Humic Substances and Efficient Microorganisms: 
Elicitation of Medicinal Plants—A Review

M. M. A. Pereira¹, L. C. Morais¹, E. A. Marques¹, A. D. Martins¹, V. P. Cavalcanti¹, F. A. Rodrigues¹, W. M. Gonçalves¹, A. F. Blank², M. Pasqual¹ & J. Dória¹

¹ Department of Agriculture, Federal University of Lavras, Brazil
² Department of Agriculture, Federal University of Sergipe, Brazil

Correspondence: M. M. A. Pereira, Department of Agriculture, Federal University of Lavras, Brazil. Tel: 55-991-004-598. E-mail: agro.maysa@gmail.com

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Abstract

In function of the green revolution the indiscriminate use of agrochemicals and pesticides in agriculture has been also shown in the production of medicinal plants, resulting in the increase of productivity but with high residual contamination and low rates in the production of secondary metabolites responsible for the biological and pharmacological activity in vegetable drugs. In another hand, new techniques of elicitation has been applied to stimulate the medicinal plants production through the organic and agroecological management, contributing for the increase of performance, quality and production. In this context, it is aimed with this review to present such as the humic substances: fulvic acid, humic acid and efficient microorganisms which influence and help the ontogeny and the secondary metabolites production of medicinal plants. The reviewed articles show that the use of fulvic acid, humic acid and efficient microorganisms in the production of medicinal plants contributes on the increase of biosynthesis, secondary metabolites production such as coumarins, alkaloids, phenylpropanoids and essential oils, as well as the increase of nutrients absorption, growth and development of species.

Keywords: biostimulation, essential oils, secondary metabolism, in vivo, humic acid

1. Introduction

Since the beginning of the green revolution the agriculture panorama has changed by the excessive use of fertilizers, pesticides, land mechanization and latifundia concentration (Lazzari & Souza, 2017; Matos & Pessoa, 2011). In order to increase productivity these agrochemicals has been indiscriminately used not only in grains and horticultural crops, but also in medicinal plants (Pereira & Raimunda, 2016).

The medicinal plants are essential source of secondary metabolites and bioactive compounds responsible for the biological and pharmacological activity of the plant in vivo (Costa & Jorge, 2011; Czelusniak, Brocco, Pereira, & Freitas, 2012; Pereira & Cardoso, 2012; Gobbo-Neto & Lopes, 2007), as alkaloids, phenolic compounds, terpenes and others. Historically they have been used by the population through homemade crops, live pharmacies, as well as acquisition in natural products stores and industrial herbal medicines (Calixto & Siqueira, 2008).

In Brazil, the cultivation of medicinal species must be totally pesticide free. However, residues of undesirable and undeclared substances such as pesticides, radioactive compounds, pathogens, mycotoxins, heavy metals and arsenic are constantly found in plant-derived drugs and phytotherapics altering the quality of the products with therapeutic purposes in the treatment of various diseases (Brasil, 2014).

Research on the cultivation of species with therapeutic purposes is still recent. Therefore, some technologies used in the production of large crops and vegetables, such as the use of elicitors (endogenous or exogenous molecules) in vivo (Canellas et al., 2015), can be applied in the organic cultivation of medicinal plants. Since research with the use of elicitor agents has been focused more in in vitro culture (Rafiee et al., 2016).

Studies carried out with plants under field grown highlights the use of plant growth promoting fungi (PGPF), plant growth promoting rhizobacterium (PGPR) (Adesemoye et al., 2009; Bhattacharyya et al., 2012), humic substances (fulvic and humic acids) (Canellas et al., 2014; Olivares et al., 2017), seaweed extracts (Craigie,
2011), hydrolyzed proteins and amino acids (Calvo et al., 2014). These bioestimulants can influence on ontogeny, secondary metabolite biosynthesis, efficiency and nutrient uptake by plants.

