Short Communication

Initial performance of *Achillea millefolium* in response of humic acids

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ABSTRACT

Owing to numerous health benefits, the cultivation of medicinal plants has significantly increased. *Achillea millefolium* L. (common names: milefoil, yarrow) is attributed important medicinal properties. Consumers of herbal medicinal products demand for production systems based on ecological farming principles, thus an interesting technology would be application biostimulant based on humic acids. The objective of this study was to evaluate the rooting and initial performance of *Achillea millefolium* in response to the use of humic acids (HA) isolated from composted cattle manure and poultry litter and applied in different concentrations (0, 10, 20, 30, and 40 mmol L^{-1} of HA) at the propagation phase. Rooting and plant biomass accumulation were analyzed. Humic acids derived from poultry litter promoted root development and increase of fresh biomass and total dry mass, proving their biostimulant effect. The optimal concentration of humic acids estimated for the initial development of *A. millefolium* was 22.25 mmol L^{-1}.

Keywords: biostimulant; medicinal plants; organic fertilization.

INTRODUCTION

The use of plants with medical functions contributes meaningly to primary health care (Halberstein, 2005), as well as providing useful information for the design of pharmacological, phytochemical, and agronomic studies on these plants (Brasileiro *et al*., 2008).

The cultivation of medicinal species has increased in the recent years (Corrêa *et al*., 1998; Arnous *et al*. 2005; Perna & Lamano-Ferreira, 2014) and relating biomass yield to plant quality is essential for the manufacture of herbal medicines (Souza *et al*., 2011).

Achillea millefolium L is a medicinal plant of the Asteraceae family, native to Europe, North America, South Australia, Asia, and widely distributed in the Brazilian flora. In Brazil, it is commonly known as mil folhas, mil-em-rama, milefolio, and erva-do-carpinteiro (Balbach, 1993; Candan *et al*., 2003). It is a perennial species, hard stem 30 to 90 cm in height, abundant fernlike leaves, and flowers pink or white. The uses of the species in medicine are mainly as antibacterial, antifungal, antitumor, healing, antioxidant, antiedematous (Rosa *et al*., 2008).

Chaves (2002) discussed that medicinal and aromatic species require soils with favorable characteristics for better development and expansion of the root system. In these conditions, organic fertilization stands out as a farming practice used to improve the physicochemical, microbiological (Kiehl, 1985), and nutritional qualities of the soil. However, fertilizers should be managed correctly, as deficiency or excess of nutrients may interfere with biomass production and the amount of active ingredients (Mapeli *et al*., 2005).

In organic fertilization practices, the use of humic acids is an alternative, especially with the gradual decrease in non-renewable natural resources, a problem for agriculture in the third millennium. Humic acids are a fraction of humid organic matter that presents bioactivity and that comprises a set of heterogeneous organic molecules organized in aggregates and stabilized by hydrophobic interactions

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and hydrogen bonding (Piccolo, 2001). A growing number of field experiments have demonstrated the benefits of using humic acids in agriculture, increasing nutrient uptake and improving soil structure, with direct effects on yield and quality of numerous crops (Silva Filho & Silva, 2002). Studies with ornamental plants indicate positive biostimulating effects of humic acids (Baldotto *et al*., 2012; Baldotto & Baldotto, 2013), suggesting a similar action on medicinal plants. However, research has shown that there are ideal levels of humic acids and that crops respond to the effect of these substances to a certain level (Silva Filho & Silva, 2002).

Humic substances have increasingly been used due to extensive research proving their effectiveness. However, there is a lack of studies on the effect of these biostimulants on medicinal plants, particularly *A. millefolium*, emphasizing the need for further investigation to increase information and efficiency in the improvement of this species to verified the origin and concentration dependence for optimization of this plant stimulant.

The objective of this work was to measure the initial performance of *Achillea millefolium* in response to the application of different concentrations of two humic acids derived from composted cattle manure or poultry litter and applied at the propagation phase.

