Evaluation of The Effect of a Vermicompost Extract in The Cultivation of Lettuce (*Lactuca sativa L.*)

Evaluación del Efecto de un Extracto de Vermicompost en el Cultivo de Lechuga (*Lactuca sativa L.*)

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Fecha de recepción: julio de 2016 / **Fecha de aceptación:** noviembre de 2016

Abstract

Nowadays, within the alternating ones of the agriculture are the liquid humic substances from organic sources of recyclable character like those coming from the compost and vermicompost; for this reason, this experiment focused on evaluating the effects of different solutions of a vermicompost extract on some biochemical-physiological indicators in organoponic conditions at two moments of its vegetative growth stage. For this, the species Lechuga (*Lactuca sativa,* L. var. Black S. Simpson) was used; the treatments were distributed in a completely randomized design, where the factor under study was spraying with vermicompost extract at 1/60 (v:v). and 1/70 (v:v); 4 replicates per treatment and one control were used. The substrate used was composed of ferralitic red leached soil, combined with cachaça in a ratio of 3:1 (s:v); where the indicators evaluated were benefited by the exogenous applications of vermicompost extract as a biostimulant; in addition of, generating important utilities from the economic and agro-ecological point of view. The best results were observed in the T3 treatment where 1/70 solution was used, since a significant increase in the number of leaves, dry mass content and the length of the same was obtained.

Key words: biostimulant, extract, lettuce, humic, aspersion.

Resumen

Hoy en día, dentro de las alternantes de la agricultura se encuentran las sustancias húmicas líquidas de fuentes orgánicas de carácter reciclable como las procedentes del compost y el vermicompost. Por esta razón, este experimento se centró en evaluar los efectos de diferentes soluciones de un extracto de vermicompost en algunos indicadores bioquímicos-fisiológicos en condiciones organopónicas en dos momentos de su etapa de crecimiento vegetativo. Para esto, se utilizó la especie Lechuga (Lactuca sativa, L. var. Black S. Simpson); los tratamientos se distribuyeron en un diseño completamente aleatorizado, en el cual el factor en estudio se pulverizó con extracto de vermicompost a 1/60 (v: v). y 1/70 (v: v). Se usaron cuatro réplicas por tratamiento y un control. El sustrato utilizado fue compuesto de suelo ferralítico rojo lixiviado, combinado con cachaza en una proporción de 3: 1 (s: v), en el que los indicadores evaluados se vieron beneficiados por las aplicaciones exógenas del extracto de vermicompost como bioestimulante, además de generar importantes utilidades desde el punto de vista económico y agroecológico. Los mejores resultados se observaron en el tratamiento T3, en el cual se utilizó la solución 1/70, ya que se obtuvo un aumento significativo en el número de hojas, contenido de masa seca y la longitud de la misma.

Palabras clave: bioestimulante, extracto, lechuga, húmico, aspersión.

Introduction

It is known that, organic matter of the soil is one of the most important resources and that its content depends on their fertility, it encompasses plant residues in varied stages of decomposition, microbial biomass and the more stable fractions known as humic substances (Guridi, 2000), the latter constituting about 80% of the soil organic matter. But recently, with the use of mineral fertilizers in an indiscriminate manner, intensive regime agriculture has resulted in a loss of the optimal levels of soil organic matter, salinity, and prolonged droughts due to the imbalance generated in agroecosystems (Ramos, 2000).

In this sense, Miklos et al. (2000) report that the decline in the use of chemical fertilizers and synthetic pesticides is one of the most important factors in the implementation of biodynamic agriculture - implementation of a group of agricultural practices such as crop rotation, green manure, organic fertilization, etc. - or of a sustainable character, humic substances being one of the alternatives used for the development of this practice; in addition, humic substances are the subject of study in several areas of agriculture, such as soil chemistry, fertility, plant physiology as well as environmental sciences, due to the multiple roles played by these materials that greatly benefit growth and development of plants (Tan, 1998).

