Article

Biostimulant Liplant®: its effect on *Solanum lycopersicum* (L.) grown on slightly saline soils

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Abstract

The application of humic substances, stimulates biochemical, physiological mechanisms, investigations have been carried out that reveal the properties and functions of these substances. The objective of this investigation was to evaluate the effect of different concentrations of Liplant® applied foliarly (1/10, 1/20, 1/30 v/v and a control-distilled water-) in the growth, production and quality of fruit of tomato, and economic feasibility. Carried out under field conditions and using a design of complete blocks at random, 5 days after the transplant was measured height, diameter of stem; 65 days later, we considered fresh and dry fruit weight, polar and equatorial diameter, number of fruits, yield, titratable acidity, total soluble solids, maturity index, vitamin C content, pH of the fruit juice, moisture loss and firmness of fruits. The economic feasibility was made considering yield, value and cost of production, net profit, cost and the benefit/cost ratio. Analysis of variance and multiple comparisons of means were made (Tukey HSD $p= 0.05$). The juice pH did not show significant differences between Liplant® concentrations. Height, stem diameter, number of fruits, polar and equatorial diameter, fresh and dry fruit weight, yield, fruit firmness, total soluble solids, vitamin C and fruit moisture loss showed higher values when the plants were sprayed with the dilution of Liplant® 1/30 (v/v). The dilution of 1/30 (v/v) of Liplant® generated a benefit of $36 753.9 thousand Cuban pesos per hectare, a benefit/cost of $3.6 Cuban pesos and a cost per weight of $0.21.

Keywords: analysis of plant growth, fruit quality, yield, salinity in the soil.

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Introduction

The abiotic stressors of water, salts and heavy metal content induce the production of reactive oxygen species in plants that consequently cause them oxidative stress, which results in severe losses in crop yield. Several studies have investigated the protective effects of agro-materials on plants under stress conditions. Applying foliar and root-level extracts of liquid humus vermicompost of cow dung have shown protective effects in various species of cultivated plants exposed to stress by salinity in the soil or irrigation water (Calderin-García *et al*., 2013).

The salinity of the soil is originated by the presence of chlorides, sulfates, carbonates and bicarbonates of Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} while the high conductivity in the irrigation water, which is due to a high concentration of Ca^{2+}, Na^{+}, Mg^{2+}, Cl^{-} and HCO^{-3} dissolved in water; on the other hand, the frequent use of agrochemicals in conventional agriculture, causes a negative effect on the quality of food, alters the physical, chemical and biological properties of soils, reduces biodiversity, increases the risk of salinization, decreases considerably the energetic reserves of the soil and the surface and underground waters are contaminated (Mendez-Guisado *et al*., 2012). According to Costales *et al*. (2007), the use of bio-products in the species of cultivated plants has economic and ecological importance, also act as stimulators or growth regulators, which in small doses increase, inhibit or modify the physiological processes of a plant.

The use of bio-products, adds to organic farming or global organic agriculture that will achieve greater productivity, sustainable and friendly to the environment. This production system suggests the use of products of natural origin as sources of fertilization and bio-stimulation, including organic fertilizers, biofertilizers and biostimulants for the growth and development of crops. This type of natural fertilizers increase the flowering and improves the fructification in quality and quantity of the species of plants cultivated for their use as food for humanity.

One of the alternatives generalized in organic farming is the use of biostimulants, which contribute to improve the quality and productivity of the cultivated species, by eliminating all or part of the chemical fertilizers and introducing biostimulants and organic fertilizers as technology to produce an organic, ecological and sustainable agriculture. Of the biostimulants, the liquid humus whose source is the vermicompost, has a high biological activity in low concentrations, which facilitates the development of the root system of the plants, the growth of the stem, the leaves and the increase in the flowering and consequently the fructification, resulting in healthier and more vigorous plants that produce more and greater yield is obtained.

The tomato (*Solanum lycopersicum* L.) is one of the most booming vegetables in the world. Classifying itself as the second vegetable of greater importance due to its high level of consumption and its multiple uses. According to the FAO (2017), the global tomato production reaches 130 million tons, with China in the first position as a producer of 40 million tons (SIAP, 2016).

Considering the premises previously exposed, the objective of the investigation was to evaluate the effect of different concentrations of Liplant® applied via foliar (1/10, 1/20, 1/30 v/v and a control -distilled water-), in the growth, production and quality of tomato fruit variety Amalia, as well as the economic feasibility of the use of this product in this species.