MATERIAL AND METHODS

Different concentrations of two humic acids (HA) (0, 10, 20, 30, and 40 mmolL^{-1}) extracted from composted cattle manure and poultry litter were applied to cuttings of *A millefolium* as biostimulants.

The waste used in composting for the extraction of humic acids to be tested came from the Cattle Sector, located in Universidade Federal de Viçosa, and from Brasília farm, located in Florestal - Minas Gerais were isolated and characterized according to the orientations described in Baldotto *et al.* (2007) and Baldotto & Baldotto (2014).

The chemical composition of the poultry litter compost were: pH = 7.92, C organic = 9.06 dag kg^{-1}, N = 1.38 dag kg^{-1}, P = 1.35 dag kg^{-1}, K = 0.84 dag kg^{-1}, Ca = 2.29 dag kg^{-1}, Mg = 0.52 dag kg^{-1}, S = 0.31 dag kg^{-1}, C/N = 6.59, Zn = 272 mg kg^{-1}, Fe = 7,588 mg kg^{-1}, Mn = 662 mg kg^{-1}, Cu = 243 mg kg^{-1}, B = 30 mg kg^{-1}. The compost of cattle manure presented pH = 7.59, C organic = 11.85 dag kg^{-1}, N = 2.75 dag kg^{-1}, P = 0.85 dag kg^{-1}, K = 1.80 dag kg^{-1}, Ca = 1.11 dag kg^{-1}, Mg = 0.58 dag kg^{-1}, S = 0.41 dag kg^{-1}, C/N = 4.33, Zn = 203 mg kg^{-1}, Fe = 12041 mg kg^{-1}, Mn = 288 mg kg^{-1}, Cu = 76 mg kg^{-1}, B = 17 mg kg^{-1}.

The work was performed at the Floriculture Section of the Universidade Federal de Viçosa - Florestal Campus (UFV-CAF). The analyses were carried out in triplicate and the data from the experiments were entered into spreadsheets.

Extraction and Preparation of Humic Acids

Composting of organic waste was performed by Baldotto *et al*. (2013) in the area of the UFV-CAF, in the second half of 2011. The organic wastes cattle manure and poultry litter were acquired from the stable at UFV-CAF and from the poultry farm Granja Brasília, respectively. Livestock and poultry production management follows the usual recommendations for the Florestal/Pará de Minas region in the state. Cattle manure and poultry liter were composted isolate, without adding other residues or additives aim to obtain two different composts and HA to the biostimulant testing.

Composting management followed the recommendations of Kiehl (2004), which mainly consisted of control of aeration and moisture to ensure aerobic conditions, remove excess carbon dioxide, and standardize the composting mass. These procedures were carried out by irrigation and/or windrow turning. HA were isolated and characterized according to Baldotto & Baldotto (2013).

Humic Acid Application

The experiment consisted of thirty *A. millefolium* plants, each with about 5 cm length root soaked in HA for 23 hours. Plants were separated into a group of 15 plants soaked in HA derived from poultry litter and a group of 15 plants soaked in HA derived from cattle manure at the concentrations of 0, 10, 20, 30, and 40 mmol L^{-1}. The treatments were run in triplicate and randomized. After soaking in HA, the cuttings were planted in a 6 m x 1m bed, at triangular spacing of 60 cm between plants and 45 cm between rows, the treatments being placed in the bed on alternate concentrations, regardless of their origin, and irrigated daily for 90 days.

Variables Analyzed

The growth measurements taken on *A. millefolium* plants treated with HA (from cattle manure and poultry litter) and irrigated for 90 days were: leaf number (LN), root length (RL, mm), shoot length (SL, mm), plant height (PH, mm) using a digital model Starret pachimeter or meter tape. The biomass measures (analytical balanced) such as: root fresh mass (RFM, g), shoot fresh mass (SFM, g), total fresh mass (TFM, g), root dry mass (RDM, g), shoot dry mass (SDM, g), and total dry mass (TDM, g), root dry matter (RDM) and shoot dry matter (SDM), after dried at 60°C in a forced air ventilation oven to constant mass.