On the other hand, Pizzeghello et al. (2000) argue that in organic agriculture it is feasible to use liquid humic substances obtained primarily from recyclable organic sources such as compost and vermicompost due to their beneficial effects on plant development and relatively low production costs. A good part of the experimental evidence obtained to evaluate the effects of humic substances has been carried out using humic substances isolated and purified from different sources. In summary, the effects of humic substances on plant growth and development point to the positive influence on ion transport by facilitating absorption, direct action on metabolic processes such as respiration, photosynthesis and protein synthesis. increased or decreased activity of various enzymes, metabolite content and hormone-like activity of these substances, mainly auxin type Nardi et al. (2002); coinciding this data with later results obtained by Canellas et al. (2004).

Thus, the objective of the present study was to evaluate the effect of different dilutions of vermicompost extract on some biochemical-physiological indicators in organoponic conditions at two moments of its vegetative growth stage.

Materials and methods

Location and soil. The experiment was carried out under natural light conditions in the "La Matilda" organoponic area, located in Nueva Paz municipality, south-east of the province of Mayabeque, Cuba.

Lechuga plants (*Lactuca sativa,* L. var. Black S. Simpson) were used as plant material; were planted on a substrate described by Hernández, et al. (2015), composed of ferralitic red leached soil combining with cachaça in proportion 3: 1 v.v. Chemical analysis of the substrate is shown in Table 1.

Table 1. Chemical analysis of the substrate used (soil: cachaça -3:1-).

COMPONENTS	Na	K	Ca	Mg	P	M.O.
	cmol * Kg				(ppm)	(%)
Soil + Cachaça	0.15	0.57	14.9	2.0	1388	5.29

Design and treatments. The treatments were distributed in a completely randomized design, where the factors studied were spraying with vermicompost extract 1/60 and 1/70 v.v., as described below:

a) **T1 (A)** witness: no vermicompost extract (with irrigation)

b) **T2 (B)** with extract vermicompost 1/60 v.v. (with irrigation)

c) **T3 (C)** with extract vermicompost 1/70 v.v. (with irrigation)

Four replicates were established for treatments, these were denoted; in the case of the Control (A1, A2, A3 and A4) for dilution 1 / 60v.v. (B1, B2, B3 and B4) and in the case of extract vermicompost 1/70 (C1, C2, C3 and C4).

Procedure and measurements. Positions of lettuce of 20 days of germinated were used, which were characterized by having at least three healthy leaves and a height that oscillated between the 7 - 10 cm of height. These were planted in two 17.5 and 24.5 m long beds by 1m wide and 0.35 m high, located from north to south, to ensure uniform luminosity throughout the plantation. The planting frame used for these cultivation conditions was 0.15 m between plants and 0.25 m between rows, to achieve optimum development of all plants and decrease the effect of competition for water, light and nutrients.

Two applications of the biostimulant (vermicompost extract) were made. The first one was carried out 5 days after transplantation by foliar spray of the product at the dilutions 1/60 and 1/70 v.v., until the drip point was reached, while the second spray was performed 15 days after the first one (5 and 20 days after transplantation).

All plants of the experiment were irrigated manually, according to the crop requirements (first 15 days daily and then until harvest, on alternate days), taking in each case to the soil up to 100% of their Capacity of Field (CF). In plants such as lettuce, where its organ of consumption is the leaves, a stress due to drought, would noticeably affect the results.

Morphological variables. *Length of roots, stems and leaves:* At the time of harvest the indicators were established, such as: length of roots, stems and leaves (in this last organ was determined the length taking as a reference the central nerve of the same) with the use of a Foot of King.

Comparison of number of sheets: The counting of the visible leaves determined the number of leaves. This was done in two moments, at 10 days after transplantation (moment I -MI-) and at the moment of harvest (moment II -MII-).

Growth and development variables: The physiological variables related to growth and development were evaluated during the vegetative period, at a rate of 20 plants per treatment and 5 per replicate, randomly selected from within the population. The variables were as follows:

Fresh mass: The evaluation of the fresh mass growth of plant organs (roots, stems and leaves, expressed in g / plant), was determined in 20 plants per treatment. For this, three measurements were made, 5 days after the first application and then with a frequency of 15 days for the remaining two measurements from the first measurement. The mass of the vegetative organs was obtained in analytical balance.