Researches have been done about the effect of bioestimulants such as microorganisms, rhizobacterium and humic substances on the production of medicinal plants in vitro (Halpern et al., 2015; Canellas et al., 2015; Olivares, 2017). Therefore, it is necessary to know if the content of secondary metabolites changes when the elicitor agents are applied in vivo and if its application in agroecological systems are economically and socially viable, overcoming the conventional agriculture and keeping the levels of productivity.

On this review, we show studies about the use of humic substances and microorganisms applied in the field (in vivo), their effect on ontogeny and on metabolites content in medicinal plants. The numbers of studies in vivo are extremely low, which makes it a scientific and social challenge for the advancement of agroecology and organic production of medicinal plants.

2. Humic Substances: Humic Acid and Fulvic Acid

The biological structure of the soil is rich in microorganisms that perform essential role in the carbon cycle and in the genesis of humic substances (Bakker et al., 2013; Fischer et al., 2017). Amongst the exudates released by the microorganisms present in plants, we can mention the formic organic acids, acetic, oxalic, tartaric and citric (Canellas et al., 2008; Zhalnina et al., 2018; Licá et al., 2018), the protons dissociate-helpers in the decomposition of minerals in soil in which middle life is too short, as well as the aromatic acids, benzen-carboxylic and phenolics (M. A. Baldotto & E. B. Baldotto, 2014).

The organic material is decomposed into new material (humus) through the microorganisms and chemical degradation of the biota constituting the biomolecules which are placed between the horizons O and A (Ebeling et al., 2011). The humic substances are organic compounds condensed that differ from biopolymers by its molecular structure and high soil persistence (Nebbioso & Piccolo, 2012). According to the International Humic Substances Society, the humic substances can be defined by macromolecules or supramolecules (Fischer, 2017).

The humic substances contain a hydrophilic portion and other one- hydrophobic (Canellas et al., 2008; Fischer, 2017) where the humic acid (HA) is founded turned to the inside part and a hydrophilic fulvic acid turned to the outside (Nebbioso & Piccolo, 2012; Drosos, 2017). These aggregate supramolecules are organised in high and low weight of organic compounds (Kawahigashi, 2005) and has varying specified structure features accordingly with the organic matter and time of transformation (Asli, 2010; Nebbioso & Piccolo, 2012).

The humic acids influence on the fruits, flowers and seeds development (M. A. Baldotto & E. B. Baldotto, 2015) that acts in several phases of the vegetable physiology (Canellas & Olivares, 2014) such as growth, development, photosynthetic structures of respiratory activity (Costa et al., 2008); changes on biochemical pathways as primary and secondary metabolism (Trevisan et al., 2011; Aminifard et al., 2012; Canellas & Olivares, 2014; Nardi et al., 2017). Due to all these beneficial effects of elicitors on plant productivity, they have attracted the interest of farmers and companies.

Researches show that humic substances contribute on the better absorption of nutrients (Halpern et al., 2015). They also perform indirect actions over the chemical and dynamic of the microorganism on the rhizosphere, altering the interaction soil-plant-microbiota (Canellas & Olivares, 2014) in relation to the assimilation of macro and micro nutrients as a function of the increase on the permeability of the plasmatic membrane with the action of the humic substances (Silva Lima et al., 2014).

Changes on the uptake of nutrients and humic substances related to the absorption rates are explained by the kinetic parameters of absorption, which are estimated through the maximum absorption rate, ion concentration and minimum ion concentration in the solution that the plant cannot absorb (Silva Lima et al., 2014).

