Initial performance of corn in response to treatment of seeds with humic acids isolated from bokashi

Marihus Altoé Baldotto[1], Lílian Estrela Borges Baldotto[2]*

10.1590/0034-737X201663010009

ABSTRACT

The humified organic matter presents bioactivity similar to the auxinic effect. As bokashi is produced by a special process of humification, information is needed about the bioactive potential of its humic acids. The objective of this work was studying the initial performance of corn-indicator plants in response to the application of different concentrations of humic acids isolated from bokashi. The corn seeds were treated for 16 hours with solutions containing 0, 10, 20, 30, 40 and 80 mmol L^{-1} of C in the form of humic acids. Then, the seeds were planted in pots of 1 dm^3 containing corrected and fertilized soil, in greenhouse. Growth characteristics of shoot and root systems were evaluated. The results showed that the humic acids extracted from bokashi had positive effects on the initial performance of corn.

Key words: organic matter, humic substances, bioactivity.

RESUMO

Desempenho inicial do milho em resposta ao tratamento das sementes com ácidos húmicos isolados de bokashi

A matéria orgânica humificada apresenta bioatividade semelhante ao efeito auxínico. Como o bokashi é produzido por um processo especial de humificação, são necessárias informações sobre o potencial bioativo de seus ácidos húmicos. Este trabalho teve como objetivo estudar o desempenho inicial de plantas indicadoras de milho em resposta à aplicação de diferentes concentrações de ácidos húmicos isolados de bokashi. As sementes de milho foram tratadas por 16 horas, em soluções contendo 0, 10, 20, 30, 40 e 80 mmol L^{-1} de carbono na forma de ácidos húmicos. Em seguida, foram plantadas em vasos de 1 dm^3, contendo solo corrigido e fertilizado, em casa de vegetação. Foram avaliadas características de crescimento da parte aérea e do sistema radicular. Os resultados obtidos permitiram concluir que os ácidos húmicos extraídos de bokashi apresentam efeitos positivos no desempenho inicial do milho.

Palavras-chave: matéria orgânica, substâncias húmicas, bioatividade.

Submitted on 05/13/2015 and approved on 11/12/2015.
[1] Universidade Federal de Viçosa, Instituto de Ciências Agrárias, Florestal, Minas Gerais, Brasil. marihus@ufv.br
[2] Universidade Federal de Viçosa, Instituto de Ciências Agrárias, Florestal, Minas Gerais, Brasil. lilian.estrela@ufv.br
*Corresponding author: marihus@ufv.br

INTRODUCTION

The tropical climate and the great territorial extension are important factors for the outstanding participation of agriculture in the Brazilian economy. An important contribution comes from the cultivation of corn, which constantly demands new technologies so that the productivity continue to increase. Some potential technological options are the increase of rooting, nutrient absorption efficiency and plant chlorophyll content (Melo *et al*., 2015). These improvements can be obtained by applying biostimulants based on humic substances isolated from soil organic matter and composted organic waste.

Humic acids, present in the organic matter of organic compounds (Piccolo, 2001), may affect the rooting of various plants of agronomic interest (Baldotto & Baldotto, 2014). Their stimulating effects in the shoot, as increments in nutrient accumulation in leaves and chlorophyll synthesis (Baldotto *et al*., 2009), are also emphasized. Recent studies reviewed by Nardi *et al*. (2002), Baldotto & Baldotto (2014) and & Canellas Olivares (2014), showed that humic acids stimulate the activity and promotion of the synthesis of H^+ - ATPase enzymes in plasma membrane of plant cells, similarly to the auxinic effect.

Synthetic auxin, also called plant growth regulators (Taiz & Zeiger, 2003), are used on a commercial scale, with concentration-dependent response. Such a response happens similarly to humic acids, indicating the need to establish the optimal concentrations for each source and for each plant species (Baldotto *et al*., 2011). A potential source, which still needs information about the extraction and use of humic acid, is the bokashi.