Biomass: The evaluation of growth in dry biomass of plant organs (roots, stems and leaves, expressed in g/plant), was determined in 20 plants per treatment in the two moments mentioned above. In all cases the plants were placed in paper envelopes, dried in an oven at 70 ºC until constant mass, which was acquired at 72 h. Analytical balance determined the control of the dry mass. For this, the same plants were used in which the fresh mass was evaluated.

Concentration analysis of photosynthetic pigments: The photosynthetic pigments analyzed were the "chlorophylls a and b". For the analysis, 20 discs of the leaves were obtained, distributed in the three levels of the plant (low, medium and superior) with a perforator of 0.93 cm in diameter. The disks were immersed in 80 % acetone solution and refrigerated for 48 h to 3 °C. Its analysis was carried out performed the Electro-photometry technique, using white acetone at 80 %. With this technique was determined the absorbance at different wavelengths (663, 647 and 470 nm respectively), recording these data in a specialized spreadsheet for this effect.

Performance Indicator: For the analysis of this indicator only the aerial organs of 20 plants per treatment were considered, manually counting the number of leaves emitted by the plants and measuring their length.

Statistical analysis. To perform the Statistical Analysis, the primary and graphic data of each of the indicators evaluated were processed using the Windows Excel tool. A completely randomized analysis of variance (STATGRAFICS v-5.1) and simple classification (ANOVA) were performed, since a completely randomized experimental design was used for each of the variables studied. In cases where the indicators showed significant differences was used for multiple comparison of means, the TUKEY test at.

Results and Discussion

When analyzing the influence of treatment, it was obtained that; the growth that was shown in the stem of the treated plants, specifically of which T2 was applied, may be due to the possible hormonal action exerted by these substances, in which the cell elongation is stimulated, and analogies between the action of the humic substances and different growth regulators such as gibberellins, cytokinins and auxins (Clapp et al., 2001; Nardi et al., 2003).

With respect at the root length, all treatments differ from each other; showing the highest values for the plants under the T3 variable; for the stem length the most outstanding values were those obtained in T2, which differed significantly from the control and T1. For the leaves, the largest values of the length were obtained in T3, which differs significantly from the remaining two, as shown in Fig. 1. The stimulation of the root development with the use of this type of biostimulant, would favor to the plants an increase in the absorption of nutrients, benefiting the growth and later development of the aerial part (stem and leaves) and of the whole plant in sense general (Guridi, 2000, Canç et al., 2002, Canellas et al., 2002 and 2004, Reyes et al., 2017).

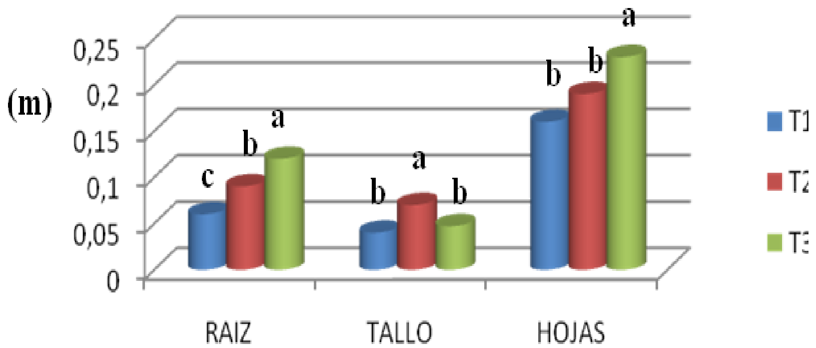

Figure 1. Length of indicators of lettuce plants, treated at different concentrations. CV = 15.3 % (root); 11.4 % (stem); 18.9 % (leaves). T1-control, T2-1/60, T3-1/70.

These results agree with what was reported in the literature (Guridi, 2000, Façanha et. al., 2002, Canellas et. al., 2002 and 2004, Reyes et. al., 2015), in which humic substances can stimulate the development of vegetables treated with liquid humus.

