substrate (Garcia-Mina *et al.* 2004) [27]. Furthermore, the ability of HS to form complexes with metallic ions improves the availability of micronutrients (zinc, manganese, copper, and iron) and macronutrients (phosphorus), particularly when these nutrients are scarce in the soil (Garcia *et al.* 2016). Humic substances bind nutrients in a molecular form, reducing their solubility in water. These binding processes reduce nitrogen leaching into the subsoil and aid in preventing volatilization into the atmosphere. Other studies have found that increased uptake of calcium (Ca), and magnesium (Mg) when plants are irrigated with humic acids (HAs) or fulvic acids (FAs). Direct action of HS, on the other hand, is associated with their localised targeted and non-targeted actions at plant cell membranes, which can activate biochemical and molecular processes at the post-transcriptional levels in roots and shoots (Van Oosten *et al.* 2017) [66]. In general, targeted actions of HS tend to increase plant absorption of macronutrients and micronutrients. Only a small percentage of 14-C labelled HS, particularly those with lower molecular weight, penetrate the root apoplastic pathway, according to Vaughan and Malcom (1985) [68]. As a result, this property can regulate the activity of HS in increasing nutrient absorption through molecular systems and signalling pathways found in root cell membranes (Asli and Neumann, 2010; Nardi *et al.* 2016) [7, 51]. The full benefit of this type of direct influence has yet to be determined. However, it is possible that the non-specific action of HS on the leaf or root surface could influence molecular and biochemical processes by influencing events at both the transcriptional and post-transcriptional stages.

**Humic Substances on plant metabolic activities**

A significant effect of HS activity in plants is due to its ability to stimulate numerous metabolic pathways. Research into the role of HS in photosynthesis control is critical because photosynthesis is the fundamental metabolic process that drives the generation of all $O_2$ and organic matter on the earth. FA stimulated more chlorophyllase (a) activity than HA, whereas HA stimulated more chlorophyllase (b) activity than FA (Yang *et al*. 2004) [70]. In maize plants (*Zea mays* L.), HA fractions from the soil were found to increase the activity of enzymes involved in C metabolism. They boosted the activity of glycolytic pathway enzymes like glucokinase (GK), phosphoglucoisomerase (PGI), PPidependent phosphofructokinase (PFK) and pyruvate kinase (PK)) as well as Krebs cycle enzymes like citrate synthase (CS), malate dehydrogenase (MDH), and isocitrate and NADP + - isocitrate dehydrogenas (Nardi *et al*. 2007) [47]. Humic molecules cause biochemical changes in plant cell membranes and numerous cytoplasmic components when they penetrate plant cells. The presence of humic substances is essential for the establishment of biotic and abiotic interactions within the plant rhizosphere (Kulikova *et al.* 2014, 2016) [34, 33]. Many enzymes have been identified that participate in various plant responses to HS, such as Fe (III) chelate-reductase (Kulikova *et al.* 2014) [34], H+-pyrophosphatase (Zancani *et al.* 2009) [72], plasma membrane H+-ATPase (Quaggiotti *et al.* 2004) [57], tricarboxylic acid cycle enzymes (Kulikova *et al.* 2016) [33]. It is concluded that HS have a wide range of regulatory activities in plants, including direct root growth stimulation and root hair proliferation, ion-uptake rate maintenance, proton release control, redox reactions, and root exudate modulation (Trevisan *et al.* 2010; Canellas *et al.* 2015) [65, 15].

