Article

Growth of tomato seedlings (*Solanum lycopersicum* L.) treated with vermicompost humate

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Abstract

The humate of vermicompost is a stimulant that improves the quality and productivity of crops and allows the total or partial elimination of chemical fertilizers, contributing to organic, ecological and sustainable agriculture. The objective of this study was to evaluate the effect of different concentrations of vermicompost humates (1/10, 1/20, 1/30 v/v and a control-distilled water-) on the growth of *Solanum lycopersicum* seedlings grown on affected soils by salinity. The experiment was carried out using a completely randomized design with four repetitions per treatment of 40 seedlings each. At 24 days after transplantation, height of seedlings, stem diameter, number of leaves, fresh and dry weight of aerial part and root were measured. The results showed significant differences between treatments and all the variables showed higher values in the dilution of 1/30 (v/v), followed by the dilutions 1/20 and 1/10, showing lower values in the control. The percentage value of increase in the dilution of 1/30 with respect to the control was 61, 68, 63, 50, 19, 30, 56 and 27 for height, stem diameter, root length, number of leaves, fresh weight of root, dry weight of root, fresh weight of aerial part and dry weight of aerial part, respectively.

Keywords: height, fresh weight, dry weight, salinity.

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Introduction

The tomato (*Solanum lycopersicum* L.) is considered one of the most important vegetables in the world because of its great demand for fresh consumption and as an industrial product (Infoagro, 2017). In Mexico, during 2015 the national area established was 49 703 ha, with a production of 3 098 329 t and a value of \$20 640 million nationwide (SIAP, 2016). In Latin America, Mexico, Cuba and the Dominican Republic stand out for the high per capita tomato consumption (Gómez *et al*., 2000). On the other hand, the salinity of soils is an adverse condition that affects the agricultural production systems of our planet and influences the settlement of human beings. It is estimated that 43% of the croplands on the planet are affected by salinity in the soil or in the water for irrigation, with referenced values of electrical conductivity that exceed the tolerance to salinity of the main cultivated species (Royo and Aragües, 2003).

Salinity increases at an average rate of 0.5% per year, mainly due to low rainfall, large area exposed to high evaporation, irrigation with saline waters and traditional farming practices that favor the increase in the concentration of salts in the soil (Royo and Abio, 2003). Soil salinization grows at a rate of 3 ha min^{-1} (González *et al*., 2005) and there are about 953 million hectares of land affected by this stress factor in different regions of the world (Nabhan *et al*., 1999) and mathematical models show that soil salinization increases year by year (FAO, 2017).

Of the 7.09 million hectares of soils that are exploited for the agricultural system of the Republic of Cuba, 46% have low fertility, 69% have low organic content; 31% are eroded by water or by wind, 24% are acidic soils, 14% are soils affected by salinity, and according to scientific data, 7.5% increases in salinated soils are estimated over the next ten years. To this are added the variations that occur in climate factors, where temperatures are higher and droughts more prolonged and intense, with a direct impact on crops (González, 2002; González *et al*., 2005). In the easternmost region of Cuba, where this research was carried out, there are salinized soils that, in total, occupy 55% of the soils of the region.

Among the most affected regions are the area of San German and Alto Cedro. Saline soils are also found around the Bay of Nipe. In the Cauto Valley there are extensive salinized areas; In addition, in the Guantanamo Valley, saline soils range from typical Solonchak, with a concentration of salts that in many cases exceed 2.5%, to slightly saline, with the eastern region where the salinization process developed most notably and exist around of 30 000 ha affected (Mesa, 2003).