Comparison of the number of leaves in both study moments. When analyzing the number of leaves emitted by the plants under study, it was found that, for the first moment the highest values were obtained for T3, treatment that only differs, at this moment of the T1; marking a much more evident difference in the second moment, in which T3 differs significantly from the rest of the treatments as shown in Figure 2. These values coincide with those obtained by Reyes (2000), in relation to the lengths of the leaves; indicators that directly affect the yield of the crop, considering that it is in the leaf where the agricultural importance is established.

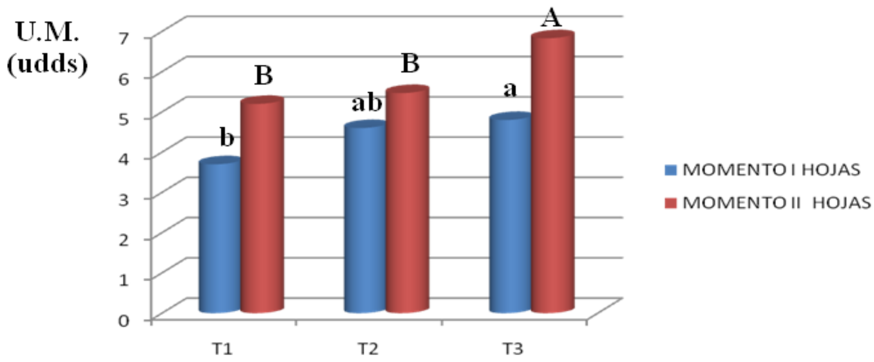

Figure 2. Number of leaves per plants under different treatments. CV = 11.2 % (MI) and 18.4 % (MII). T1-control, T2-1/60, T3-1/70.

In the same way, Reyes (2000) argues that with the application of vermicompost, the number of leaves evaluated in horticultural species such as tomato, basil, lettuce and celery increased notably the number, size of leaves and height of the plant.

According to Chen et al. (1990), these results can be attributed to the fact that the humic substances applied by foliar route show stimulating effects given the possible almost immediate absorption, which results in a rapid development of apical meristems because it influences some biochemical processes of the cell wall. In addition, it points out that it can act in the transport of nutrients by interacting with the phospholipids of cell membranes.

Furthermore, the main components of this extract are mainly humic substances, known for their effects and participation in the different physiological processes biochemical in plants; with positive influence on respiration and the activity of enzymatic systems of the Krebs cycle that leads to a higher production of Adenosine Triphosphate (ATP); as well as, selective effects on protein synthesis and increase or inhibition of the activity of other enzymes, etc. (Nardi et. al, 2002).

Growth and development variables. Next, the behavior of some variables that reflect more clearly the effect of treatments on lettuce plants and the response of these to the application of vermicompost extract in two different dilutions.

Fresh mass (FM). For FM analysis of vegetative organs (root, stem and leaves) it was necessary to make a separation by plant organs in each of the treatments, see figure 3. At the root, the largest increase of FM was presented in T2, differing significantly from the two remaining treatments. In the stem, the highest values were presented in T3 opposite the control and T2. For the leaves, the highest results were obtained in T2, presenting a significant difference with respect to the other treatments. Therefore, the highest values of FM were presented in plants that were treated with T2 evidenced in the root and leaves. The results of this study were like those reported by other authors (Días et al., 2002, Caru 2004, Besú et al., 2005) with the use of liquid humus in the root mass index in different crops, such as broccoli, radish, carrot, chard, and corn.