**Influence of Humic substances on plant growth and development**

The effect of HS on plant growth is affected by the source, concentration, and molecular weight of the humic fraction. While a low molecular size (LMS 3500 Da) fraction easily reaches the plasmalemma of higher plant cells and is partially absorbed, a high molecular size (HMS > 3500 Da) fraction is not absorbed and can only interact with the cell wall. As a result, an LMS fraction is the most likely choice for assessing the beneficial effects of HS on plant development. The latter effects are mediated in part at the plasma membrane level by positively impacting the absorption of certain nutrients, particularly nitrate (Nardi *et al.* 2002) [50]. Mindari *et al.* (2018) [41] found that using HSs, boosted crop yield by improving plant growth in terms of shoot length, root length, shoot dry weight, root dry weight, and chlorophyll content. Humic acids generated from vermicompost increased the growth of marigold and pepper roots, as well as the quantity and weight of strawberry fruits and tomato fruits (Arancon *et al.* 2006) [6]. These might be due to humic compounds (humic acid) bind positive ions, form chelates with micronutrients, and slowly release them when needed by plants. They also operate as chelating agents, preventing precipitation, fixing, leaching, and oxidation of micronutrients in soil (Kadam *et al*. 2010) [31]. Humic compounds (humic acid and fulvic acid) with auxin activity boost nutrient absorption and dry matter production by inducing a hormonal influence on catalytic activity and cell permeability, there by it also improved the development and nutritional quality young plants (Eshwar *et al.* 2017) [25]. Furthermore, humic acid has many effects because it increases cation exchange capacity, which affects nutrient retention and availability, as well as due to a hormonal effect, or a combination of both (Chunhua *et al*. 1998) [19]; as a result, it can be used to solve many soil problems, such as soil nutrient availability and chemical reactions that affect the loss or fixation of almost all nutrients (Abourayya *et al.* 2020) [1]. Regulating activities of humic substances in different crops and its effect on plant growth and development is represented in Table 1 and Figure 1.

Foliar spray of humic compounds to young actively growing leaves enhance plant growth more than foliar applications to older plant leaves. Actively growing plant parts that are involved in cell divisions and other growth processes easily integrate various trace minerals and growth regulating compounds into on-going metabolic processes, whereas older plant parts that have slowed metabolic processes are unable to efficiently utilise added humic substances and associated nutrients. Suh *et al*. (2014) [62] observed that foliar application of fulvic acid affects tomato plant growth, fruit quality, and yield. Foliar treatment promotes crop productivity in areas such as olives, wheat, tomato, papaya, maize, creeping bent grass, and others (Cooper *et al*. 1998; Delfine *et al*. 2005; Zaller 2006; Cavalcante *et al*. 2011; Canellas *et al*. 2013) [20, 22, 71, 18, 13]. Humic and fulvic acids (HAs and FAs) are effective foliar fertiliser transporters and activators. It increases plant development processes within the leaves, resulting in a rise in the carbohydrates content of the leaves and stems. Enhanced carbohydrate synthesis can result in either higher product quality or higher yields. Increased vegetative growth can be attributed to the positive effect of humic substances on both plants and soil in increasing microbial activity and improving soil effectiveness in nutrient uptake as a chelating agent, as well as bio-stimulation of plant growth, which improves vegetative characteristics, nutritional status, and crop yield (Abourayya *et al*. 2020) [1].