The stress caused by salinity affects the yield of the cultivated species, prevents their development and in some cases the death of the plant. When tomato plants are subjected to stress due to salinity, they deposit solutes such as proline, fructose, glucose and sucrose (Pérez-Alfocea *et al*., 1996, Balibrea *et al*., 1997) and the alteration of the synthesis of these osmoregulators is carried out in the plant with a high-energy cost (Heuer, 1994, Heuer and Nadler, 1998) negatively affecting the development of plants and yield (Mizrahi *et al*., 1988). More recent studies indicate that the main effects of salinity on plants is the decrease in water absorption, absorption of ions that cause toxicity, nutritional imbalance, physiological changes, among others (Tarchoune *et al*., 2013; Ghulam-Abbas *et al*., 2015; Postnikova and Nemchinov, 2015).

To counteract the effect of salinity on plants, different alternatives have been sought that allow organic, ecologically sustainable nutrition that has as a main condition, in addition to production to meet human needs, to improve and conserve the environment. According to Wencomo and Lugo (2013), one of the alternatives is to use natural stimulants that contribute to the increase of the productivity and quality of the products derived from the species of cultivated plants, eliminating partially or totally the use of fertilizers of origin chemical. One of the most used biological stimulants to mitigate the effect of salinity is the humate of vermicompost, a product that has its origin in the Agrarian University of Havana, Cuba and is registered under the commercial name of Liplant® (Garcés, 2000).

This biostimulant presents a high biological activity at low concentrations of the product, facilitates the root development of the plants, the growth of the stem and the leaves, higher flowering index with an increase in the fructification effects that produce more healthy and vigorous plants, which achieve production and greater yield per crop area (Garcés, 2000). Biostimulants also represent a viable and potential alternative for the sustainable production of food, especially nowadays, where the development of organic agriculture is a reality that allows achieving stability in the biological cycle “climate-soil-plant” to obtain benefits greater resources available and protect and conserve the environment (Arteaga *et al*., 2007).

That is why they are developed and used organic compounds, such as organic fertilizers, green fertilizers, the combination of fertilizers with crop rotation, crop residues, stubble, animal waste as well as more elaborate forms such as compost, the vermicompost, the earthworm humus, which are increasingly used because they contribute to the establishment and development of a sustainable agriculture (Vilches and Núñez, 2000; Fonseca de la Cruz *et al*., 2011; Mariña de la Huerta *et al*., 2012).

As mentioned above, the eastern region of Cuba, specifically the Granma province, presents problems in soils, since in some cases they are affected by salts and cause that some cultivated species, such as tomatoes, do not reach their maximum growth potential in conditions of seedbed or nursery, due to the stress caused mainly by NaCl, so it is necessary to use alternatives to increase the tolerance of the seedlings that will later be transplanted to the field for fruit production. In this context, the objective of this study was to evaluate the effect of different concentrations of vermicompost humate on the growth and development of tomato seedlings grown in soils affected by salinity.

Materials and methods

Edapho-climatic study and characterization site

The research was carried out in nursery or nursery conditions, in the Jiguani municipality, located at coordinates 176 100 N and 506 000 E (Academy of Sciences of Cuba, 1989) specifically with a group of producers of the UBPC # 1 “Ernesto Che Guevara”. The seeds were sown in the month of October and were deposited at a distance of 1 cm between them and 5 cm between rows, in soil type Fluvisol (Hernández *et al*., 1999), maintaining the right conditions for germination and development of the seedlings, according to Gomez *et al*. (2000). The characteristics of the soil at the depth of development of the seedlings (0-20 cm) showed an electrical conductivity of 3.50 dS

m^{-1}, a pH of 7.5, an organic matter content of 3.25%. During the development stage of the seedlings, the average humidity was 83%, an average temperature of 26.3 °C and a total precipitation of 45.2 mm.

Treatments with vermicompost humate

Four treatments consisting of three dilutions of vermicompost humate of the product known as Liplant® and a control without application of this product were used. Prior to the transplant, the Amalia variety tomato seedlings were imbibed in the different dilutions of the vermicompost humate. Four equal volume portions were prepared, consisting of the three (v/v) dilutions of humate (1/10, 1/20 and 1/30 and the fourth portion was used as the control, using distilled water). The seedlings were placed for 6 hours in immersion in each dilution and the control (distilled water) were subsequently dried for 24 h at room temperature and in the shade. Later they were transplanted in the field.