The word bokashi, of Japanese origin, means “fermented organic matter”. It is also a Japanese painting technique that means “dilute” or “blur”, adapted by farmers to the practice of mixing organic matter to forest soil, leaving it to ferment before mixing it into the soil of crops. Some farmers still employ this technique using surface soil of the forest to make the fermentation of organic materials (Siqueira & Siqueira, 2013).

Bokashi is a balanced mixture of organic materials under controlled fermentation process. The bokashi nutrients are available as organic chelates, most often by increasing their availability. The production of bokashi, therefore, requires, in addition to balanced organic matter, the use of compost accelerator yeasts. There are several formulations of bokashi, but usually, it is made from plant waste, such as rice, wheat, barley brans and castor bean, cakes of sunflower, cotton and palm oil, enriched with animal meal (meat, bones and fish flour) and some minerals (natural phosphate, rock powder, limestone) in adequate amounts to not disrupt the lactic fermentation process, which is of the acid type (Siqueira & Siqueira, 2013).

In addition to being a nutrient source, bokashi introduces beneficial microorganisms into the soil, which trigger a process of fermentation in the available biomass, providing favorable conditions for fast multiplication and performance of beneficial microflora existing in the soil. Among these are fungi, bacteria, actinomycetes, mycorrhizal fungi and organic nitrogen fasteners that are part of the complex process, improving the chemical, physical and biological characteristics of the soil, contributing to plant nutrition and health. For these reasons, bokashi has been used by conventional, organic and farmers who want to do the agro-ecological transition (Siqueira & Siqueira, 2013).

Even with the preparation technology and use of bokashi relatively widespread, there are still technological opportunities, including, the isolation of humic acids (Baldotto & Baldotto, 2014) and use of its bioactivity, that is, its action as a plant growth regulator. Additionally, developing technology from the extraction of humic acids helps to increase the income of the bokashi producer. Therefore, we start from the assumption that the quality of bokashi, which puts it among highly preparation processes of organic compounds, both from the point of view of supplying nutrients and beneficial microorganisms, also leads to the production of humic acids containing bioactive substances capable of increasing the plant performance.

The aim of this study was to evaluate the initial performance of corn in response to the application of increasing concentrations of humic acids isolated from bokashi applied via seed.

MATERIAL AND METHODS

The experiment was conducted in the campus *Florestal* of the Universidade Federal de Viçosa, during the second semester of 2014.

The study factor was the application of humic acids isolated from bokashi in AG1051 corn seed, used as indicator plant, by evaluating its initial performance. Corn seeds were treated with concentrations of 0, 10, 20, 40 and 80 mmol L^{-1} of organic carbon in the form of humic acids isolated from bokashi. The humic acids were isolated and characterized according to the International Humic Substances Society (IHSS, 2015).

The application of the biostimulant was performed in the seeds, which remained submerged in humic acid solution in the concentration corresponding to each treatment for 16 h before planting. The seeds that were not treated with biostimulants were soaked in distilled water so that similar conditions of water absorption could be given to all treatments.

The experimental unit consisted of 1 dm^3 pots, filled with soil from 20 to 40 cm layer of a Typic Dystrophic Red Latosol. The soil received liming and fertilization, determined as recommended by Ribeiro *et al*. (1999). The corrected and fertilized soils were transferred into plastic pots that received five corn seeds each. After seedling emergence, thinning was carried out, and the experimental unit remained with two plants.

The experiment was conducted in a controlled environment, in a completely randomized design, with five repetitions, totaling 25 experimental units. The pots were monitored throughout the experiment. Daily irrigations kept the water holding capacity between 80 and 100%. These values were archieved on a volumetric basis (moisture equivalent) bymeans of weighing the vessels. Other cultural practices such as weed control, plant protection treatments, lighting, were carried out and standardized in all experimental units.