Therefore, the humic and the fulvic acid can influence directly and indirectly in the plants metabolism (Canellas et al., 2014; Vaccaro et al., 2015), altering the metal complexation, increasing the capacity of cationic exchange (Ateia et al., 2017; Lee et al., 2017; Kwiatkowska-Malina, 2018), nutrients supply and humidity retention, interfering on the ions transportation, respiratory activity, chlorophyll levels, nucleic acid synthesis and on the activity of several enzymes (Muscolo et al., 2013; Ozfidan-Konakci et al., 2018; Shahabivand et al., 2018).
Table 1. Overview of the medicinal plants species elicited by humic substances: fulvic and humic acid

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<td>Hibiscus sabdariffa L.</td>
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<td>Gerbera planta cv. Malibu</td>
<td>soil</td>
<td>Antioxidant activity, malondialdehyde</td>
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<tr>
<td>Anethum graveolens L.</td>
<td>Leaf pulvization</td>
<td>Essential oil (carvone, dihydrocarvone, limonene, dill apiole piperitone)</td>
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3. The Influence of Humic Substances on the Ontogeny, Primary and Secondary Metabolism

Studies evaluating the application of humic substances in medicinal plants in vivo (Table 1) demonstrate that those substances can contribute to the increase of the biosynthesis of secondary metabolites and to the activity of bioactive substances from different classes, such as flavonoids, coumarins, phenylpropanoids, total phenols and anthocyanins (Ahmed et al., 2011; Khazaie et al., 2011; Aminifard et al., 2012; El-Gohary et al., 2014; Ghasemi et al., 2015; Hendawy et al., 2015; Said-Al et al., 2016).

There are different ways of applying humic substances: via soil, spray or fertigation (Kołodziej et al., 2013; Hendawy et al., 2015; Ghasemi et al., 2015). Nevertheless, those substances significantly increased the biosynthesis of the metabolism of medicinal plants and production of metabolites with biological and
pharmacological properties independent of the way they were applied on the plants (Andrade et al., 2001; Khazaie et al., 2011; Kołodziej et al., 2013).

Humic acid can also be applied via homeopathy techniques promoting a 55.10% increase in coumarin content in Justicia pectoralis Jacq. (Andrade et al., 2001) when dynamized as well as demonstrated great variability in the electromagnetic field of these plants.

Although the application procedure does not show difference on the performance and biosynthesis of metabolites on medicinal species, humic substances applied via soil act as soil conditioners. They interact with great number of functional groups which allows wide espectro of reactivity with other molecules (Drosos et al., 2017), hydrophilicity forming hydrogen bonds with water and the structural malleability wich is the capacity of intermolecular association and changes on pH and redox values (Caron et al., 2015; Fischer, 2017).

These changes in the physical, chemical and microbiological structure of the soil have the purpose of greater water retention, aeration, directly influencing root growth, nutrient absorption, primary and secondary metabolic pathways (Canellas & Olivares, 2014; Canellas et al., 2015; Olivares et al., 2017; Ozfidan-Konakci et al., 2018), hormone signaling and, consequently, higher yield of the medicinal plants.

In this context, the application of humic and fulvic acid in vivo increased growth, development and organogenesis of the plants in the field, influencing on the differentiation of the vegetative tissues in the production of flowers (Costa et al., 2008). Higher content and concentration of active ingredients of the essential oils of Hyssopus officinalis L., Mentha piperita L, Mintha piperita var. Citrate, Anethum graveolens L. was also observed, showing a positive corelation between the increase of dry or fresh biomass with the essential oil content (Hendawy et al., 2015; El-Gohary et al., 2014).

Anthocyanins and phenolic compounds (monoterpene, terpene, phenylpropanoids, flavonoids) are the main classes of secondary metabolites influenced by the application of humic substances found in the literature (Haghighi et al., 2016; Said-Al et al., 2016). In fruits of Capsicum annum var. Red chili humic acid increased the concentration of flavonoids, capsaicin, carotenoids (lycopene and B-carotene) and antioxidant activity (Aminifard et al., 2012).

Humic substances improves the root system development, which contributes to higher nutrient uptake efficiency, leading to changes on the root exudation profile (Canellas & Olivares, 2014), lateral and adventitious roots formation. These responses involve mechanisms of cellular stimulation and differentiation that act as messenger inducing physiological effects, as well as influence on the production of chlorophyll A and B and carotenoid content (Ghasemi et al., 2015).