Experimental design

The experiment was developed in a completely randomized design with four repetitions per treatment. The treatments consisted of applying to the seedlings, the three dilutions of vermicompost humate (1/10, 1/20, 1/30 and a control-distilled water-). The 640 seedlings were used per treatment.

Composition of the vermicompost humate

The humate of vermicompost was obtained by donation from the Agrarian University of Havana, Havana, Cuba. This product is a plant stimulator and carrier of minerals such as calcium (20.2 mg L^{-1}), copper (0.164 mg L^{-1}), magnesium (6.52 mg L^{-1}), manganese (0.492 mg L^{-1}), potassium (18.30 mg L^{-1}), iron (11.4 mg L^{-1}), sodium (5.70 mg L^{-1}), zinc (1.11 mg L^{-1}), phosphorus (1-28%), nickel (0.032 mg L^{-1}) and chromium (0.225 mg L^{-1}). It also contains free amino acids (9-10 mg L^{-1}), polysaccharides, carbohydrates, inorganic elements, humic substances (25-30% p/v), beneficial microorganisms, plant hormones such as auxins (AIA, AIP, from 0.5 to 2 mg L^{-1}), gibberellins (GA3, from 0.5 to 2 mg L^{-1} and cytokinins (adenine, from 0.01 to 0.5 mg L^{-1}) and soluble humus, whose composition by chemical fractions correspond to a pH of 7.0 to 7.5, 53.4 % C, 4.85% H, 35.6% O, 3.05% N, 0.72% S, an H/C ratio of 0.08, an O/C ratio of 0.62, a C/N ratio of 18.4, 4.82 humic acids and 7.17 fulvic acids in an E4/E6 ratio of their optical coefficient.

Growth variables

For the evaluation of the tomato seedling growth variables, 40 seedlings were selected at random for treatment and repetition, for a total of 160 seedlings per treatment. At 24 days after the transplant (DDT), the following variables were measured:

Height of seedling (cm). It was measured with a flexometer from the base of the stem below the first internode to the top of the branches or crown of the seedling.

Stem diameter (cm). It was measured with a vernier or vernier caliper.

Number of leaves per seedling. The number of true leaves of each seedling was counted directly.

Fresh weight of aerial part and root (g). Each seedling was taken and divided into aerial part (stem and leaves) and root and weighed separately, using a precision balance (Mettler Toledo® PR2002).

Dry weight of aerial part and root (g). The leaves and stems (aerial part) and root, after registering the fresh weight, were placed in paper bags and placed in a drying oven (Shel-Lab®, model FX-5, serie-1000203) at 65 °C, until constant weight. The weight was determined by precision balance (Mettler Toledo® PR2002).

Statistical analysis

Analysis of variance was performed and when significant differences between treatments were found, a multiple means comparison test (Tukey HSD, $p \leq 0.05$) was used. The number of leaves was transformed by square root of each data by means of the equation $X = \sqrt{n}$, in order to comply with the assumptions of variance homogeneity (Sokal and Rohlf, 1988). All statistical analyzes were performed with Statistica® v. 10.0 for Windows (StatSoft®, Inc. 2011).

Results and discussion

All the tomato seedling growth variables showed significant differences between the dilutions of the vermicompost humate used (Table 1). Likewise, all the variables showed higher values in the dilution of 1/30 (v/v), followed by the dilutions 1/20 and 1/10, showing lower values in the control treatment. The percentage value of increase in the dilution of 1/30 with respect to the control treatment was 61, 68, 63, 50, 19, 30, 56 and 27 for height, stem diameter, root length, number of leaves, fresh weight of root, dry weight of root, fresh weight of aerial part and dry weight of aerial part, respectively.