At the end of the experiment, 45 days after planting, the following variables were evaluated: number of leaves, length of the larger leaf, width of the median part of the larger leaf and diameter. Then, the plants were cut at soil level and the fresh matter of the shoot was weighed on a precision scale. The roots of the plants were isolated from the soil, carefully crumbled, and washed for determining their fresh matter. Both shoot and root systems were placed in paper bags and remained in a forced ventilation oven at 60 °C until reaching constant weight to determine the shoot and root dry matters. With the results of dry and fresh weigth of roots and shoots, dry and fresh, the ratio between the dry and fresh matters was calculated.

The results were submitted to analysis of variance through the program Genes and the effects of the treatments were analyzed by regression. The application of the F test, to the unfolding of the factors, was performed at 10, 5 and 1% probability and the regression models to explain the biological behavior were tested when they presented coefficient of determination higher than 0.60, according to Baldotto *et al*. (2011).

RESULTS

The results of the initial performance of corn plants treated with increasing concentrations of humic acids isolated from bokashi are shown in Table 1.

Table 2 provides the regression equations adjusted among the dependent variables, estimates of the performance of corn plants and increasing concentrations of humic acids isolated from bokashi, revealing a curvilinear behavior, with quadratic variations. In the same Table, we can see the coefficient of determination of each regression equation.

It is observed that for all variables analyzed, there was an initial increase, following the increase in the concentration of humic acid, followed by stabilization and decreasing increments in the highest concentrations used, characterizing a concentration-dependent behavior (Tables 1 and 2).

The concentration of humic acid of maximum physical efficiency, that is, the one that resulted in higher biomass accumulation of indicator corn plants, was obtained by means of the regression adjusted to the total dry master production. Through the derivatives from the regression equation, it was observed that the concentration of 45.70 mmol L^{-1} C in the form of humic acids (C_{HA}) maximized the initial performance of corn, resulting in a dry master accumulation of 3.37 g/plant. Compared to the control treatment with 2.39 g/plant of total dry matter, in the best concentration of humic acid, there was an increase of 0.98 g/plant (41%).

Replacing the value 45.70 mmol L^{-1} C_{HA} in the other regression equations of Table 2, the values of other variables were estimated to the condition of maximum physical efficiency, that is shown in Table 3, which also shows the increments in the initial performance of indicator corn plant in this concentration comparatively to the control treatment.

In Table 3, all the variables were positively incremented by the treatment with humic acids. However, the ratio root/shoot, both in fresh and dry matter, decreased with the application of humic acids, that is, the treatments with biostimulant had proportionally less investment in root biomass compared with the control treatment.

DISCUSSION

It is considered that the decomposition of plant residues in the soil does not destroy, at least fully, their bioactive substances or cannot change them and/or produce new molecules, which would persist and stabilize along with the humic substances. The structural conceptual models of the humic substances (macromolecular, and supramolecular) reviewed by Baldotto & Baldotto (2014) supports the hypothesis and the experimental data provide indications to accept the bioactivity mechanism, because humic substances notably alter plant development as observed in this study.

The cellular and molecular bases of humic acids action result from studies that point out the activity and promotion stimulation of the synthesis of H^+-ATPases enzymes in the plasma membrane, similar to the auxinic effect (Façanha *et al*., 2002). Façanha *et al*. (2002) showed that humic acids isolated from vermicompost and sewage sludge, promoted root development of corn and coffee seedlings and the activation of H^+-ATPase of the plasma membrane. The H^+-ATPases are transmembrane enzymes that are able to hydrolyze ATP to generate energy and an electrochemical gradient that is directly involved in two fundamental mechanisms for plant growth:

(i) energizing the secondary system of ion translocation, which is crucial for macro and micronutrients absorption (Sondergaard *et al*., 2004), a mechanism that can be explained by the action of H^+-ATPase in the plasma membrane depolarization and, therefore, in the activation of carriers;

(ii) promoting increase of cell wall plasticity by acidifying the apoplast, which is fundamental for the process of growth and elongation of plant cell (Cosgrove, 1997). This latter mechanism is associated with the *acid growth theory*, which postulates that an increase in the proton extrusion, mediated by the H^+-ATPase of the plasma membrane, induces the action of specific enzymes, which act on the cell wall, increasing its plasticity and, consequently, allow the elongation of the cell (Rayle & Cleland, 1992).