Therefore, the application of humic substances in the in vivo cultivation of medicinal plants opens up the opportunity for the development of organic fertilizers for agroecological systems, aiming at good quality raw material without pesticides with increased concentration of secondary metabolites biologically and pharmacologically interesting (Canellas et al., 2015; Halpern et al., 2015, Olivares et al., 2017).

4. Application of Plant Growth Promoting Microorganisms

It is estimated that living microorganisms, aggregate to organic matter and rhizosphere, occupy less than 5% of the total space of soil. What makes this environment a dynamic and living space, with a central role in processes of decomposition and cycling of nutrients available to the plants through the mineralization (Chavarria et al., 2018; Lehmann et al., 2018; Zheng et al., 2018).

The microorganisms applied on agroecological and organic systems have influence on the health and microbial activity of the soil (Chavarria et al., 2018). Soil community is diverse, constituted by fungi and bacterium that perform as promoters of plant growth (Leite et al., 2016; Silva et al., 2017; Trinh et al., 2018). Thus the microorganisms of the rhizosphere shape efficient systems for absorption and catabolism of organic compounds existent in the exudate roots (Bakker et al., 2013; Ahemad & Kibret, 2014).

Plant growth promoting bacterium can influence directly or indirectly the general morphology of plants, as they have the ability to colonize tissues and different mechanisms of action (Pandya et al., 2013; Chagas et al., 2018). They can act on iron sequestration and phytoremediation (Karami & Shamsuddin, 2010), phosphorus solubilization, atmospheric nitrogen fixation (Santi et al., 2013; Ahemad & Kibret, 2014), hormone production, organic siderophore compounds (Bhattacharyya & Jha, 2012), systemic resistance to pathogens, tolerance to abiotic and biotic stress (Etesami & Maheshwari, 2018; Shameer & Prasad, 2018).

The main efficient microorganisms associated to rhizosphere capable of exerting a beneficial effect on plant growth and physiology are the Azospirillum spp., Alcaligenes spp., Artrobacter spp., Acinetobacter spp., Bacillus
spp., *Burkholderia* spp., *Enterobacter* spp., *Erwinia* spp., *Flavobacterium* spp., *Pseudomonas* spp., *Rhizobium* spp., *Serratia* spp., *Streptomyces* spp. and mycorrhizal fungi as the Arbuscular mycorrhizal fungi present in 80% of the roots (Bulgarelli et al., 2013; Gupta et al., 2018).

Bacterium of the genus *Azospirillum* spp., *Bacillus* spp. and *Enterobacter* spp. are effective for promoting better performance of plants acting on the regulation of physiological process (Bhattacharyya et al., 2012), stimulating the ability of producing and degrading plant growth regulators like gibberellins, auxins, cytokinins and ethylene phytohormones (Calvo et al., 2014).

The role of microorganisms applied to agriculture are diverse, for example, mycorrhizal arbuscular fungi have the ability to solubilize phosphorus (P) by secretion of phosphatases which degrade the P organic or the acids that dissolve inorganic compounds of P (Richardson et al., 2009; Yousefi et al., 2011). The free-living diazotrophic microorganisms (*Azospirillum*, *Psudomonas* and *Cyanobacterium*) can fix nitrogen from the atmosphere to the soil to be mineralized and made it available to the plants in the form of ammonia they can also synthesize phytohormones (Fukami et al., 2018).

Besides, microorganisms can act as elicitors and increase the production of secondary metabolites involved in the biological and pharmaceutical activity of medicinal plants (Mohammadi et al., 2017), such as flavonoids and terpenes content, phenolic compounds, microbial activity, chlorophyll content, nutrient uptake, plant growth and development (Banchio et al., 2008; Cappellari et al., 2013; Zhao et al., 2016; Damam et al., 2016; Swamy et al., 2016).

Most of the studies regarding the use of microorganisms on medicinal plants aiming phytochemical and biochemical changes on secondary metabolites production were carried out *in vitro* (Rafiee et al., 2016; Du et al., 2013; Vafadar et al., 2014; Çetin et al., 2016). However, researches about the use of elicitors agents in the field are very important due to its great and innovative potential on the organic grown of medicinal plants, once uncontrolled edaphoclimatic factors may influence the colonization and the mechanisms of action of growth promoting microorganisms.

