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Role of humic acid or humic substances in agriculture: A Review

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Abstract
Humic material is very large and complex molecules extracted from organic matter, have been used in many ways for plant and seed production. There are numerous reports of plant response to these materials. This reviews their use in agriculture and points to consider before using humic materials. Since the beginning of Human civilization, the soil organic matter has been used as plant growth promoter or regulator. Indeed, early in plant science history, even before the auxin concept has been established, the term "auximones" was coined to describe plant growth promoting humic acids derived from peat. Despite of this, until the end of the 20th century, humic substances remained as some of the most neglected environment signals in plant physiology research. However, this scenario has changed in last decade with the discovery that the major systems of energy transduction of the plant cell membranes, the proton pumps, can be tightly orchestrated by humic substances just as elicited by a hormonal signaling. Differential activations of both plasma membrane (PM H+-ATPase) and vacuolar pumps (V-ATPase and H+-PPase) are modulated by humic substances triggering ion signatures related to specific patterns of plant growth and development. Phytohormones have been found to be associated with this humus bioactivity. The key role of organic matter in the sustainable agriculture will also be highlighted from a biochemical perspective of the plant cell responses to biofertilization, specially in tropical environments.

Keywords: environments, bio fertilization, vacuolar pumps, plasma membrane

Introduction

Plants grown on soils which contain adequate humin, humic acid (HA), and fulvic acid (FA) are less subject to stress, are healthier, produce higher yields and the nutritional quality of harvested foods and feeds are superior. The value of humic substances in soil fertility and plant nutrition relates to the many functions these complex organic compounds perform as a part of the life cycle on earth. The life death cycle involves a recycling of the carbon containing structural components of plants and animals through the soil and air and back into the living plant. Humates are an accessible raw material that can be used in agriculture. Humates are a part of soil humus and play an important role in the living organism. Modern farming practices have stripped the soil of its humus and no replacements have been used. Humic substances have not been appreciated and as a result not addressed in fertilizing practices. Today, our soils are low on these humic substances and as a result, animals and man are not receiving adequate amounts in their normal diet. Research has indicated that when soil humus percentages fall below 2%, the soil cannot provide sufficient quantities of humic materials into the crops grown for the amounts needed by the living organism.

Effect of humic acid on plant growth and yield

Enhancement of photosynthesis, chlorophyll density and plant root respiration had resulted in greater plant growth with humate application (Sladky, 1959 and Smidova, 1960) [68]. David *et al.,* (1994) [26] studied the effect of humic acid on nutrient accumulation and growth of tomato seedling in a solution of limited nutrient availability in a greenhouse. Humic acid were added to the nutrient solution in a rates of 640, 1280 or 2560 mg / l. Results showed that addition of 1280 mg / l humic acid produced significant increases in shoots accumulation of P, K, Ca, Mg, Fe, Mn and Zn as well as increased accumulation of N, Ca, Cu and Zn in roots. Padem *et al.,* (1997) [55] studied the effect of humic acid application as foliar fertilizer on seedling quality and nutrient content of eggplant and pepper. They found that seedling stem diameter, number of leaves, shoot and dry matter were significantly increased by foliar application in both vegetables. El-Desuki (2004) mentioned that growth (number and fresh weight of leaves) and yield of onion were gradually and significantly increased with increasing the level of humic acid application from 0 to 6 l /fed.

Zaky *et al.,* (2006) [75] studied the effect of humic acid on growth and productivity of common bean plants. They found that the number of shoots / plant, leaf area, total yield, average pod fresh weight and P content were increased by application of humic acid as foliar fertilizer at rate of 1g /l. Abd El-Mawgoud *et al.* (2007) [1] studied the effect of GrowthvPlex SP (a water soluble fertilizer with humic acid) in rates of 0, 60, 90 or 120 g/100 l of water on tomato plants fertilized with different rates of chemical fertilizer of NPK (0, 50, 75 or 100% of recommend dosages). The application of Growth-Plex Spin 90 g / 100 l with 75 and 100 % NPK increased the number of leaves and fresh and dry weights of plants. Total and marketable yield showed a similar positive trend with the same treatment.

Fathima and Denesh 2013 The results indicated Increased branches, number of fruits per plant and red chilli yield in treatment T3-humic acid sprayed @ 4 ml L-1 twice, once at

30 DAP and another at 50 DAP. The pooled data of red dry chilli yield for two years showed significant increase due to the application of humic acid sprayed twice @ 4 ml L-1 once at 30 DAP and another at 50 DAP, respectively over control and was at par with humic acid sprayed @ 6 ml L-1 twice(at 30DAP and 50 DAP). The highest B:C ratio was recorded in T3 compared to other treatments. The present study indicated that application of two sprays of humic acid @ 4.0 ml L-1 at 30 and 50 DAP, respectively (at 50% flowering and fruit formation stage) in chilli is economical and significantly increased the dry chilli yield.