The disenssed above converger to several studies that have reported the presence of auxins on the supramolecular structure of the humic acids (Canellas *et al*, 2002; Trevisan *et al*., 2010).

The results lead, therefore, to technological opportunities with optimization and operation of the extraction, dilution, dosage and application procedure (Baldotto & Baldotto, 2014) of humic acids of bokashi for tropical agriculture, in convergence with the need for development of new inputs. The recognized high chemical and biological quality of bokashi to be applied to the soil, as well as all its fertility corollary (chemistry, physics and soil biology) (Siqueira & Siqueira, 2013), is added to the potential for complementarity with the biostimulant application. Thus, fertilization via soil enabled nutrient supply and the bioactivity stimulated the growth and development of corn plants.

It can be considered that the cost of both the bokashi and the extraction of humic acid is low, since they can be handmade with recyclable organic waste, including by family agriculture. The extraction procedures are simple and can be adapted to a rural company, without further waste impacts of the production process. As low concentrations are applied, the extraction process, even if operated by commercial companies, will reach the producer with affordable prices, which will not overtax the cost of production. The cost/benefit is low and, for

Table 1: Initial performance of corn in response to application of increasing concentrations of humic acids (HA) isolated from bokashi

Treatment	Variables[1]											
	NL	LL	WL	SD	SFM	RFM	SDM	RDM	TFM	TDM	FRS	DRS
AH (mmol L^{-1})	mm				g/plant							
0	3	50.7	15.0	7.7	11.26	11.20	1.30	1.09	22.46	2.39	1.23	0.83
10	4	60.2	24.8	9.0	12.78	11.18	1.52	1.20	23.96	2.73	0.84	0.84
20	3	60.1	26.0	8.8	13.62	10.98	1.65	1.24	24.60	2.89	0.95	0.74
40	4	69.0	27.3	9.2	16.88	12.12	1.98	1.45	29.00	3.43	0.78	0.60
80	4	66.6	26.7	8.6	13.96	12.62	1.55	1.06	26.58	2.61	1.05	0.77

[1] Variables: NL: number of leaves; LL: length of the longest leaf; WL: width of the largest leaf; SD: stem diameter; SFM: shoot fresh matter; RFM: root fresh matter; SDM: shoot dry matter; RDM: root dry matter; TFM = total fresh matter; TDM = total dry matter; FRS = fresh root/shoot ratio; DRS = dry root/shoot ratio.

Table 2: Regression equations and coefficient of determination (R^2) for estimates of the initial performance of corn in response to the application of increasing concentrations of humic acids (HA) isolated from bokashi

Variable[1]	Regression Equation	R^2
NL	$\hat{y} = 3.1462 + 0.0228\ x - 0.0002^{o}\ x^2$	0.8830
LL (mm)	$\hat{y} = 51.5820 + 0.6564\ x - 0.0059^{**}\ x^2$	0.9349
WL (mm)	$\hat{y} = 17.3450 + 0.4832\ x - 0.0046^{**}\ x^2$	0.8077
SD (mm)	$\hat{y} = 7.9498 + 0.0611\ x - 0.0007^{o}\ x^2$	0.7253
SFM (g/plant)	$\hat{y} = 10.8670 + 0.2264\ x - 0.0023^{**}\ x^2$	0.9200
RFM (g/plant)	$\hat{y} = 11.0260 + 0.0179\ x - 0.00005^{(17)}\ x^2$	0.8456
SDM (g/plant)	$\hat{y} = 1.2691 + 0.0289\ x - 0.0003^{o}\ x^2$	0.9616
RDM (g/plant)	$\hat{y} = 1.0588 + 0.0168\ x - 0.0002^{o}\ x^2$	0.9017
TFM (g/plant)	$\hat{y} = 21.8920 + 0.2442\ x - 0.0023\ x^2$	0.8841
TDM (g/plant)	$\hat{y} = 2.3278 + 0.0457\ x - 0.0005^{*}\ x^2$	0.9384
FRS	$\hat{y} = 1.1544 - 0.0183\ x + 0.0002^{o}\ x^2$	0.7298
DRS	$\hat{y} = 0.8786 - 0.0105\ x + 0.0001^{o}\ x^2$	0.7900