Thus, the use of efficient microorganisms as phytoremediators, bioprotectors and biofertilizers are potential tools for organic and agroecologic agriculture, improving plant physiology, intermediating the production of substances of the primary and secondary metabolism, which are related to yield, production of active ingredients and essencial oil with good quality for therapeutic use.

5. Influence of Microrganisms on Phytochemical Composition

Microorganisms (fungi and bacterium) applied in the cultivation of medicinal plants *in vivo* (Table 2) had great influence on plant growth and development, photosynthetic rate, production of secondary metabolites, abiotic stress tolerance and increased synthesis of active compounds (Aseri et al., 2008; Shahmohammadi et al., 2014; Akhazari et al., 2015; Tahami et al., 2017).

Interactions between microorganisms and medicinal plants can lead to neutral, negative or positive effects on plants (Ahemad & Kibret, 2014). In this review, we verified the use of different genera of microorganisms such as *Bacillus* spp., *Azotobacter* spp. and *Azospirillum* spp., heterotrophic and nitrogen-fixing aerobic bacterium, being that *Azospirillum* genera (facultative endophytes) the most commonly applied on the plants (Mohammad et al., 2012; Shahmohammadi et al., 2014; Tahami et al., 2017).

Facultative endophytic bacterium have the ability to colonize the interior and surface of the roots as a function of the chemotaxis of rhizosphere exudates (Ahemad & Kibret, 2014). They can convert atmospheric N to ammonium, produce phytohormones such as cytokinins, gibberellins and auxins (El-Hadi et al., 2009), increase water and mineral absorption, tolerance to abiotic stresses (Shameer & Prasad, 2018; Fukami et al., 2018; Silva & Pires, 2017), and are able to colonize the endorizosphere and xylem vessels (Fatma et al., 2008). Thus, there is a growing interest in inoculating microorganisms *in vivo* as they not only increase the productivity and phytochemical profile of medicinal plants but also reduce the use of chemical fertilizers (Adesemoye et al., 2009).
<table>
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<th>Scientific Name</th>
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<td>&gt; Plant height, nº of branches, fresh and dry weight and weight frasco and usefulness of fruits</td>
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<td>Microral activity, chlorophyll and nutrients rates</td>
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<td>A. brasiliensis and G. monn ease</td>
<td>&gt; Leaf area and dry weight</td>
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<tr>
<td>Punica granatum</td>
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<td>Mentha arvensis L.</td>
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<td>Mentha spicata L.</td>
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<td>Azotobacter and Azospirillum &gt; Essential oil usefulness</td>
<td>&gt; Growth, usefullness and density of the roots</td>
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<tr>
<td>Carum copticum L.</td>
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<tr>
<td>Coriandrum sativum L.</td>
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<td>Rauwolfia serpentine</td>
<td>Soil and coal</td>
<td>Growth, alkaloid rates, microbial biomass</td>
<td>Dry roots</td>
<td>Azotobacter chroococcum, Azospirillum brasilense and Pseudomonas putida</td>
<td>&gt; Growth</td>
<td>Bai et al. (2017)</td>
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</table>
The number of umbels and seeds increased with the application of *Azotobacter chroococcum* and *Azospirillum lipoferum* in plants of *Coriandrum sativum* L. (Akhani et al., 2012), which has fungicidal, bactericidal and anthelmintic properties (Shahmohammadi et al., 2014). As for *Anethum graveolens* (L.) seed production increased as well as the yield of essential oil. This may be related to the better development of the ontogenic phase of the species, as well as a higher uptake of nitrite, nitrate and phosphate (Cappellari et al., 2013), influencing on growth, increased leaf area, higher photosynthetic rates that may contribute to yield, essential oil production and increased concentration of active principles (Damam et al., 2016; Rai et al., 2017).