A sequence of works discussing the effects of HS on plant growth and physiology. Schnitzer and Poapst (1967) [64] have showed an effect dose dependent revealed in a wide range concentration of fulvic acids extracted from a Spodosol on bean root initiation. The chemical functional groups of these HS, such as carboxylic acid and both phenolic and alcoholic hydroxyls, were reputed as responsible for part of the stimulus on plant growth.

The use of humic acid as a fertilizer has increased with increasing agricultural production and could be applied directly to the soil or as foliar spray to plants. Bioorganic fertilizers are used to reduce environmental pollution along with reducing the production cost and to improve crop quality (Asik *et al.,* 2009) [6]. Helmy (2015) evaluates the beneficial effects of soybean foliar spray with humic acid (HA) at 2000 mg L-1.Morard *et al.,* (2011) who reported positive response to HA spray on various plants.

The action of humic acid on plant growth can be divided into direct and indirect effects. It affects plant membranes increasing the transport of plant nutrients, enhancing protein synthesis, photosynthesis, microbial activity and solublization of micronutrients, reducing the active levels of toxic elements (Saruhan *et al.,* 2011) [62]. In a study on effects of bio and mineral fertilizers and humic substances on growth and yield of cowpea (Magdi *et al.,* 2011) [45] reported that, chemical fertilizer with humic substances improve growth and yield of cowpea. (Mahmoud, 2006) found that treatment of humic acid increased straw and seed yields as well as oil and protein content in peanut. The term “humus” shall be considered as synonymous of humified organic matter or HS, the relatively stabilized organic compounds in soil, sediments or water, resulted from plants and animals residues transformed through interactions with microorganisms and minerals. Humic acid (HA) is one of the main organic substances, which is an important component of humic substances.

Sodium humate, humic and fulvic acids have been found to promote root initiation of Pelargonium hortorum cuttings possible via an auxin-like activity (O’Donell 1973) [64].

In this regard, HS of both relative high and low molecular mass isolated from different sources in several exposure times and concentrations were able to exert its hormone-like activity in maize, tomato, Arabidopsis and cucumber (Dobbss *et al.* 2007, Zandonadi *et al.* 2007, Carletti *et al.* 2008, Elena *et al.* 2009, Mora *et al.* 2010, Schiavon *et al.* 2010, Zandonadi et al. 2010, Trevisan et al. 2011, Mora *et al.* 2012) [44, 76, 77, 20, 32, 50].

Humus effects on plant physiology are justified only by the auxin-like effect. Indeed there seems to be a complex signals network interaction, at some extent independent of auxin (Dobbss et al. 2007, Schmidt et al. 2007, Zandonadi et al. 2010, Mora et al. 2012) [44, 77, 50, 63]. Water-extractable soil HS altered development in Arabidopsis roots, inducing an increase in root hair density in IAA-independent manner as shown by the lack of responsiveness of the DR5 constructs containing auxin-response element (Schmidt et al. 2007) [63]. Arabidopsis root development was also shown to be altered by both high (>14,000 Da) and low (<700 Da) molecular weight HS, in an auxin-like mode (Dobbss et al. 2007) [44]. Authors have found an unusual auxin response (growth of primary root), as previously reported (Zandonadi *et al.,* 2007) [76]. In fact, other important signal, partially independent of auxin, such as nitric oxide, is induced by HA (Zandonadi *et al.,* 2010, Mora *et al.,* 2012) [77, 50]. Possibly, the root hair enhancement induced by HS observed by Schmidt *et al.,* (2007) [63] did not affect auxin report genes due to nitric oxide-induced hair emergence rather than auxin directly. Since nitric oxide is also induced by Alkamides in sites of adventitious root proliferation (Campos-Cuevas *et al.,* 2008) [18] and lateral root site (Méndez-Bravo *et al.,* 2010), HS might have chemical groups similar to these molecules.

Potassium bound to soil humic substances is mobile and can be readily changed into compounds available to the plants by mineralization and exchange reactions (Adherkhin and Belyager, 1991). Nakagawa *et al.,* (1966) [52] reported that the correlation between plant yield and K was positive and highly significant. The K uptake by the root, leaf, stem and seeds of green gram were estimated at 0.6, 5.0, 7.0 and 14 kg ha-1 (f.w.) respectively.