Statistical Analysis

The results of the evaluations after ninety days were subjected to regression analysis between the variable means and the HA concentrations tested. The F test was performed at 5% probability level. Regression equations were used to determine which HA (cattle manure or poultry litter) resulted in the best plant development and dose of maximum physical efficiency for total dry mass. Regression equations were considered acceptable when the F test were significant and the coefficient of determination (R^2) was greater than 0.60 ($R^2 > 0.60$).

RESULTS AND DISCUSSION

Tables 1 and 2 present the means, Tukey test and regression equations for measurements of growth and accumulated biomass of *Achillea millefolium* in response of increase on the humic acids concentration.

The results varied with the type of HA and the concentration tested. This variation may be due to the different origins of the composts (Carmo & Silva, 2012). Previous studies also pointed out that humic substances extracted from different sources have different effect on plant development (Silva *et al*., 1998; Façanha *et al*., 2002; Eyheraguibel *et al*., 2008; Baldotto & Baldotto, 2013). The regression equations for plant growth and biomass characteristics as a function of humic acid concentrations allowed the estimation of the concentration of maximum physical efficiency of *A. millefolium* TDM, which was 22.25 mmol L^{-1} of C for HApl resulting in the significant plant production of 72, 9251 g of biomass. This result corroborates the report of enhanced response of plants to humic acids in concentrations between 10 and 20 mmol L^{-1} by Baldotto & Baldotto (2013).

At the highest concentration (40 mmol L^{-1}), the growth and biomass measurements of the plants treated with poultry litter were negative and resulted in the lowest plant performance. The reason for that is that the effect of the biostimulant at high concentrations stabilizes and tends to decrease, sometimes being harmful when compared with no application, analogously to the auxinic effect which its high concentrations results on the stimulation of ethylene production with senescence effects (Baldotto *et al*., 2009; Baldotto & Baldotto, 2013).

Table 1: Means, mean square error (MSE), coefficient of variation (CV), Tukey's least significant difference (5%) for the characteristics leaf number (LN), root length (RL), plant height (PH), shoot length (SL), total fresh mass (TFM), shoot fresh mass (SFM), root fresh mass (RFM), total dry mass (TDM), root dry mass (RDM), and shoot dry mass (SDM) of *Achillea millefolium* in response to humic acids extracted from composted poultry litter (HApl) and cattle manure (HAcm)

Treatment	LN	RL	PH	SL	TFM	SFM	RFM	TDM	RDM	SDM
		-mm-			g					
HApl (0)	63	248	283	531	155.4	88.7	66.7	16.5	16.6	33.1
HApl (10)	44	200	282	483	155.5	82.1	63.4	20.8	21.6	42.4
HApl (20)	79	243	336	579	295.0	184.9	116.0	40.5	39.4	79.9
HApl (30)	95	242	397	639	272.8	170.9	101.9	36.6	35.8	72.4
HApl (40)	35	230	259	489	77.2	42.7	35.4	10.6	11.1	21.7
HAcm (0)	62	330	290	619	295.3	156.0	139.2	57.3	29.5	27.9
HAcm (10)	61	210	330	544	375.2	207.3	167.9	82.3	46.8	35.5
HAcm (20)	61	240	300	541	180.2	106.3	75.2	50.1	26.3	23.8
HAcm (30)	101	250	410	658	263.6	240.9	136.5	103.0	50.2	52.8
HAcm (40)	50	320	340	667	191.3	106.8	84.5	56.4	32.3	24.1
MSE	559	48	121	39	21771.8	7607.3	3458.6	1352.2	436.0	284.6
CV (%)	36	28	19	19	65.2	62.8	59.6	61.4	67.4	58.3
Tukey (5%)	40	12	19	11	250.9	148.3	100.0	62.5	35.5	28.6