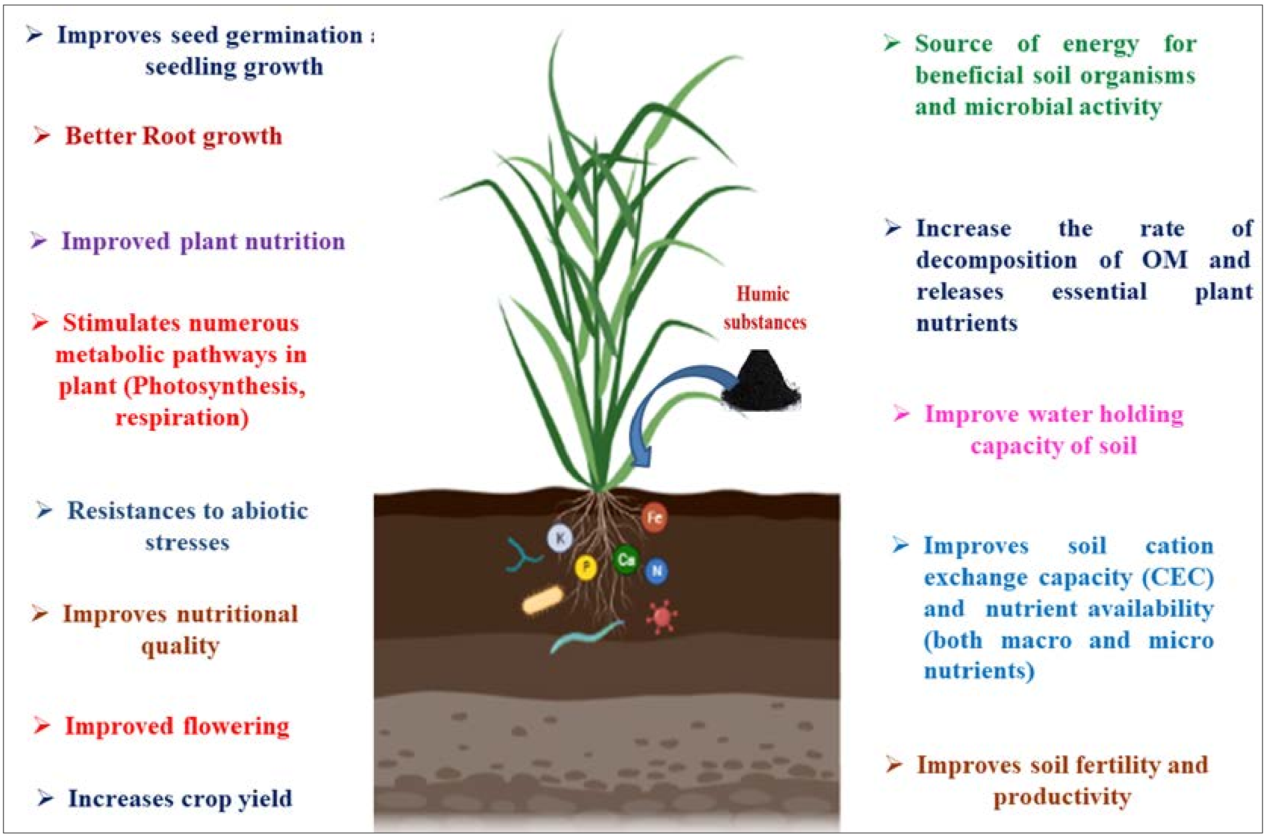


**Fig 1:** Effect of Humic substances on plant growth and development

**Table 1:** Regulating activities of humic substances in different crops.

| Parameter | Mechanism of Humic substances | Crop | References |
|---|---|---|---|
| Seed germination and seedling growth | As the humic material enters the seed cells, the rate of respiration rises and cell division processes speed up. These same respiratory mechanisms promote root meristem growth and the activation of additional growing sites in seedlings. | Spring-wheat, barley and sugar beet | Braziene *et al.* 2021 [11] |
| | | Papaya | Cavalcante *et al.* 2011 [18] |
| | | Wheat | Qin *et al.* 2016 [56]; Litvin *et al.* 2020 [36] |
| | | Rice | Adetumbi *et al.* 2019 [2] |
| | | Maize | Nandhini *et al.* 2018; [46] |
| Root growth | Humic substance application to roots enhances plasma membrane H+-ATPase activity, shoot mineral nutrient concentration, and ABA concentrations in roots. Plants treated with HA had an increase in the quantity, diameter, and length of maize roots | Cucumber | Mora *et al.* 2012 [42] |
| | | Maize | Nardi *et al.* 2016 [51]; Nunes *et al.* 2019 [53] |
| | | Broad bean | Tamer *et al.* 2015 [63] |
| | | Gerbera | Nikbhakt *et al.* 2008 [52] |
| | | Wheat | Ali *et al.* 2014 [5] |
| Plant nutrition | The presence of HS in soil promotes root and shoot development by enhancing mineral nutrition under the soil surface | Wheat | Bezuglova *et al.* 2017 [10] |
| | | Rice | Mindari *et al.* 2019 [40] |
| | | Peanut | Li *et al.* 2019 [35] |
| | | Maize | Khaled and Fawy, 2011 [32] |
| | | Apple | Cansu and Erdal *et al.* 2016 [17] |
| Plant metabolism | HSs stimulates numerous metabolic pathways in plants, namely photosynthesis and respiration etc., | Maize | Nardi *et al.* 2007 [47] |
| | | Bent grass | Chunhua *et al.* 1998 [19] |
| | | Chlorella | Toropkina *et al.*2017 [64] |

| | | | |
|---|---|---|---|
| | | Wheat | El-Bassiouny *et al.* 2014 [24] |
| | | Rapeseed | Lotfi *et al.* 2018 [37] |
| Growth and development | Increased vegetative growth attributed to increasing microbial activity and improving soil effectiveness in nutrient uptake as a chelating agent, as well as bio-stimulation of plant growth, resulting in a rise in the carbohydrates content of the leaves and stems. Enhanced carbohydrate synthesis can result in either higher product quality or higher yields | Wheat | Baris *et al.* 2009 [9] |
| | | Strawberry | Arancon *et al.* 2006 [6] |
| | | maize | Canellas *et al.* 2013 [13] |
| | | Tomato | Suh *et al.* 2014 [62]<br>Arancon *et al.* 2006 [6]<br>Suarez-Estrella *et al.* 2008 [61] |
| | | Rice | Sivakumar *et al.* 2007 [59] |