Bakry *et al.,* 2015 [10]. Results indicated that, humic acid foliar treatment with 20 (mg/l) in addition to sulfur fertilizer at rate of (250 and 500 kg/fed) gave significant increases in seed, straw and oil yield / fed. These increases due to the recorded increases in morphological criteria (plant height and root length, fresh and dry weight of shoot and root), photosynthetic pigments, (chlorophyll a, chlorophyll b and carotenoids), carbohydrate constituents (total carbohydrates, total soluble sugars and polysaccharides), IAA, phenol, free amino acids and proline contents. Meanwhile, lipid peroxidation decreased significantly in response to the above mentioned treatment as compared with control plants. Interaction between humic acid (20 cm/L) with sulfur at rate of (500 kg/fed) was the most effective treatment as it gave the highest increases in all morphological criteria, biochemical parameters, yield and yield attributes compared with the other treatments. Humic acid and sulfur fertilizer at all levels caused marked decreases in total saturated fatty acids accompanied by marked increases in total unsaturated fatty acids. The essential fatty acids (Linoleic acid C 18:2 + Linolenic acid C18:3) were increased by all applied treatments. It is worthy to mention that, the enhancement effects of humic acid (20 mg/l) and sulfur fertilizer (500 kg/fed) were the most pronounced treatment on flax plant.

Humic acid treatment increased significantly all yield and their related traits except technical length. humic acid with the rate of (20 cm/l) gave the highest values of seed, straw and oil yields/fed. This superiority may be due to the increases in plant height, fruiting zone length, the number of fruiting branches/plant, number of capsules/ plant and seed yield/ plant. Moreover, the superiority in oil yield/fed may be attributed to the increase in seed yield/fed and the increase in seed oil %. The trend of these results are supported by of (Bakry *et al.,* 2012; Unlu, *et al.,* 2011; Shehata *et al.,* 2012; Shafeek, *et al.,* 2013 and Abdel-Razzak and ElSharkawy, 2013) [71, 67, 66, 2]. The effect of humic acid on flax yield components could be attributed to

presence of plant growth regulators, which are produced by increased activity of microbes such as fungi, bacteria, yeasts, actinomycetes and algae (Chris *et al.,* 2005) [23]. It could be summarized that humic acid caused an increment in total seeds yield and caused an enhancement in some physical properties of pods.
The integrated effect of humic acid and bio effect of microorganisms on increasing available nutrients for plant growth and accordingly maximizing the biological yield and grain quality (Ewees and Abdel Hafeez, 2010) [33]. Bakry *et al.,* (2013) [8] pointed out that foliar application of humic acid and/or ascorbic acid and their combinations shows significant differences and gave the highest protein % and protein yield of wheat grown under newly reclaimed sandy soil.