[1] Variables: NL: number of leaves; LL: length of the longest leaf; WL: width of the largest leaf; SD: stem diameter; SFM: shoot fresh matter; RFM: root fresh matter; SDM: shoot dry matter; RDM: root dry matter; TFM = total fresh matter; TDM = total dry matter; FRS = fresh root/shoot ratio; DRS = dry root/shoot ratio; **, *, º and (^{p}) = significant at 1, 5, 10 and p% probability, respectively.

Table 3: Estimates of corn initial performance in response to the application of the concentration of humic acid (HA) isolated from bokashi of maximum physical efficiency for total dry matter (45.70 mmol L^{-1} C_{HA})

Variable[1]	MPE Value	Control Value	Difference (%)[2]
NL	3,8	3,0	23
LL (mm)	69,8	50,7	38
WL (mm)	30,3	15,0	102
SD (mm)	9,3	7,7	21
SFM (g/plant)	16,4	11,3	45
RFM (g/plant)	12,6	11,2	13
SDM (g/plant)	1,97	1,30	52
RDM (g/plant)	1,41	1,09	29
TFM (g/plant)	28,37	22,46	26
TDM (g/plant)	3,37	2,39	41
FRS	0,74	1,23	66
DRS	0,60	0,83	38

[1] Variables: NL: number of leaves; LL: length of the longest leaf; WL: width of the largest leaf; SD: stem diameter; SFM: shoot fresh matter; RFM: root fresh matter; SDM: shoot dry matter; RDM: root dry matter; TFM = total fresh matter; TDM = total dry matter; FRS = fresh root/shoot ratio; DRS = dry root/shoot ratio; Difference [2] (%) = [(highest mean – lowest mean) / lowest mean] x 100.

the economic analysis, the cost of application is practically used, in face of which the cost of preparation can be neglected (Baldotto & Baldotto, 2014).

As the root biomass increases, the use efficiency of fertilizers is higher, having all the benefits of chemical, physical and biological improvements of the soil if combined with bokashi applications directly to the soil (Siqueira & Siqueira, 2013). Also, the direct application of bokashi to the soil does not result in biostimulant effect similar to the effect of isolated humic acids, mainly because the isolation solubilizes and provides the bioactive substances optimally. This fact is very important because even in high-productivity agriculture, it is imperative to seek new fertilizers, given the high cost and the finitude of raw materials that come from non-renewable natural resources. Finally, it is inferred that it is strategically appropriate to encourage the use of humic acids, both for agriculture of large areas and productivity and for organic farming, commonly familiar, and that the use of humic acid is suitable for all these modalities, without incompatibility with their production systems, stimulating the nutrient absorption from the restitution with mineral and organic fertilizers, or both, including additional effects of metabolic, conditioner and protective order.

CONCLUSIONS

The growth response of indicator corn plants to the treatment of seeds with humic acids isolated from bokashi had mostly positive and quadratic increments.

For the humic acids extracted from bokashi, the concentration 45.70 mmol L^{-1} of C resulted in maximum dry matter accumulation of indicator corn plants, with an increase 41% higher than the control.