**Conclusion**

The use of HS as a bio-stimulant for plant growth is a helpful and environmentally sustainable method. Plant morphological and biochemical changes in the root system caused by HS are the primary variables responsible for increased nutrient absorption, however an increase in nutrient availability caused by chelation and partially by auxin-like effects is another way HS contributes to plant development. HS may have a favourable effect on higher plant metabolism, resulting in increased cell division and plant development. LMS humic fractions appear to do this job more easily because they can penetrate the plasma membrane of root cells and subsequently be translocated. As a result, the use of HS not only promotes plant growth and development but also improves soil conditions and contributes to sustainable agricultural production Author.

**References**


1. Abourayya MS, Kaseem NE, Mahmoud, TSM, Rakha AM, Eisa RA, Amin OA. Impact of soil application with humic acid and foliar spray of milagro bio-stimulant on vegetative growth and mineral nutrient uptake of Nonpareil almond young trees under Nubaria conditions. Bulletin of the National Research Centre; c2020, 44(1). https:// DOI.org/10.1186/s42269-020-00296-x.
2. Adetumbi JA, Orimadegun IO, Akinyosoye ST, Akintayo OT, Agbeleye OA. Enhancing Planting Value of Rice Seed through Priming with Humic Substance. Journal of Experimental Agriculture International. 2009;29(6):1-8. https:// DOI.org/10.9734/jeai/2019/46194.
3. Aguirre E, Diane L, Eva B, Marta F, Roberto B, Zamarreño AM. The root application of a purified leonardite humic acid modifies the transcriptional regulation of the main physiological root responses to Fe deficiency in Fe-sufficient cucumber plants. Plant Physiology and Biochemistry. 2008;47:215-223. DOI: 10.1016/j.plaphy.2008.11.013.
4. Aiken GR, Mcknight DM, Wershaw RL, Maccarthy P. Humic substances in soil, sediment, and water. Soil Science. 1986;142(5):323.
5. Ali H, Akbar, Razaq A, Muhammed D. Effect of humic acid on root elongation and percent seed germination of wheat seeds. International Journal of Agriculture and Crop Sciences. 2014;7(4):196-201.
6. Arancon NQ, Edwards CA, Lee S, Byrne R. Effects of humic acids from vermicomposts on plant growth. European Journal of Soil Biology; c2006. https:// DOI.org/10.1016/J.EJSOBI.2006.06.004
7. Asli S, Neumann PM. Rhizosphere humic acid interacts with root cell walls to reduce hydraulic conductivity and plant development. Plant Soil. 2010;336:313-322. DOI: 10.1007/s11104-010-0483-2.
8. Ayuso M, Hernandez T, Garcia C, Pascual JA. Stimulation of barley growth and nutrient absorption by humic substances originating from various organic materials. Bioresource Technology. 1996;57(3):251-257.
9. Baris BA, Murat AT, Hakan C, Ali VK. Effects of Humic Sustances on plant growth and mineral nutrients uptake of wheat (Triticum durum cv. Salihi) under conditions of salinity. Asian Journal of Crop Science. 2009;1(2):87-95.
10. Bezuglova OS, Polienko EA, Gorovtsov AV, Yhman VAL, Pavlov PD. The effect ofhumic substances on winter wheat yield and fertility of ordinary chernozem. Annals of Agrarian Science. 2017;15:239-242.
11. Braziene Z, Paltanavicius V, Avizienytė D. The influence of fulvic acid on spring cereals and sugar beets seed germination and plant productivity. Environmental Research. 2021;195:1-5. https:// DOI.org/10.1016/j.envres.2021.110824.
12. Calvo P, Nelson L, Kloepper JW. Agricultural uses of plant biostimulants. Plant and soil. 2014;383(1):3-41.
13. Canellas LP, Martinez-Balmori D, Médici LO, Aguiar NO, Campostrini E, Rosa RCC, *et al.* A combination of humic substances and Herbaspirillum seropedicae inoculation enhances the growth of maize (*Zea mays* L.). Plant Soil. 2013;366:119-132. DOI:10.1007/s11104-012-1382-5.
14. Canellas LP, Olivares FL. Production of border cells and colonization of maize root tips by Herbaspirillum seropedicae are modulated by humic acid. Plant and Soil. 2017;417(1):403-413.
15. Canellas LP, Olivares FL, Aguiar NO, Jones DL, Nebbioso A, Mazzei P. Humic and fulvic acids as biostimulants in horticulture. Scientia Horticulturae. 2015;196:15-27. DOI: 10.1016/j.scienta.2015.09.013.
16. Canellas LP, Olivares FL, Okorokova-Façanha AL, Façanha AR. Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H+-ATPase activity in maize roots. Plant Physiology. 2002;130:1951-1957. DOI: 10.1104/pp.007088.
17. Cansu M, Erdal I. Effect of humic substance applications on mineral nutrition and yield of granny smith and jersey Mac apple varieties. Journal of Agricultural Sciences. 2016;24:162-169.
18. Cavalcante IHL, Silva RRS DA, Albano FG, Lima FN, Marques AS. Foliar spray of humic substances on seedling production of papaya. Journal of Agronomy. 2011;10(4):118-122.
19. Chunhua L, Cooper RJ, Bowman DC. Humic acid application affects photosynthesis, root development and nutrient content of creeping bentgrass. Horticultural Science. 1998;33:1023-1025.
20. Cooper RJ, Liu C, Fisher DS. Influence of humic substances on rooting and nutrient content of creeping bentgrass. Crop Science. 1998;38(6):1639-1644.