Beneficial effect of humic acid on environment and soil ecological functions

Rima *et al.,* (2011) [60] humic substances may enhance uptake of nutrients through stimulation of soil microbiological activity. In other words, HA application could be a hormone substance enhancing root development and proliferation. Humic acid is complex substances derived from organic matter decomposition. Humic acid are the most significant constituents of organic matter in both soils and municipal waste compost, and have a relevant role in the cycling of many elements in the environment and in soil ecological functions (Senesi *et al.,* 1996) [65].
The effects of endogenous plant hormone auxin during seedling growth and the dark-colored humus from natural earthworm action were first reported almost simultaneously (Darwin 1880, 1881) [24, 25], and its role in humic substances (HS) bioactivity was established in the following century (Bottomley 1917, Guminski 1968, Dell'Agnola and Nardi 1987) [16, 40, 30].
Humic substances are the major components of stabilized organic matter, widely spread in terrestrial and aquatic environments (Stevenson 1994) with several functions on ecological systems, affecting biochemical and biogeochemical pathways (Steinberg *et al.,* 2006) [69].
Regarding the lifecycle of higher plants, the possible linkage of auxin and organic matter content in soils has been long recognized (Whitehead 1963, O'Donnell 1973) [73, 54].
HS are endowed with hormone-like activities (Guminski 1968, Schnitzer and Poapst 1967, Cacco and Dell'Agnola 1984, Muscolo *et al.,* 1998, Nardi *et al.* 2000, Canellas *et al.,* 2002, Elena *et al.,* 2009, Zandonadi *et al.,* 2010) [77, 32, 40, 17, 64, 51, 19]. Even after a number of evidences have shown that both low molecular weight (LMW) HS as well as high molecular weight (HMW) HS could regulate the plant growth and physiology, the biological activity/molecular weight paradigm remains alive.
It is also argued that HS could not reach the interior of plant cell, and their observed effects should be regarded to other factors such as the HS release of ions and nutrient availability (Chen and Aviad 1990, Chen *et al.,* 2004) [22, 21].
In this regard, HS affect this enzyme activity, protein expression, proton extrusion (Canellas *et al.* 2002, Façanha *et al.,* 2002) [19, 34] and mRNA levels (Quaggiotti *et al.,* 2004, Elena *et al.,* 2009) [59, 32] of plasma membrane proton ATPase (PM H+-ATPase), in a similar way of those effects of auxin on PM H+-ATPase reported in maize (Frias *et al.,* 1996) [38]. This enzyme plays a crucial role on nutrient uptake and root growth, as confirmed by its abundance in root tissues (Palmgren 2001) [56].
This material was incubated with a mixed culture of aerobic soil organisms and apparently the material called "humic acid" in the peat was converted into "soluble humates" (Bottomley 1914) [14] This material was essentially described as "an aqueous extract of the alcohol-soluble material of the bacterised peat" (Bottomley 1914) [14], considered as an excellent culture medium for nitrogen fixing bacteria Azotobacter chroococcum and wheat seedlings growth, apparently containing the so called "accessory factors" besides the mineral factors used.
Guminski (1968) [40] reviewed the main information available in that time on the physiological effects of HS on plants. Guminski analyzed the results of literature in two main trends; one that claimed that HS could affect plant physiology directly and other, which stated that humic compounds may only affect plant indirectly. The direct effects would be the ones that modify plant metabolism, while the indirect effects would affect other characteristics on plant environment, such as ion uptake. Most of discussed works presented were focused on whether humus could enter plant cell or not. In this review, Guminski clarifies that even though a given fraction of HS does not penetrate the cell, it still does not mean that its fraction has no physiological activity. In addition, the discussion of direct and indirect HS action should take into account another factor, the intervention of microorganisms.
Some authors have showed HS as very important in modifying plant growth, ions uptake and enzymes activity (Vaughan 1974, Malcolm and Vaughan 1979) [47, 72], and some of them observed the auxin-like mode of action (Schnitzer and Poapst 1967, O'Donell 1973) [64]. Plants were treated with humates (unfractionated), HA (with apparent HMW) or fulvic acids (with apparent LMW), and the effects on plant growth and metabolism appeared to be independent of its molecular weight at some extent.
The auxin-like activity of low molecular HS observed earlier by Dell'Agnola and Nardi (1987) [30] was related to the presence of IAA (Muscolo *et al.*, 1998) [51]. These authors showed that the concentrations of IAA in HS were estimated to be 0.5% (w/w) by enzyme immunoassay and 2 to 3.7% by radioimmunoassay. The hormone-like activity of HS may also be associated to other molecules (Young and Chen, 1997) [74].
David *et al.,* (1994) [26] opined that increased surface area of roots due to the action of humic acid facilitated the efficient nutrient uptake by plants. Balanced nutrient uptake by plants could be attributed to the chelating nature of humic acid. In general, nutrient status is an important deciding factor judging the total dry matter accumulation in plants.
Ananthi and Vanangamudi, 2014 [5] reported foliar spray of 0.1 % HA + 0.1 ppm BR (T5) increased the P content (0.42) over control (0.22 at 45 DAS). However, the favourable nutrient status was shown by BR spray, which was followed by BA and SA. Hence, it can be inferred that brassinosteroid plays an important role in enhancing the water and nutrient uptake in addition to enhanced morphological growth and physiological / biochemical parameters. Similar result was reported by Sairam (1994) [61]. This higher nutrient status was believed to be responsible for more chlorophyll content and palisade cells per unit area of leaf tissue which ultimately increased the photosynthesis and yield (Dean and Knavel, 1969) [28].
Phosphorus mobilization in the soil was increased by humic

acid by forming humo-phospho complex. This can be easily absorbed by the plants (Balasubramanian *et al.,* 1989a) [11]. The stimulating activity of humic acid on respiration might have increased the demand for inorganic phosphorus for ATP synthesis, thus leading to increased P uptake (Smidova, 1960) [68].

Humic seem to have a particular favorable effect on the nutrient supply. Foliar sprays of these substances also promote growth, and increase yield and quality in a number of plant species (Karakurt *et al.,* 2009) [43] at least partially through increasing nutrient uptake, serving as a source of mineral plant nutrients and regulator of their release (Atiyeh, *et al.,* 2002) [7]. Moreover, humic acid influence respirationprocess, the amount of sugars, amino acids and nitrate accumulated (Boehme *et al.,* 2005) [13].