Materials and methods

Study area

The investigation was carried out under field conditions, on the planting date recommended for the area (october-january) of the municipality of Jiguani, eastern province of Granma, Cuba, in Unit BPC No. 1 “Ernesto Che Guevara” of the Company Cauto The Yaya, located at 176° 100’ north latitude and 506° 000’ LE (Academy of Sciences of Cuba, 1989). The average temperature and humidity during the experimentation period were 24.5°C and 77.8%, respectively according to Hernández *et al*. (2013), the soil of the area is Fluvisol type, whose composition at a depth of 21-40 cm showed a pH of 7.6; an electrical conductivity of 2.45 dS m^{-1}, an organic matter content of 2.8% and a slope of less than 1%, with low fertility and slightly saline.

Experiment management

The Amalia tomato variety was used. Soil preparation and cultural practices were carried out in accordance with Gómez *et al*. (2000). Irrigations were applied according to the water needs of tomatoes in the eastern region of Cuba. Hilling and weeding were done manually.

Experimental design

The experiment was established in a randomized complete block design with four repetitions. The treatments applied were three dilutions of Liplant®, 1/10, 1/ 20, and 1/30 v/v and a control without application of this product (distilled water). The transplant was performed at a distance of 1.4 m between rows and 0.25 m between plants, in plots of 8 m^2, with a total experimental area of 164.45 m^2. The useful plot consisted in selecting the plants located in the three central furrows of each experimental unit, except for two plants at the ends of each furrow.

The Liplant® was applied in a foliar way using a Senior model sprinkler with a conical nozzle, which was previously calibrated. The applications were made 10 and 25 days after the transplant (ddt) and 15 days after the first application.

Composition of the Liplant®

The Liplant® is considered a biostimulator plant and carrier of nutrients (Ca, Mg, Na, P_2O_5, K, N), free amino acids, polysaccharides, carbohydrates, inorganic elements, humified substances, beneficial microorganisms, plant hormones and soluble humus, whose composition by chemical fractions correspond to a pH of 8.7, 53.4% of C, 4.85% of H, 35.6% of O, 3.05% of N, 0.72% of S, an H/C ratio of 0.08, an O/C ratio of 0.62 , a C/N ratio of 18.4, 4.82 of humic acids and 7.17 of fulvic acids in an E4/E6 ratio of their optical coefficient.

Growth variables

For the evaluation of the growth variables, ten plants were used per treatment and repetition, randomly selected, while, for the fruit quality variables, ten fruits were randomly selected by treatment and repetition. The height of the plant (cm). It was measured 5 days after the transplant, with a flexometer from the base of the stem below the first internode to the top of the branches or crown of the plant. The stem diameter (cm). It was measured 5 days after the transplant, with a vernier or vernier caliper.

Production variables

Fresh weight of fruits (g). At 65 days after the transplant, the fruits were harvested and weighed on a precision scale (Mettler Toledo® PR2002). The polar and equatorial diameter of fruits (mm). These variables were determined using a vernier or vernier caliper (VWR® modelo 62379-531, S/N/ 61581129, USA). The dry weight of fruits (g). The fruits were placed in paper bags and placed in a drying oven (Shel-Lab®, modelo FX-5, serie-1000203) at 65 °C, until constant weight. The weight was determined by precision balance (Mettler Toledo® PR2002). The number of fruits per plant. The quantification of the fruits per plant was carried out, when 50% of the fruits of each plant appeared by treatment and repetition. The yield of fruits (t ha^{-1}). The fruits were harvested and weighed in each plot and with this the yield was estimated.

Fruit quality variables

Total soluble solids or sugar content (%). It was determined by manual refractometer (Atago® N-1 alfa, Atago® Co., LTD. Itabachi-ku, Tokio, Japan; °Brix 0-32%), placing two drops in the prism of the refractometer, according to the equipment instructions. The titratable acidity (expressed as percentage of citric acid). It was determined by the AOAC method (1990), homogenizing 10 g of tomato fruit pulp in a blender (Hamilton Beach®, model 58149-MXR) with 50 mL of distilled water and once the juice or extract was obtained, it was filtered, aliquots of 10 mL were taken and 0.01 N NaOH was added until neutralization was achieved. The maturity index. It is the value that relates the total soluble solids and the titratable acidity. It was determined by dividing the values of the total soluble solids and the titratable acidity.