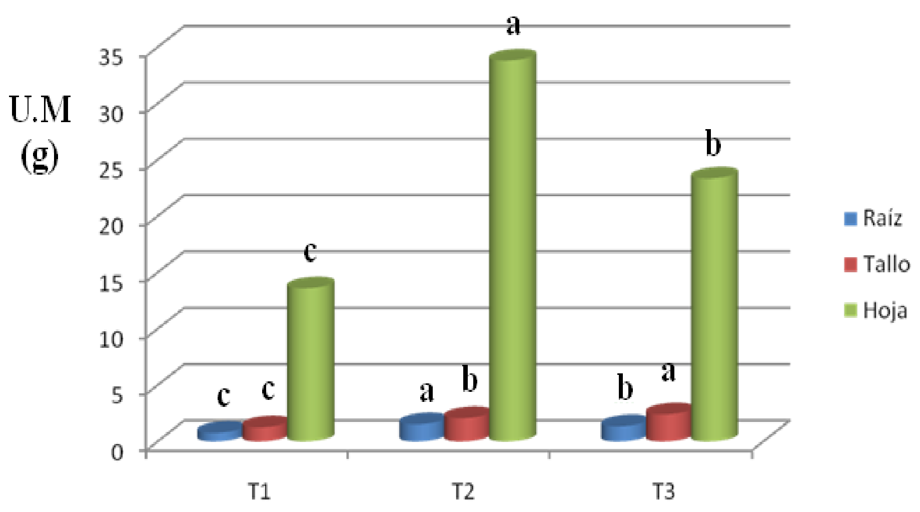

Figure 3. Variation of the fresh mass (FM) of the vegetative organs of the plants (root: CV = 10.9 %, stem: CV = 6.4 %, leaves: 15.2 %).

The applications were done foliar form until the point of drip, showing for the treatment T2 (the less diluted) an increase of the mass of the roots; which is of interest since when analyzing the total fresh mass of the aerial part with respect to that of the root and comparing it with the previous treatments, this one presents a greater proportion, since the root development is greater for T2, making it possible for the plant to be in a better position to adsorb the nutrients contained in the soil necessary for your metabolism. Authors such as, Guridi (2000), Façanha et al. (2002); Canellas et al. (2002 and 2004); Reyes et al. (2015) have related the stimulation of root development using humic acids isolated from Vermicompost, which are incorporated into the rhizosphere, as well as the ones expressed by Reyes et al. (2017), where roots of different plants in contact with humic acids of the extract vermicompost, stimulated the sites of mitosis in them, resulting also in a greater emission of secondary roots.

In terms of stem length, there was a slight increase in T3 plants (1/70 v.v.) compared to the others. Similar results were obtained by De Grazia et al., (2001), in nutritional work for this crop, which showed that biostimulants derived from vermicompost have many phytohormones that are directly related to stem growth, aspects that in lettuce, according to the same author, is related to the number of leaves.

Dry mass (DM) of roots, stems and leaves. As for the dry matter (DM) indicator of the root, stem and leaves, it can appreciate (see Figure 4) that at the first moment (M1) the greatest accumulation was in the plants treated in T3, a behavior that remained in the second moment (MII), being in the latter more prominent the accumulation of DM. It should be noted that only in the stem did not show significant differences between the two treatments and control for the parameter under study.