DOI:10.2135/cropsci1998.0011183X003800060037x.

21. De Hita D, Fuentes M, Fernández V, Zamarreño AM, Olaetxea M, García-Mina JM. Discriminating the short-term action of root and foliar application of humic acids on plant growth: Emerging role of jasmonic acid. Frontiers in Plant Science. 2020;11:1-14. https:// DOI.org/10.3389/fpls.2020.00493.
22. Delfine S, Tognetti R, Desiderio R, Alvino A. Effect of foliar application of N and humic acids on growth and yield of durum wheat. Agronomy for Sustainable Development. 2005;25:183-191. DOI:10.1051/agro:2005017.
23. Eisa RA, Thanaa SM, Nabila EK, Abou Rayya MS. Foliar application of low-biuret urea and humic acid influences on the growth and leaf mineral composition of Nonpareil almond young trees s under South Sinai conditions. Journal of Innovations in Pharmaceuticals and Biological Sciences. 2016;3:143-153.
24. El-Bassiouny HSM, Bakry BA, Attia AAE, Allah MMA. Phsiological role of humic acid and nicotinamide on improving plant growth, yield and mineral nutrient of wheat (Triticum durum) grown under newly reclaimed sandy soil, Agricultural Sciences. 2014;5(8):687-700.
25. Eshwar M, Srilatha M, Bhanu Rekha K, Harish Kumar Sharma S, Eshwar CM. Effect of humic substances (Humic, Fulvic acid) and chemical fertilizers on nutrient uptake, dry matter production of aerobic rice (*Oryza sativa* L.). Journal of Pharmacognosy and Phytochemistry. 2017;6(65):1063-1066.
26. García AC, Santos LA, de Souza LGA, Tavares OCH, Zonta E, Gomes ETM. Vermicompost humic acids modulate the accumulation and metabolism of ROS in rice plants. Journal of Plant Physiology. 2016;192:56-63. DOI: 10.1155/2016/3747501.
27. Garcia-Mina JM, Antolin MC, Sanchez-Diaz M. Metal-humic complexes and plant micronutrient uptake: a study based on different plant species cultivated in diverse soil types. Plant and Soil. 2004;258(1):57-68.
28. Hager A, Debus G, Edel HG, Stransky H, Serrano R. Auxin induces exocytosis and the rapid synthesis of a high turnover pool of plasma-membrane H+-ATPase. Planta. 1991;185:527-537.
29. Ihsanullah D, Bakhashwain AA. Effect of humic acid on growth and quality of maize fodder production. Pakistan Journal of Botany. D. L Sparks. Environmental Soil Chemistry Second Edition. (Academic Press, USA, 2003). 2013;45:21-25.
30. Jindo K, Soares TS, Peres LEP, Azevedo IG, Aguiar NO, Mazzei P. Phosphorus speciation and high-affinity transporters are influenced by humic substances. Journal of plant nutrition and soil science. 2016;179:206-214. DOI: 10.1002/jpln.201500228.
31. Kadam SR, Amrutsagar VM, Deshpande AN. Influence of organic nitrogen sources with fulvic acid spray on yield and nutrient uptake of soyabean on inceptisol. Journal of Soils and Crops. 2010;20(1):58-63.
32. Khaled H, Fawy HA. Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. Soil Water Resources. 2011;6:21-29.
33. Kulikova NA, Abroskin DP, Badun GA, Chernysheva MJ, Korobkov VI, Beer AS. Label distribution in tissues of wheat seedlings cultivated with tritium-labeled leonardite humic acid. Scientific Reports. 2016;6:28869. DOI: 10.1038/srep28869.