Vitamin C (mg 100^{-g}). It was determined by means of an oxide-reduction volumetry, using an iodine solution as an oxidizing agent that constitutes the standard titrant (Ciancaglini *et al*., 2001). To measure the pH of the fruit juice, a sample of 10 g of fruit pulp was taken and homogenized in a blender (Hamilton Beach®, model 58149-MXR) adding 100 mL of distilled water; Once the juice or extract was obtained, it was filtered and the pH was measured with a portable potentiometer (Orion Stara A3215 Thermo Scientific®, USA). Loss of fruit weight (%). The fruit weight was obtained using an analytical balance (Mettler Toledo®, modelo AG204). The water loss was determined with respect to the initial weight of the fruit. Firmness of fruits with peel (lb $pulg^{-1}$). It was determined by means of penetrometry, using a penetrometer or portable durometer (Mitotuyo® Tamex Precision, Japan).

Economic valuation

To determine the economic effect of the use of the vegetable bioestimulant Liplant®, an accounting analysis was carried out based on the yield (t ha^{-1}) and considering the following indicators: value of production in thousands of pesos (MP) per hectare (VP); cost of production of one hectare in MP (CP); net profit in MP (B); cost per weight for one hectare of tomato (C/P) and benefit/cost ratio in pesos (B/C), which were calculated with the following equations:

VP= R×Vm. Where: VP= value of production in MP per hectare; R= agricultural yield in tons per hectare; Vm= value of a ton of tomato.

CP= Cc+Cct. Where: CP= production cost of one hectare in MP; Cc= common cost for one hectare in MP; Cct= cost of harvest and transport of one hectare in MP.

B= VP-CP. Where: B= net profit in MP; VP= value of production in MP per hectare; CP= production cost of one hectare in MP.

C/P= CP/B. Where: C/P= cost per weight for one hectare of tomato; CP= production cost of one hectare in MP; B= net profit in thousands of pesos MP.

B/C= B/CP. Where: B/C = benefit/cost ratio in pesos; B= net profit in MP; CP= production cost of one hectare in MP.

Statistical analysis

Analysis of variance was carried out and when significant differences were found between treatments, multiple comparison tests of means were performed (Tukey HSD, $p\leq 0.05$). In order to comply with the assumptions of homogeneity of variance, data transformations were made when necessary. The variables expressed as a percentage were transformed using arcosine (Sokal and Rohl, 1989). The analyzes were performed with Statistica® v. 10.0 for Windows StatSoft®, Inc. (2011).

Results and discussion

Growth variables

Stem height and diameter showed significant differences between treatments, observing that both variables increased their values in the 1/30 dilution of Liplant® (Table 1). Napoles-Vinent *et al*. (2016), reported results with positive response of tomato variety Amalia before the action of Liplant®, where the most effective treatment proved to be that of the dilution of the product in a concentration of 1/30 (v/v). Fonseca de la Cruz *et al*. (2011), in tomato, it reports positive results with the use of arbuscular mycorrhizols by imbibition to the seeds during 12 hours and three treatments of earthworm humus (Liplant®).

Studies using vermicompost humus as a foliar fertilizer agree that the positive effect of this product on plant growth is due to the biochemical composition of Liplant® (Nardi *et al*., 2002). The presence of minerals and phytohormones such as auxin, which are in greater concentration in the Liplant®, stimulate the height of plants (Pierik, 1990, Mayhew, 2004). In other species, such as tobacco, the Liplant® also significantly increased the agricultural yield, by spraying at a dilution of 1/60 in correspondence with the greater length and width of the leaf and biomass accumulated in the leaf and stem (Mariña de la Huerta *et al*., 2012).

Production variables

Number of fruits per plant, polar and equatorial diameter of fruits, fresh and dry weight of fruits and fruit yield, showed significant differences between the dilutions of Liplant® and in all the mentioned variables, they increased their values in the dilution of 1/30 (Table 1).

The average fruit dry weight was higher in the 1/30 dilution, with 86.2 g, surpassing the control by 81%; similar to the rest of the production variables, fruit yield was higher in the 1/30 dilution, with 21.6 t ha^{-1}, surpassing 62.5% in the control (Table 1). Similar results reported Napoles-Vinent *et al*. (2016), when using Liplant® in tomato, showing a significant increase in plant height, stem diameter, number of fruits and polar and equatorial fruit diameter, as Liplant® dilutions were increased from 1/10 to 1/30 v/v, with respect to the control. In lettuce, Hernandez *et al*. (2013). There are several reports of the increase in the number of fruits caused by the presence of phytohormones (Alfonso *et al*., 2010; Falcón *et al*., 2010; Terry *et al*., 2012; De la Huerta *et al*., 2012).