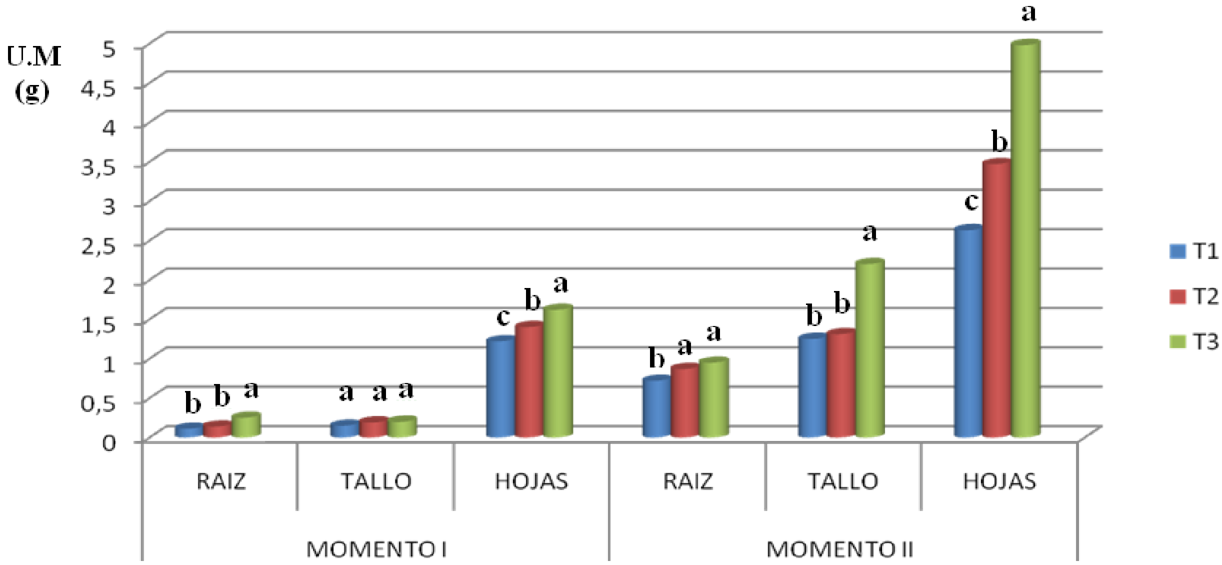

Figure 4. Dry mass of indicators of lettuce plants at different concentrations in two moments. MI-(root: CV = 21.4 %, stem: CV = 26.149 %, leaves: CV = 17.8 %); MII-(root: CV = 17.3 %, stem: CV = 18.19 %, leaves: CV = 24.56 %).

These results do not show the same trends as the MF indicator, which seems to indicate that the elaborated substances have been stored, but not consumed in the growth processes carried out by the plants under T3. A greater accumulation of MS is related to a higher nutritional value. These results are like those found in other crops by different authors (Díaz et al., 2002; Caru 2004; Besú et al.; 2005, Huelva et al., 2004), showed that humic acid (HA) and fulvic acids (FA) extracted from vermicompost of bovine manure achieved an increase in the dry foliar mass of grains and vegetables.

Content of chlorophyll a and b in plants treated with vermicompost extract. The results obtained when evaluating the content of photosynthetic pigments, chlorophyll a and b, in each treatment can be seen in figure 5; there it is observed that the content of total chlorophylls (a + b) found in T2-1/60 v.v., surpassed only those treated under the T3-1/70 standards; not showing significant differences between any of the treatments for the physiological indicators evaluated. Results that differ from those obtained by Vaughan et. al. (1985), Nardi et. al. (2002); Huelva et al. (2004), who have expressed that the use of the humic substances brings with it the increase in chlorophylls; furthermore Yang et al. (2004), using HA, FA and Huminas extracted from different soils, evaluated the effect of these three humic substances (HS) on the activity of the enzymes chlorophyllase "a" and chlorophyllase "b" and found that the FA stimulated the activity of chlorophyllase "a" more than the other two HS and that HA further stimulated the activity of chlorophyllase "b".

So, it seems that other metabolic processes were favored with the applications of vermicompost extract. Nardi et. al., (2002) state that the extract has, among others, SH, of which its effects and participation in different physiological and biochemical processes in plants are known, with positive intervention in respiration, speed of enzymatic reactions of the Krebs cycle, which leads to a higher production of ATP, as well as to have selective effects on protein synthesis and increase or inhibition of the activity of various enzymes, etc.