Table 1. Growth variables of tomato seedlings grown in saline soil and subjected to different dilutions of vermicompost humate.

Dilutions of humate of vermicompost (v/v)	Plant height (cm)	Stem diameter (cm)	Num. of leaf	Fresh weight of aerial part (g)	Aerial part dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)
1/10	13.67 c	0.2 c	2.67 c	1 c	0.1 b	0.08 c	0.05 c	6.44 c
1/20	14.92 b	0.22 b	3.45 b	1.2 b	0.11 b	0.12 b	0.08 b	7.47 b
1/30	18.12 a	0.25 a	4.62 a	1.4 a	0.15 a	0.16 a	0.1 a	8.73 a
Control	10.98 d	0.17 d	2.3 d	0.79 d	0.04 c	0.03 d	0.03 d	5.54 d
Sig. level	0.05	0.001	0.05	0.001	0.001	0.001	0.001	0.013

Mean with different letters in a column differ statistically (Tukey HSD $p \leq 0.05$).

According to Casanova *et al*. (2003), for the production of seedlings of tomato in nursery or nursery, it should be taken into account the length and diameter of the stem and the number of leaves, because these variables intervene in the management of the seedlings at the time of the transplant, increasing or decreasing the resistance to stress that constitutes this work for the seedlings, in addition to the establishment of them in the production area. In this context, Casanova *et al*. (2003) established desirable characteristics of tomato seedlings for transplantation, values between 12 to 14 cm in height, 3 to 4 leaves per plantlets and a stem diameter greater than 3 mm, in addition, care must be taken with the health and the nutritional status of the seedlings.

The results of this study in relation to all the measured variables indicate that the applied vermicompost humate was effective in the soil type conditions in the seedling or nursery stage, when significant increases were achieved with respect to the control, which allowed the seedlings, present desirable characteristics and in accordance with the values established by Casanova *et al*. (2003). Also, Wencomo and Lugo (2013) reported positive effects of this biostimulant demonstrating that it favors the growth of the stem, among other variables related to the growth of seedlings. On the other hand, Ortega and Fernández (2007) point out that the humic substances of the vermicompost humate have a biostimulant effect and they are also deposited in small amounts in the leaves, achieving a nutritional effect, but a greater availability is guaranteed when applied through of the soil, because it is absorbed by the roots.

Results similar to those found in this study were reported by Arteaga *et al*. (2006) in a field experiment and using tomato plants to which he applied different dilutions of vermicompost humate via foliar. Also in tomato, Fonseca de la Cruz *et al*. (2011) used different dilutions of vermicompost humate, including 1/30, and found increases in vegetative growth variables. In corn, Calderin-García *et al.* (2009) reported that the 1/30 v:v dilution of earthworm humus stimulated the growth, the biomass in the root and the stem, while the 1/20 v:v dilution exerted a greater effect on the length and the area of the root.

In a study with tomato variety Vyta and using three dilutions of vermicompost humate (1/10, 1/20 and 1/30), Reyes-Pérez (2009) reported larger increases in the growth variables in the dilution of 1/30. Other dilutions such as 1/60 of the humate of vermicompost, have been shown to mitigate the negative effects caused by NaCl in plant species such as basil (*Ocimum basilicum* L.) in the emergency stage, reporting that the percentage and rate of emergence, length of radicle, seedling height, fresh and dry radicle and aerial biomass increased both in the tolerant NaCl (Napoletano) and sensitive (Sweet Genovese) variety when using the biostimulant, even in conditions of stress due to salinity (Reyes -Pérez *et al*., 2014).

In the same species, but in the initial vegetative growth stage, Reyes-Pérez *et al*. (2016) showed that at this stage, the vermicompost humate at a dilution of 1/60, also mitigates the harmful effect caused by NaCl, increasing tolerance to salt stress of both the tolerant and the NaCl sensitive varieties.