34. Kulikova NA, Badun GA, Korobkov VI, Chernysheva MG, Tsvetkova EA, Abroskin DP. Accumulation of coal humic acids by wheat seedlings: direct evidence using tritium autoradiography and occurrence in lipid fraction. Journal of Plant Nutrition and Soil science. 2014;177;875-883. DOI: 10.1002/jpln.201300648.
35. Li Y, Fang F, Wei J, Wu X, Cui R, Li G, *et al*. Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut:a three-year experiment. Scientific Reports. 2019;9:12014. https:// DOI.org/10.1038/s41598-019-48620-4.
36. Litvin, VA, Deriy SI, Plakhotniuk LN, Abi NR. Effects of humic substances on seed germination of wheat under the influence of heavy metal. Cherkasy University Bulletin: Biological Sciences Series. 2020;1:42-52.
37. Lotfi R, Kalaji HM, Valizadeh GR. Effects of humic acid on photosynthetic efficiency of rapeseed plants growing under different watering conditions. Photosynthetica. 2018;56:962-970. https:// DOI.org/10.1007/s11099-017-0745-9.
38. MacCarthy P, Malcolm, RL, Clapp CE, Bloom PR. An introduction to soil humic substances. Humic substances in soil and crop sciences*:* Selected readings; c1990. p. 1-12.
39. Maggioni A, Varanini V, Nardi S, Pinton R. Action of soil humic matter on plant roots: stimulation of ion uptake and effects on ($Mg^{2+}$+$K^{+}$) ATPase activity. Sci. Total Environ. 1987;64:334-336. DOI: 10.1016/0048-9697(87)90257-90259.
40. Mindari W, Sasongko, Kusuma Z, Syekhfani, Aini N. Efficiency of various sources and doses of humic acid on physical and chemical properties of saline soil and growth and yield of rice, AIP Conference Proceedings; c2019. https:// DOI.org/10.1063/1.5061854.
41. Mindari W, Sasongko PE, Kusuma Z, Syekhfani, Aini N. Efficiency of various sources and doses of humic acid on physical and chemical properties of saline soil and growth and yield of rice. AIP Conference Proceedings; c2018. https:// DOI.org/10.1063/1.5061854.
42. Mora V, Baigorri R, Bacaicoa E, Zamarreño AM, García-Mina JM. The humic acid-induced changes in the root concentration of nitric oxide, IAA and ethylene do not explain the changes in root architecture caused by humic acid in cucumber. Environmental and Experimental Botany. 2012;76:24-32. DOI: 10.1016/j.envexpbot.2011.10.001.
43. Muscolo A, Felici M, Concheri G, Nardi S. Effect of earthworm humic substances on esterase and peroxidase activity during growth of leaf explants of Nicotiana plumbaginifolia. Biology and Fertility of Soils. 1993;15:127-131.
44. Muscolo A, Sidari M, Nardi S. Humic substance: relationship between structure and activity. Deeper information suggests univocal findings. Journal of geochemistry exploration. 2013;129:57-63. DOI: 10.1016/j.gexplo.2012.10.012.
45. Mustafa NS, El-Shazly SM. Impact of some biostimulant substances on growth parameters of Washington Navel orange trees. Middle East Journal of Applied Science. 2013;3:156-160.
46. Nandhini RS, Shelishiyah R, Prakash P. Effect of humic acid on seed germination of *Zea mays*. Indian Journal of Environmental Protection. 2018;38(10):862-866.