Several studies point out the importance and interest of applying *Azotobacter* spp. and *Azospirillum* spp. *in vivo* in order to improve the physiology and phytochemistry of medicinal plants. They can increase yield and content of active constituents of the essential oil in various medicinal species like *Foeniculum vulgare* Mill. (Mahfouz et al., 2007). *Rauwolfia serpentina* had better performance and anetol rates; higher alkaloids rates were found in *Carum copticum*. Rai et al. (2017); alpha-pinene content increased in *Coriandrum sativum* L. (Ghilavizadeh et al., 2013); on the cultivation of, had increased the alpha-pinene rates (Akhani et al., 2012); greater content of the essential oil of the *Matricaria chamomilla* L. (Dastborhan et al., 2012). When the Bacillus spp. is applied in the production, it’s verified the increase on the radicular system and better usefulness of the essential oil (Tahami et al., 2017).

In addition to the rhizobacterium applied as elicitors, arbuscular mycorrhizal fungi (AM) can help to improve the physical, chemical and structural properties of the soil. By increasing the availability of phosphorus through its specialized hyphae, the organic matter content and reduction of the apparent soil density (Yousefi et al., 2011; Akhazari et al., 2015), water retention by stimulating the plant’s root system to exploit greater soil volume and to assist in the accumulation of secondary metabolites (Swamy et al., 2016).

Among the species evaluated by Bharti et al. (2016) and Hashemzadeh et al. (2013) the AM fungi *Gladus* spp. and *Glomus* spp. can interfere in the physiological development, increasing the total content of chlorophyll, the accumulation of higher levels of nutrients, total phenols and amino nitrogen by increasing the activities of dehydrogenase, alkaline phosphatase and nitrogenase that causes alteration in the mineralization of nutrients present in organic matter (Aseri et al., 2008).

Studies have shown that application of fungi (AM) in the production of *Mentha arvensis* L. in saline and non-saline conditions may induce tolerance and/or resistance to abiotic stress like water and salt stress (Bharti et al., 2016). Therefore, agronomic characteristics can be improved, such as biomass, height, stem and leaf ratio, higher nitrogen and phosphorus absorption due to the increase of mycorrhizal colonization.

On the other hand, species of fungi (AM) under abiotic stress conditions such as *Halomonas* spp. reduced rhizosphere colonization and yield in essential oil content (Bharti et al., 2016). Similar results were found with the application of photosynthetic bacterium, actinobacterium, lactic acid bacterium, fermentation fungi and yeast in the cultivation of *Chamomilla recutita* L. Rausch. The authors did not find significant differences for essential oil yield and flavonoid content (Kwiatkowski et al., 2015).

Thus, the application of fungi and bacterium as elicitors in the *in vivo* cultivation of medicinal plants is an important tool in organic and agroecological production, which contributes to the production of raw material with quality and pharmacological interest. They can also improve the performance and optimization of the use of nutrients, opposing the application of chemicals in the production of medicinal plants, conferring quality production with respect to the man and to the environment (Adesemoye et al., 2009; Olivares et al., 2017).

**6. Final Considerations**

Based on the reviewed studies, the use of eliciting agents in the cultivation of medicinal plants through the application of humic substances (fulvic and humic acid), efficient microorganisms *in vivo*, it opens opportunities for the development of organic fertilizers and biofertilizers that can be applied in organic and agroecological cultivation for a sustainable agriculture. The use of humic substances and microorganisms improved the physicochemical characteristics of the soil, increased availability of nutrients for medicinal species, contributed to growth and development through the production of phytohormones, induction of the root system, release of exudates, influence on phytochemistry, primary and secondary metabolism by increasing the production of active principles and essential oil for therapeutic, biological and pharmacological use in the treatment of numerous diseases. The use of these elicitor agents in the field, focusing on medicinal species, is a scientific and social potential in improving the yield and quality of the production of medicinal plants without contamination by pesticides and chemical fertilizers that, besides the social and environmental benefits, potentiate the production of metabolites plants.
References


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