The positive effect of Liplant® on tomato was evident in this study, considering that this species is demanding in nutrient levels (Hernández and Chailloux, 2001). Torres-Rodríguez *et al*. (2016), used three dilutions of Liplant® (1/40, 1/50, 1/60 v/v and a control treatment) in tomato plants, checking positive results 24 days after sowing.

Fruit quality variables

The variables fruit firmness, titratable acidity, total soluble solids, vitamin C, maturity index and moisture loss, showed significant differences between the treatments based on dilutions of Liplant®, while the pH of the fruit juice showed no differences significant (Table 1) and showed average values with a minimum difference (4.35 and 4.36), which coincide with those reported by Cantwell (2006) and Navarro-López *et al*. (2012). The highest values of fruit firmness, total soluble solids, vitamin C, maturity index and moisture loss, were presented in the dilution of 1/30 (v/v) and exceeded the control in 84.2, 84.11, 91.56, 64.00 and 90.34%, respectively.

The highest value of the titratable acidity was presented in the control and this decreased in the dilution of 1/30. Accord with Infoagro (2017). Other studies report that liquid humus in dilutions of 1/30 and 1/40, in tomato variety Amalia, did not cause significant variations in the pH and acidity of the fruit (Arteaga *et al*., 2006).

The pH of tomato fruit juice usually does not vary significantly between the different factors or sources of variation that are reported (Cantwell, 2006; Casierra-Posada and Aguilar-Avendaño, 2008; Navarro-López *et al*., 2012; Napoles-Vinent, 2016), which coincides with the results of this research; however, for fruits that are intended for industry, a pH of 4.4 is suggested (Hidalgo-González *et al*., 1998).

Table 1. Variables of plant growth, production and quality of tomato fruits grown in slightly saline soils and subjected to different dilutions of vermicompost humates. Averages with different letters in a column differ statistically (Tukey HSD $p\leq 0.05$).

Treatments of Liplant® (v/v)	Plant height (cm)	Stem diameter (cm)	Number of fruits per plant	Polar diameter (cm)	Equatorial diameter (cm)	Fresh weight fruits (g)	Dry weight fruits (g)	Yield (t ha^{-1})
Variables of plant growth and fruit production								
1/10	43.1 c	0.85 c	18.72 c	5.25 c	5.73 c	109.8 b	80 c	16.5 c
1/20	46.1 b	1.01 b	22.2 b	6.44 b	5.77 b	110 b	85 b	18.9 b
1/30	48.36 a	1.3 a	26.17 a	7.46 a	5.82 a	120 a	86.2 a	21.6 a
Control	40 d	0.75 d	15.77 d	4.52 d	5.35 d	103.2	69.5 d	13.5 d
Significance level	0.012	0.001	0.015	0.013	0.003	0.01	0.019	0.012

Treatments of Liplant® (v/v)	Firmness of fruits (lb in^{-2})	Titratable acidity (% citric acid)	Total soluble solids (°Brix)	Vitamin C (mg 100 g^{-})	Maturity index (SST/acidity ratio)	Loss of moisture of fruits (%)	pH of the fruit juice
Fruit quality variables							
1/10	6 c	0.43 b	5.11 c	18.25 c	11.8 c	4.51 c	4.36 a
1/20	6.5 b	0.4 c	5.21 b	19.32 b	13.02 b	4.75 b	4.35 a
1/30	7 a	0.35 d	5.35 a	19.81 a	15.28 a	4.87 a	4.36 a
Control	5.9 d	0.46 a	4.5 d	18.14 d	9.78 d	4.40 d	4.36 a
Significance level	0.002	0.001	0.001	0.001	0.001	0.001	0.3

The range of the titratable acidity values of this study was from 0.36 to 0.46. It is reported that the titratable acidity in tomato differs a lot depending on the factors such as the variety, cultivation conditions, temperature or weather conditions in general. Thus, Arias *et al*. (2000) reports an average titratable acidity value in tomato of 0.63%, while Dobricevic *et al*. (2007) in his research found a range of 0.19 to 0.45%, while Navarro-López *et al*. (2012) reported a range of 0.27 to 0.45%. Values less than 0.25 are required for tomato industrialization (Hidalgo-González *et al*., 1998). In relation to the content of soluble solids (°Brix), loss of humidity and vitamin C, also Arteaga *et al*. (2006), reported percentage increases in tomato in these variables, when applying liquid humus in a dilution of 1/30, with values of 14-24, 22.5-37.0 and 11-26.2%, respectively. The values of total soluble solids presented by the fruits in this study (4.5-5.35%), are located in the range of those reported by Navarro-López *et al*. (2012).