ACKNOWLEDGMENT

This work counted with financial support from CNPq, FAPEMIG and FUNARBE. The authors thank the students, staff and faculty of the Campus Florestal of UFV who contributed to the realization of this project.

REFERENCES

Baldotto LEB, Baldotto MA, Giro VB, Canellas LP, Olivares FL & Bressan-Smith R (2009) Desempenho do abacaxizeiro 'Vitória' em resposta à aplicação de ácidos húmicos durante aclimatação. Revista Brasileira de Ciência do Solo, 33:979-990.

Baldotto MA, Muniz RC, Baldotto LEB & Dobbss LB (2011) Root growth of *Arabidopsis thaliana* (L.) Heynh. treated with humic acids isolated from typical soils of Rio de Janeiro state, Brazil. Revista Ceres, 58:504-511.

Baldotto MA & Baldotto LEB (2014) Ácidos húmicos. Revista Ceres, 61:856-881.

Canellas LP, Façanha AO, Façanha AR & Olivares FL (2002) Humic acids isolated from earthworm induces root mitotic sites and plasma membrane H^+-ATPase. Plant Physiology, 30:1951-1957.

Canellas LP, Olivares FL, Okorokova-Facanha AL & Facanha AR (2002) Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H-ATPase activity in maize roots. Plant Physiology, 130:1951-1957.

Canellas LP & Olivares FL (2014) Physiological responses to humic substances as plant growth promoter. Chemical and Biological Technologies in Agriculture, 1: 3-14.

Cosgrove DJ (1997) Relaxation in a high-stress environment: The molecular bases of extensible cell walls and cell enlargement. Plant Cell, 9:1031-1041.

Façanha AR, Façanha ALO, Olivares FL, Guridi F, Santos GA, Velloso ACX, Rumjanek VM, Brasil F, Schripsema J, Braz-Filho R, Oliveira MA & Canellas LP (2002) Bioatividade de ácidos húmicos: Efeito sobre o desenvolvimento radicular e sobre a bomba de prótons da membrana plasmática. Pesquisa Agropecuária Brasileira, 37:1301-1310.

IHSS - International Humic Substances Society (2015) Products. Disponível em: <http://www.ihss.gatech.edu/products.html>. Acessado em: 10 de maio de 2015.

Melo RO, Baldotto MA & Baldotto LEB (2015) Desempenho inicial do milho em resposta a ácidos húmicos isolados de esterco bovino e cama de aviário. Semina: Ciências Agrárias, 36:1863-1874.

Nardi S, Pizzeghello D, Muscolo A & Vianello A (2002) Physiological effects of humic substances on higher plants. Soil Biology and Biochemistry, 34:1527-1536.

Rayle DL & Cleland RE (1992) The acid growth theory of auxin induced cell elongation is alive and well. Plant Physiology, 99:1271-1274.

Ribeiro AC, Guimarães PTG & Venegas VHA (1999) Recomendações para uso de corretivos e fertilizantes em Minas Gerais: 5ª Aproximação. Viçosa, Comissão de Fertilidade do Solo do Estado de Minas Gerais. p.25-32.

Siqueira APP & Siqueira MFB (2013) bokashi: adubo orgânico fermentado. Niterói, Programa Rio Rural. 16p. (Manual Técnico 40).

Sondergaard TE, Schulza A & Palmgren MG (2004) Energization of transport processes in plants. Roles of plasma membrane H^+-ATPase. Plant Physiology, 136:2475-2482.

Piccolo A (2001) The supramolecular structure of humic substances. Soil Science, 166:810-832.

Taiz L & Zeiger E (2003) Fisiologia vegetal. 3ª ed. Porto Alegre, Artmed. 719p.

Trevisan S, Francioso O, Quaggiotti S & Nardi S (2010) Humic substances biological activity at the plant-soil interface from environmental aspects to molecular factors. Plant Signaling & Behavior, 5:635-643.