ISSN impreso: 0258-5936
ISSN digital: 1819-4087
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

AGRONOMIC TOMATO CROP RESPONSE TO BIOPRODUCT QUITOMAX®

Respuesta agronómica del cultivo de tomate al bioproducto QuitoMax®

Elein Terry Alfonso[1]✉, Alejandro Falcón Rodríguez[1], Josefa Ruiz Padrón[1], Yudines Carrillo Sosa[1] and Hugo Morales[2]

ABSTRACT. The bioactive products, have various beneficial effects on plants, such as, induction of defense mechanisms and stimulation of plant growth, besides it having the advantage of not being harmful to plants and the environment; the oligosaccharines and inside them the chitosan and its derivatives are an example, they exert positive effect on growth and development of plants. This work was carried in field condition with the overall objective of evaluating the effect of different concentrations (0,1, 0,5 and 1,0 g L^{-1}), and its application by imbibition and foliar spray product QuitoMax® on growth, development and yield and its components of tomato (cultivar Mara). The results showed a positive effect of byproduct from the seeds imbibition on concentration of 1,0 g L^{-1} stimulating growth variables that were evaluated in the nursery; moreover, the combination soaking plus foliar spray at a dose of 0,3 g ha^{-1} at 7 days after transplantation, stimulated yield components of plants and increased crop yields by 55 % compared to control treatment.

Key words: biostimulant, growth, chitosan, yield, *Solanum lycopersicum*

RESUMEN. Los productos bioactivos, ejercen diversos efectos beneficiosos en las plantas, tales como, la inducción de mecanismos defensivos y la estimulación del crecimiento vegetal, además de tener la ventaja de no ser dañinos a las plantas ni al medio ambiente; siendo un ejemplo, las oligosacarinas y dentro de ellas la Quitosana y sus derivados, las cuales ejercen efecto positivo en el crecimiento y desarrollo de las plantas. El presente trabajo se desarrolló en condiciones de campo, con el objetivo general de evaluar el efecto de diferentes concentraciones (0,1; 0,5 y 1,0 gL^{-1}), y su aplicación por imbibición y aspersión foliar del bioproducto QuitoMax® en el crecimiento, desarrollo y rendimiento del cultivo del tomate (cultivar Mara). Los resultados mostraron un efecto positivo del bioproducto a partir de la imbibición de las semillas en la concentración de 1,0 g L^{-1} estimulándose las diferentes variables del crecimiento que fueron evaluadas en semillero; por otra parte, la combinación imbibición más la aspersión foliar con la dosis de 0,3 g ha^{-1} a los siete días después del trasplante, estimuló los componentes del rendimiento de las plantas e incrementó el rendimiento agrícola en un 55 % con respecto al tratamiento control.

Palabras clave: bioestimulante, crecimiento, quitosano, rendimiento, *Solanum lycopersicum*

INTRODUCTION

In order to conserve the agroecosystem and taking into account the growing demand for food, as well as the high prices of technological packages, there is a need to look for new technologies to increase production and offer products free of toxic waste to consumers (1).

Governments today reinstate the idea of efficient recycling of waste and the use of biological products such as biostimulants to minimize the use of chemicals that cause toxicity to human health and the agroecosystem in general. These biostimulants are a range of products containing active principles, which act on the physiology of plants, increasing plant growth and development, as well as yield and quality of crops (1).

In the specific case of Cuba, the use of these bioproducts is gradually increased in agriculture and its application is frequent and almost essential in the agrotechnical management of crops,

[1] Instituto Nacional de Ciencias Agrícolas (INCA). Gaveta postal No.1, San José de las Lajas. Mayabeque, Cuba. CP 32700
[2] Universidad Autónoma de Chihuahua (UACH). Cd. Delicias Carretera a Rosales km 2½. Chihuahua, México. C.P. 33000
✉ terry@inca.edu.cu

with the aim of increasing agricultural yields and ensuring biological and economic sustainability of production systems (2).

Generally, these products are applied by foliar route through the spray, they allow to quickly correct nutrient deficiencies at times critical to the development of crops. However, other forms of application such as the treatment of seeds via imbibition and addition to the substrate have been reported as positive in the biological benefit of the crops but have been little studied (3).

In the current context of agricultural biostimulants, the development of products that have Chitosan as main active ingredients has been booming in the last decade. These are polymers and oligomers of glucosamine which are obtained by basic deacetylation of the chitin polymer, which in turn is extracted from the exoskeleton of crustaceans (4, 5).