Table 2: Regression equations of the characteristics leaf number (LN), root length (RL), plant height (PH), root fresh mass (RFM), shoot fresh mass (SFM), total fresh mass (TFM), root dry mass (RDM), shoot dry mass (SDM), and total dry mass (TDM) of *Achillea millefolium* in response to humic acids extracted from composted poultry litter (HApl) and cattle manure (HAcm)

Variable	Unfolding	Regression Equation	R^2
RL (mm)	HAcm	$\hat{y} = 31.969-1.0477x + 0.0268^{*}x^2$	0.862
RFM (g)	HApl	$\hat{y} = 53.934+ 5.2766x-0.\ 138^{*}x^2$	0.654
SFM (g)	HApl	$\hat{y} = 63.103+ 10.252x -0.2571^{*}x^2$	0.617
TFM (g)	HApl	$\hat{y} = 120.04+ 15.409x -0.395^{*}x^2$	0.667
RDM (g)	HApl	$\hat{y} = 12.727+ 2.343x-0.\ 0578^{*}x^2$	0.780
SDM (g)	HApl	$\hat{y} = 12.128 + 2.4462x-0.\ 0601^{*}x^2$	0.756
TDM (g)	HApl	$\hat{y} = 24.855+4.7892x-0.\ 1179^{*}x^2$	0.768

* = significant F test at 5% probability.

The physiological effect of humic acids is similar to that of the plant-hormone auxin (Façanha *et al*., 2002; Canellas *et al*., 2006; Zandonadi *et al*., 2007; Silva *et al*., 2011; Baldotto *et al*., 2012a). It promotes root development owing to the increased ATPase activity, which favors root expansion (Façanha *et al*., 2002), as observed in plants treated with HApl. The adequate initial development of the root system brings advantages to the plants, allowing for the exploration of a larger soil volume (Melo *et al*., 2015).

Although HA produced no significant increase in plant growth, there was increase in biomass of HApl-treated plants, proving the stimulating effect of these acids. This type of organic compost may have influenced soil moisture retention and contributed to increase fresh biomass of both root and shoot (Baldotto & Baldotto, 2013). However, the accumulation of total dry mass indicates good use of solar radiation, which is available at the beginning of the cycle (Halberstein, 2005). The positive effects on biomass increase indicate the biostimulating potential of humic acids, especially HApl. This humic acid present smaller C/N ratio, a humification index (Baldotto *et al*., 2007: Baldotto & Baldotto, 2018) correlated with the bioactivity of humified organic matter. Baldotto & Baldotto (2018) confirmed a relationship between the humification degree and the bioactivity of humic substances. The authors indicated that humic substances presents bioactivity in part because they contain residues like plant hormones in their structure and the aromatic and hydrophobic nature of stable carbon in combination with the aliphatic and hydrophilic character of humic substances results in a supramolecular structure with a large ion retention capacity. They also contain plant growth regulators (Taiz & Zeiger, 2004) which are not degraded during organic debris humification. The redox index of carbon stability is a sensitive index of conformational and structural changes in humic substances. It increases in proportion to the accumulation of stimulant biopolymers such as residues of auxins, which are not decomposed by microorganisms as are other polymers like carbohydrates, proteins, cellulose, etc., whose breakdown products remain within the supramolecular structure of humic substances. The organic matter solubilization allows it to be reused by plants, and the isolation of humic substances makes them bioactive and biostimulant in plants (Baldotto & Baldotto, 2014: Baldotto & Baldotto, 2018).

CONCLUSIONS

The concentration applied to plants and the source of organic matter influence the action of humic acids in plant development.

Humic acids extracted from poultry litter compost proved to be more bioactive than those extracted from cattle manure to the *Achillea millefolium* L. initial performance and present more humification degree (compost with smaller C/N ratio).

The concentration of maximum physical efficiency was 22.25 mmolL^{-1} of C for humic acids of poultry litter resulting in the significant plant production of 72, 9251 g of biomass.

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