47. Nardi S, Muscolo A, Vaccaroa S, Baiano S, Spaccini R, Piccolo A. Relationship between molecular characteristics of soil humic fractions and glycolytic pathway and krebs cycle in maize seedlings. Soil Biology and Biochemistry. 2007;39:3138-3146.
48. Nardi S, Concheri G, Dell AG. Biological activity of humic substances. In: piccolo, a, (ed.), humic substances in terrestrial ecosystems, Elsevier, Amsterdam; c1996. p. 361-406.
49. Nardi S, Concheri G, Dell Agnola G, Scrimin P. Nitrate uptake and ATPase activity in oat seedlings in the presence of two humic fractions. Soil Biology and Biochemistry. 1991;23:833-836. DOI: 10.1016/0038-0717(91)90094-Z.
50. Nardi S, Pizzeghello D, Muscolo A, Vianello A. Physiological effects of humic substances on higher plants. Soil Biology and Biochemistry. 2002;34(11):1527-1536. https:// DOI.org/10.1016/S0038-0717(02)00174-8.
51. Nardi S, Pizzeghello D, Schiavon M, Ertani A. Plant biostimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. *Scientia Agricola*. 2016;73:18-23. DOI: 10.1590/0103- 9016-2015-0006.
52. Nikbhakt A, Kafi M, Babalar M, Xia YP, Luo A, Etemadi NA. Effect of Humic Acid on Plant Growth, Nutrient Uptake and Postharvest Life of Gerbera. Journal of plant nutrition. 2008;31:2155-2167.
53. Nunes RO, Domiciano GA, Alves WS, Melo ACA, Nogueira FCS, Canellas LP, *et al*. Evaluation of the effects of humic acids on maize root architecture by label-free proteomics analysis. Scientific Reports. 2019;9(1):1-11. https:// DOI.org/10.1038/s41598-019-48509-2.
54. Omaima MH, Malaka A Saleh, Mostafa EAM, El-Shamma MS, Maksoudd MA. Improving pollination efficiency, yield and fruit quality of two date palm cultivars using growth activator. International Journal of Agricultural Research. 2014;9:29-37.
55. Pinton R, Varanini Z, Vizzotto G, Maggioni A. Soil humic substances affect transport properties of tonoplast vesicles isolated from oat roots. Plant Soil. 1992;142:203-210. DOI: 10.1007/BF00010966.
56. Qin Y, Zhu H, Zhang M, Zhang H, Xiang CB. GC-MS analysis of membrane-graded fulvic acid and its activity on promoting wheat seed germination. Molecules; c2016, 21(10). https:// DOI.org/10.3390/molecules21101363.
57. Quaggiotti S, Ruperti B, Pizzeghello D, Francioso O, Tugnoli V, Nardi S. Effect of low molecular size humic substances on nitrate uptake and expression of genes involved in nitrate transport in maize (*Zea mays* L.). Journal of Experimental Botany. 2004;55:803-813. DOI: 10.1093/jxb/erh085.
58. Shoba SA, Salimgareeva OA, Gorepekin IV, Fedotov GN, Stepanov AL. Stimulation of seed germination by humic substances: on the nature of the phenomenon. Доклады Академии Наук. 2019;487(3):342-345. https:// DOI.org/10.31857/S0869-56524873342-345.
59. Sivakumar KL. Devarajan K, Dhanasekaran D, Venkatakrishnan, Surendran U. Effect of humic acid on the yield and nutrient uptake of rice, Oryza. 2007;44(3):277-279.
60. Stevenson FJ. Organic matter—micronutrient reactions in soil. In: Mortvedt, J.J, Cox, F.R, Shuman, L.M, Welch, R.M. (Eds.), Micronutrients in Agriculture, Soil Science Society of America, Madison; c1991. p. 145-186.
61. Suarez-Estrella F, Vargas-Garcia MC, Lopez MJ, Moreno J. Effect of humic substances extracted from compost to plant growth and soil microorganisms, Dynamic Soil, Dynamic Plant. 2008;2(1):96-102.
62. Suh HY, Yoo KS, Suh SG. Effect of foliar application of fulvic acid on plant growth and fruit quality of tomato (*Lycopersicon esculentum* L.). Horticulture, Environment, and Biotechnology. 2014;55(6):455-461.