Foliar treatment with humic acid on some growth parameters of flax plant. The results illustrated that flax plant of Opal cultivar treated with humic acid (20 mg/l) recorded significant increases of shoot and root length, fresh and dry weight of shoot and root compared with control plants. These obtained results are in agreement with those obtained by Peymaninia *et al.,* (2012) [57] on wheat, Bakry, *et al.,* (2013) [8] on flax and El-Bassiouny *et al.,* (2014) [31] on wheat plant. These obtained increases in response to humic acid due to that, humic acid is considered to increase the permeability of plant membranes and enhance the uptake of nutrients (Piccolo, *et al.,* 1992) [58]. It could be concluded that this increases may be due to the role humic acid in increasing endogenous hormone as IAA (Table-4) and the role of these hormones in stimulating cell division and/or the cell enlargement and this in turn improve plant growth (Abdel Mawgaud *et al.,* 2007) [1]. Furthermore, humic acid increases the porosity of soil and improve growth of root system which leads to increase the shoot system (Garcia, *et al.,* 2008) [39]. Mataroiev (2002) [48] describe the role of humats in improving soil physical and chemical characteristics by reaction with soil minerals then improving watery, aerial soil characteristics and nutrient mineral adsorption.

The increases in photosynthetic pigments content due to humic acid application are in agreement with those obtained by (Ameri and Tehranifar, 2012; Bakry *et al.,* 2013; El-Bassiouny *et al.,* 2014) [4, 8, 31] on different plants. This positive effect of humic acid on photosynthetic pigments could be attributed to an increased in CO2 assimilation and photosynthetic rate which increased mineral uptake by the plant (Ameri and Tehranifar, 2012) [4]. These increases may be due to that, HA probably caused an increase in the synthesis of the chlorophyll and/or delayed chlorophyll degradation in the leaves of flax plant (Bakry *et al.,* 2012a) [9].

The percent of increases reached 65.97, 67.27 and 23.99% for total carbohydrate, polysaccharides and total soluble sugar, respectively as compared to control plant obtained by Bakry *et al.,* 2013 [8] and El-Bassiouny *et al.,* 2014) [31] on flax plant. The significant increases in total carbohydrates, in shoots of flax plant concomitantly with the increased growth rate led to the conclusion that the photosynthetic efficiency was increased in response to HA treatments and thus led to enhance biosynthesis of carbohydrates which are utilized in growth of flax plants. This positive effect of HA has also been observed on the main photosynthetic metabolism in maize leaves, where a decrease in starch content was accompanied by an increase of soluble sugars (Ferrara and Brunetti, 2008) [37].

Humic acid foliar application caused significant increases in free amino acids, proline, while caused significant decrease in lipid peroxidation compared with control. These results of free amino acids and proline content increments are in agreement with those obtained by Farahat, *et al.,* 2012 [35] and El-Bassiouny *et al.,* 2014 [31]. Many functions have been postulated for proline accumulation in plant tissues, proline and free amino acids could be involved in the osmotic adjustment of plants (Delavari, *et al.,* 2010) [29] and could also be a protective agent of enzymes and membranes (Gzik, 1996) [41]. When plant subjected to unfavorable conditions as the newly reclaimed sandy soil, plants maintain their water content by accumulation of compatible organic solutes act as osmoprotectants, as proline, in their cytoplasm (Bandurska, 1993) [12].

Inhibitory effect of unfavorable conditions on flax plant was alleviated by humic acid treatments through increasing proline synthesis and/or enhancing the biosynthesis of other amino acids and their incorporation into protein. With regard to lipid peroxidation, as malondialdehyde (MDA) contents the obtained data are in harmony with those obtained by Bakry *et al.,* (2013) [8] on flax plants. Higher level of this substance is found in plants subjected to high levels of oxidative stress. So any stress is known to damage the cell, and alter most of its components and functions (Harinasut, *et al.,* 2000; Zhang and Kirkham, 1996) [42, 78]. The reduction in MDA contents caused by humic acid treatment may be as a result of their role in removing the stress of peroxidative, as they affecting the antioxidant enzymes and lipids peroxidation, would protect flax plant against stress.

Conclusion

Humic acid is better option for soil health and plant growth, as compare to other chemical substances. It is completely eco-friendly for agriculture. Humate is a safe material and exists in all soils, plants and animals. It is natural to the food chain and plays a role in the composting of dead matter into nutrients, transfer of minerals, and other roles within the living organism.

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