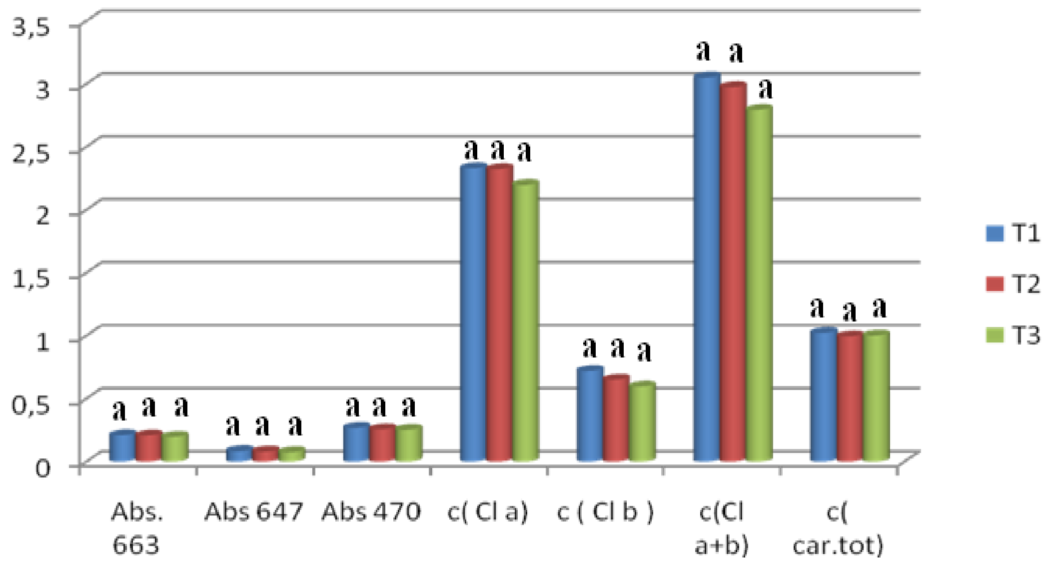

Figure 5. Chlorophyll content "a" and "b" (Cla and Clb) in mg / ml extract in plants treated at different concentrations. CV = 14.9 %.

Moreover, there are reports indicating that HS activity in the alkaline extracts of vermicompost exert a similar action to phytohormones such as auxins or polyamines (Yang et al., 2004). On the other hand, this humus contains minerals and amino acids, chemical fractions that by themselves or combined with previous ones that could provoke synergistic nutritional or biostimulation effects in the growth and development of plants (Garcés et al., 2003).

Thus, the application of vermicompost extract via leaf, according to Caro et. al.,

(2002) is more efficient in the short term than the root canal; although this last one is the advisable one to favor the rooting after the transplant, mainly in crops of vegetables. That is why this result could be of interest to producers who are engaged in lettuce cultivation, as it is a biostimulator that besides being of national production, is a product with a high stability in its composition and demonstrated effects.

Conclusions

Given the application of the extract, the length of roots, stems and leaves was stimulated; and the production of fresh and dry mass; resulting in the most promising solution.

No similar effects were found on photosynthetic pigment content; but in both treatments (1/60 and 1/70) stimulation was favored in the indicator number of leaves per plants. Additionally, due to the applications of the extract, an additional harvest was achieved in the same period of time, presumably due to the stimulating effect on the growth and development of the plants.

References

Besú, D. & Ruíz, Y. (2005). *Efectos de diferentes concentraciones de Liplant en el cultivo del rábano rosado*. Taller de investigación científica estudiantil en ciencias agropecuarias. Evento de Ciencias Universitarias. CAG04, 56-68.

Canellas, L.P. & Façanha, A.R. (2004). Chemical nature of soil humified fractions and their activity. *Pesquisa Agropecuaria brasileira. 39* (3), 233-240.

Canellas, L.P., López. O.F., Okorokova, N.A. & Rocha, F.A. (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.

Caro, I. (2004). *Caracterización de algunos parámetros químico - físico del humus líquido obtenido a partir de vermicompost de estiércol vacuno y su evaluación sobre algunos indicadores biológicos y productivos de dos cultivos*. (Tesis de Maestría). UNAH, La Habana.

Caro, I., Garcés, N., Guridi, F., Huelva, R., Mesa, S. & Arteaga, B. (2002). *Efecto bioestimulante del Liplant en el cultivo del maíz (Zea mayz)*. XIV Seminario Científico-Técnico INCA. ISBN: 959-7023-22-9. (CD).

Chen, Y. & Aviad, T. (1990). Effects of humic substances on plant growth. In: Humic substances in Soil and Crop Sciences. *Selected readings*, 161-186.

Clapp, C.E., Chen, Y., Hayes, M.H.B. & Cheng, H.H. (2001). *Plant growth promoting activity of humic substances*. In: Swift RS, Sparks KM, eds. Understanding and managing organic matter in soils, sediments and waters. Madison, WI: IHSS, 243.

De Grazia, J., Tittonell, P. & Chiesa, A. (2001). Efecto de la época de siembra, radiación y nutrición nitrogenada sobre el patrón de crecimiento y el rendimiento del cultivo de lechuga (Lactuca sativa L.). *Invest. Agr. Prod. Prot. Veg.* 16(3).