Most studies that use vermicompost humate as a plant growth biostimulant and as an abiotic stress reliever, agree that the positive effect exerted by humate is due to the humic and fulvic acids contained in this stimulant (Arteaga *et al*., 2006) of which its hormonal action is known, generally auxin type, which implies the increase of the activity of the H^+-ATPase and consequently the increase in the extrusion of protons, which would correspond with the growth theory (Canellas *et al*., 2002; Quaggiotti *et al*., 2004; Canellas and Façanha, 2004) that brings about a greater absorption capacity of the nutrients by the root system of the seedlings, which allows a greater fixation on the soil when these are transplanted in countryside.

The humic and fulvic acids of the vermicompost humate have a positive effect on the root and on the aerial part of the plants (Vaughan and Malcolm, 1985; Van de Venter *et al*., 1991; Façanha *et al*., 2002; Canellas *et al*., 2002; Canellas and Façanha, 2004), an effect that is achieved through the physiological-biochemical processes in plants, with positive intervention in respiration, speed of the enzymatic reactions of the Krebs cycle, which favors a production of Higher ATP, as well as in selective effects on protein synthesis and increase or inhibition of the activity of various enzymes (Nardi *et al*., 2002). The application of humic substances in species that develop under stress conditions, including saline, reduces the negative effects of this abiotic stress (Varanini and Pinton, 1995; Dubbini, 1995; Chukov *et al*., 1996).

Apparently the humic substances reduce the Na^+ absorption because they act on the plasma membrane H^+-ATPases (Canellas *et al*., 2002, Canellas and Façanha, 2004) and H^+ -pyrophosphatases stimulating the natural process of exclusion of Na^+ by the plant. The main and specific toxicity mechanism caused by NaCl in plants is the high generation of free radicals that cause oxidative stress in mitochondria (Hernández *et al*., 1993). In this sense, humic substances affect enzymatic activity, protein expression, proton extrusion (Canellas *et al*., 2002, Façanha *et al*., 2002) and messenger RNA levels (Quaggiotti *et al*., 2004; Elena *et al*., 2009) of the proton of the plasmatic membrane of ATPase (PM H+-ATPase) in a similar way to the effects exerted by auxins on PM H+-ATPase reported in corn (Frias *et al*., 1996). This enzyme plays a crucial role in nutrient uptake and root growth, which is confirmed by its abundance in root tissues (Palmgren, 2001).

The liquid humus of vermicompost also contains amino acids, minerals and chemical fractions, which activate the production of metabolic energy, which is used in the formation of new structures in plants (Mayhew, 2004). According to Pierik (1990), the high ratio of auxin/cytokinin induces the formation and elongation of roots and in the case of the humate of vermicompost, the auxin content is higher than that of cytokinin, which favors the response of the plant when applying this biostimulant.

Although in general terms the response of plants with and without stress is favorable, according to Zandonadi *et al*. (2013) it is important to have a standard method that is used to achieve an efficient comparison of results in a variety of sources of humic substances and plant species, especially direct efforts towards the development of a biochemically standardized method, technically accessible and economically viable to qualify the bioactivity of organic matter.

Conclusions

The variables of tomato seedling growth, height, stem diameter, root length, number of leaves, fresh weight of root, dry weight of root, fresh weight of aerial part and dry weight of aerial part, reached maximum values in the 1/30 dilution (v/v) of the vermicompost humate, with percentage values with respect to the control treatment of 61, 68, 63, 50, 19, 30, 56 and 27 for height, stem diameter, root length, number of leaves, fresh weight of root, dry weight of root, fresh weight of aerial part and dry weight of aerial part, respectively.

In general, the obtained results confirm the positive effect of the vermicompost humate on the growth and development of tomato seedlings, by significantly increasing the growth variables with respect to the untreated seedlings; In addition, this biostimulant is an ecological option to obtain quality seedlings, grown in fragile and degraded agroecosystems such as saline soils, whose use does not pollute the environment, also conserves soil fertility and biodiversity.

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