The accumulation of dry matter due to loss of moisture of fruits was greater in the fruits harvested in the plants to which the Liplant was applied, which increased as the concentration of the product increased (Yang *et al*., 2004). Garcés (2002) points out that the minerals K and P present in the

Liplant® when absorbed via foliar and found in appropriate concentrations, increase the dry matter of the fruit, by increasing the size and therefore the diameters of this. While vitamin C increased as the dose increased.

The maturity index, that is, the ratio of total soluble solids and titratable acidity is an important criterion to evaluate the quality of the tomato fruit, because the application of liquid humus, caused lower acidity (better flavor) and therefore higher nutritional quality (Arteaga *et al*., 2006). Similar results were found in this study, other reports indicate that a value greater than or equal to 4 °Brix for fresh consumption (Gómez *et al*., 2000; Arteaga, 2004; Navarro-López *et al*., 2012). Although in this study, enzymes associated with salinity stress were not determined, it is important to mention that the application of humic substances derived from vermicompost, specifically Liplant® under conditions of abiotic stress, increases the proline content and the accumulation of biomass of the corn plants (Huelva *et al*., 2009; Reyes-Pérez *et al*., 2009; Reyes-Pérez *et al*., 2011).

In rice grown under drought conditions, the application of humic substances in concentrations of 30 and 40 mg L^{-1} increased the activity of peroxidase (Schiavon *et al*., 2010, García *et al*., 2012). The application of humic substances in corn, causes an effect in the production of reactive oxygen substances and increases the activity of catalase (Cordeiro *et al*., 2011; Aydin *et al*., 2012).

Economic valuation

The results of the economic analysis showed that the greatest economic benefit was obtained by applying Liplant® at a dilution of 1/30 (v/v), which reported a benefit of $36 753.9 thousand Cuban pesos per hectare, with a higher value in the benefit/cost ratio of $3.6 Cuban pesos. The benefit was lower in the control, with $16 264 thousand Cuban pesos and a value in the benefit/cost ratio of $1.6 Cuban pesos (Table 2). In this study, it is emphasized that the introduction of Liplant® in agriculture is economically viable.

Table 2. Economic valuation of tomato plants treated with Liplant and grown on soils slightly affected by salinity (values expressed in Cuban pesos).

Economic indicators	Control	1/10	1/20	1/30
Yield (t ha^{-1})	12.16	15.03	17.86	21.63
Production value (MP ha^{-1})	26 387.2	32 615.1	38 756.2	46 937.1
Cost of production (MP ha^{-1})	10 123.2	10 183.2	10 183.2	10 183.2
Benefit (MP ha^{-1})	16 264	22 431.9	28 573	36 753.9
Cost per weight ($)	0.38	0.31	0.26	0.21
Benefit/cost ($)	1.6	2.2	2.8	3.6

MP= thousands of pesos.

Conclusions

The variables of growth, plant height and stem diameter showed higher values when the plants were sprayed with the dilution of Liplant® of 1/30 (v/v). Also, the variables related to the production of tomato fruits, number of fruits per plant, polar and equatorial diameter of fruits, fresh and dry fruit weight and yield, showed higher values when the 1/30 Liplant® dilution was applied.

Likewise, the variables associated with fruit quality in tomato, fruit firmness, total soluble solids (°Brix), vitamin C and loss of fruit moisture, showed higher values in the 1/30 dilution, while the titratable acidity, showed lower values in this dilution.

The use of the vermicompost humatos improved both the growth of the tomato plants, as well as the production and the quality of the fruits, even though the experiment was carried out in slightly saline soils. From the economic point of view, the dilution of 1/30 (v/v) of Liplant®, in tomato plants under unfavorable growing conditions, generated a profit of $36 753.9 Cuban pesos per hectare, a benefit/cost of $3.6 Cuban pesos and a cost per peso of $0.21.

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