Díaz, M., Terán, Z. & Garcés, N. (2002). *Efectos de compuestos obtenidos a partir de vermicompost sobre vegetales*. I Encuentro Provincial de Agricultura Orgánica, ACTAF. *Resúmenes*, 104.

Façanha, A.R., Façanha, A.L.O., Olivares, F.L., Guridi, F., Santos, G.A., Velloso, A.C.X., Rumjanek, V.M., Brasil F., Schripsema, J., Braz-filho, R., Oliveira, M.A. & Canellas, L.P. (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.

Garcés, N., Marbot, R., Ramos, R. & García, L. (2003). *Sustancias con actividad biológica sobre las plantas en el producto Liplant (Humus l*íquido). V Encuentro de la Agricultura Orgánica de la Actaf. La Habana, Cuba, 71.

Guridi, F. 2000. *O fósforo, a materia orgánica y a micorriza no cafeeiro (C. Arabica L.)*. (Tesis de Doctorado). UFRRJ, Brasil.

Hernández, A., Pérez, J.M., Bosch, D. & Castro, N. (2015). *Clasificación de los suelos de Cuba. Mayabeque*. Cuba: Instituto Nacional de Ciencias Agrícolas, Instituto de suelos, Ediciones INCA.

Huelva, R., Garcés, N., Ramos Amarilys. y León, P. (2004). *Evaluación de la bioactividad del humus líquido obtenido a partir de vermicompost y sus fracciones húmicas en el cultivo de soya (Glycine max; var: INCASOY-24)*. XIII Congreso del INCA, CD-ROM. ISBN 959-7023-22-9.

Nardi, S., Pizzeghello, D., Bragazza, L., y Gerdol, R. 2003. Low-molecular-weight organic acids and hormone-like activity of dissolved organic matter in two forest soils in N Italy. *Journal of Chemical Ecology*, 9(7), 1549-1564.

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

Primavesi, A. (2003). Conferencia impartida en el V Encuentro de la Agricultura Orgánica de la ACTAF. La Habana, Cuba.

Ramos, R.R. (2000). *Aplicación de sustancias húmicas como productos de acción bioestimulantes. Efectos frente al estrés salino*. (Tesis de doctorado). Valencia, España.

Reyes, A.J.C. (2000). *Micorriza arbuscular y vermicomposta en el desarrollo y fisiología de un portainjerto de aguacate raza mexicana (Persea americana Mill.) en un sustrato alternativo de vivero*. (Tesis de Maestría). Colegio de Postgraduados, Montecillo, México.

Reyes, J.J.P., Abasolo, F.P., Yépez, Á.J.R., Luna R.A.M., Zambrano, D.B., Vázquez, V.F.M., Cabrera, D.A.B., Guzmán, J.A.A., Torres, J.A.R. & Rodríguez, W.O.M. (2017). Ácidos húmicos y su efecto sobre variables morfométricas en plantas de zanahoria (*Daucus carota L*). *Biotecnia* 19(2), 25-29.

Reyes, J.J.P., Torres, J.A.R., Murillo, B.A., Herrera, M.F.H., Fernando, G.I., Luna, R. A.M., López, R.J.B. y Real, G.E.G. (2015). Humatos de vermicompost y su efecto en el crecimiento de plántulas de tomate *(Solanum lycopersicum l.)*. *Biotecnia*. 17(2), 9-12.

Tan, K.H. (1998). Colloidal chemistry of organic soil constituents. In: Tan, K.H., (ed). *Principles of soil chemistry*. New York: Marcel Dekker, 177-258.

Vaughan, D. y Malcolm, R.E. (1985). Influence of humic substances on growth and physiological processes. In: Soil organic matter and biological activity. Vaughan, D., Malcolm, R. E. (eds). Dordrecht: Martinus Nijhoff Pub, 37-76.

Yang, C.M., Wang, M.C., Lu, Y.F., Chang, I.F. y Chuou, C.H. (2004). Humic substances affect the activity of chlorophyllase. *Journal of Chemical Ecology*. 30 (5), 1057-1065.