63. Tamer B, Şener A, Ahmet EE. Effects of Humic Acid on Root Development and Nutrient Uptake of Vicia faba L. (Broad Bean) Seedlings Grown under Aluminum Toxicity, Communications in Soil Science and Plant Analysis. 2015; 46:277-292.
64. Toropkina MA, Ryumin AG, Kechaikina IO, Chukov SN. Effect of humic acids on the metabolism of *Chlorella vulgaris* in a model experiment. Eurasian Soil Science. 2017;50:1294-1300. https:// DOI.org/10.1134/S1064229317110126.
65. Trevisan S, Francioso O, Quaggiotti S, Nardi S. Humic substances biological activity at the plant-soil interface: from environmental aspects to molecular factors. Plant Signal and Behaviour. 2010;5:635-643. DOI: 10.4161/psb.5.6. 11211.
66. Van OMJ, Pepe O, De PS, Silletti S, Maggio A. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chemical and Biological Technologies in Agriculture. 2017;4:5. DOI: 10.1186/s40538-017-0089-5.
67. Varanini Z, Pinton R. Humic substances and plant nutrition. In: Lu¨ttge, U, (Ed.), Progress in Botany, vol. 56. Springer, Berlin; c1995. p. 97-117.
68. Vaughan D, Malcom RE. Influence of humic substances on growth and physiological processes. In: Vaughan, D, Malcom, R.E. (Eds.), Soil Organic Matter and Biological Activity, Martinus Nijhoff/ Junk W, Dordrecht, The Netherlands; c1985. p. 37-76.
69. Visser SA. Effetto delle sostanze umiche sulla crescita delle piante. In: Burns, R.G, Dell'Agnola, G, Miele, S, Nardi, S, Savoini, G, Schnitzer, M, Sequi, P, Vaughan, D, Visser, S.A. (Eds.), Sostanze Umiche. Effetti sul Terreno e sulle Piante, Ramo Editoriale degli Agricoltori, Roma; c1986. p. 96-143.
70. Yang C, Wang M, Lu Y, Chang I, Chou C. Humic substances affect the activity of chlorophyllase. Journal of Chemical Ecology. 2004;8:1561-1573.
71. Zaller JG. Foliar spraying of vermicornpost extracts: effects on fruit quality and indications of late-blight suppression of field-grown tomatoes. Biological Agriculture & Horticulture. 2006;24(2):160-185. DOI:10.1080/01448765. 2006.9755017.
72. Zancani M, Petrussa E, Krajňáková J, Casolo V, Spaccini R, Piccolo A. Effect of humic acids on phosphate level and energetic metabolism of tobacco BY-2 suspension cell cultures. Environmental and experimental botany. 2009;65:287-295. DOI: 10.1016/j.envexpbot.2008.09.012.
73. Zandonadi DB, Canellas LP, Façanha AR. Indolacetic and humic acids induce lateral root development through a concerted plasmalemma and tonoplast H+ pumps activation. Planta. 2007;225:1583-1595. DOI: 10.1007/s00425-006-0454-452.
74. Zandonadi DB, Santos MP, Busato JG, Peres LEP,

Façanha AR. Plant physiology as affected by humified organic matter. Theoretical and experimental plant physiology. 2013;25:13-25. DOI: 10.1590/S2197-00252013000100003.

75. Zandonadi DB, Santos MP, Caixeta LS, Marinho EB, Peres LEP, Façanha AR. Plant proton pumps as markers of biostimulant action. *Scientia agricola*. 2016;73:24-28.
76. Zanin L, Tomasi N, Cesco S, Varanini Z, Pinton R. Humic substances contribute to plant iron nutrition acting as chelators and biostimulants. Frontiers in Plant Science. 2019;10:675. DOI: 10.3389/fpls.2019.00675.
77. Zanin L, Tomasi N, Zamboni A, Sega D, Varanini Z, Pinton R. Water-extractable humic substances speed up transcriptional response of maize roots to nitrate. Environmental and experimental botany. 2018;147:167-178. DOI: 10.1016/j.envexpbot.2017.12.014.