Chitosan polymers and oligomers may have broad agricultural application based on the biological potentialities of these compounds, such as the promotion of plant growth and development of several crops of economic importance (6, 7).

The Bioactive Products Group (GPB, according its acronyms in Spanish) of the National Institute of Agricultural Sciences (INCA) has developed a liquid product based on chitosan polymers obtained from chitin present in the lobster exoskeleton, whose trade name is QuitoMax®. This product is evaluated and extended in several crops of economic importance such as tobacco (*Nicotiana tabacum* L.), potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.), bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.), among others, with positive and promising results that have determined a current demand in national agriculture (8).

The tomato (*Solanum lycopersicum* L.) is one of the most industrialized and industrialized vegetables in the world and the most economically valuable. Its demand increases continuously and with it its cultivation, production and trade, annual averages exceed 152 956 115 t which was referred in different reports. In Cuba, this crop accounts for 50 % of the total area devoted to vegetables and production ranges around 750 000 t; however, the yields recorded in several productive areas of the country are low, due, among some causes, to the unfavorable prevailing edaphoclimatic conditions, the lack of inputs and the scarcity of alternatives to guarantee crop requirements (9).

The objective of this study was to evaluate the effect of different doses and forms of application of QuitoMax® on the growth, development and agricultural yield of the tomato crop.

MATERIALS AND METHODS

This research was carried out in experimental areas of the Department of Agricultural Services of INCA, located in the municipality of San José de las Lajas, Mayabeque province, Cuba, during the period from December 2014 to March 2015.

The plants grew on Ferralitic Compound Red soil, according to the New Classification Version of the Soils of Cuba (10). The chemical characteristics were determined following the techniques described in the manual for analysis of soil, foliar, organic fertilizers and chemical fertilizers (11). The results of the chemical analysis (Table I) are shown below; where, with the exception of organic matter, which was low, the pH and interchangeable cations, are in the range suitable for the normal development of the tomato crop (11).

Table I. Chemical soil characteristics

Depth (cm)	pH H_2O	OM (%)	Exchangeable cations (cmol kg^{-1})				
			Ca	Mg	Na	K	$\sum$
0-12	7,5	1,61	16,0	2,0	0,1	0,5	18,6
12-22	7,4	1,67	17,5	2,5	0,1	0,5	20,6

Later, to carry out the experiment, tomato seeds of the 'Mara` cultivar, with a germinative power superior to 90 %, obtained through the Program of Genetic Improvement of the INCA were used. The biostimulator QuitoMax® whose active ingredient is a Chitosan polymer, obtained by the INCA Bioactive Products Group (GPB), was used from the basic deacetylation (NaOH) of the chitin present on the lobster cover (12). A stock solution of 1 % QuitoMax® was prepared, which was diluted in sterile distilled H_2O to obtain the desired concentrations for the experiments.

The seeds were embedded (Emb) for 30 minutes at the corresponding QuitoMax® concentrations and were planted in traditional open-air seedlings on stonecutters (13); which were composed of a substrate composed of organic manure (bovine manure) and soil, where each treatment occupied an experimental area of 1,0 m², under a completely randomized design with three replicates.

Seedlings with 30 days old in the nursery were taken to the field, establishing them in plots of 28 m^2, to a plantation frame of 1,40 x 0,30 m distributed under a random block design with four replicates and six treatments (Table II). The cultural attention was realized according to those established in the technical instructions of the crop[A].

Table II. Description of treatments (T)

T	Seed bed	T	Field
1	Control$_{(without\ QuitoMax)}$	1	Control$_{(without\ QuitoMax)}$
2	0,1 g $L^{-1}$$_{(Imb)}$	2	-----
		3	(AF*)
3	0,5 g $L^{-1}$$_{(Imb)}$	4	-----
4	1,0 g $L^{-1}$$_{(Imb)}$	5	-----
		6	(AF)

* Asparagus Foliar

The foliar application (AF) of the product was performed seven days after transplantation (beginning of floral primordia) with a dose of 0,3 g ha^{-1} (4), in early morning hours (8: 00-9:00 a.m.) using the stomatal opening of the leaves, using a 16 liters of manual sprinkler, which was calibrated before use.

At 10, 15 and 25 days after germination (DAG) under seed conditions, destructive sampling of the whole plant with a sample size was carried out, which allowed 95 % certainty (15 seedlings per plot of each treatment) (14), who were given the growth assessments referred to in other studies (1), such as:

- Height of seedlings (cm): measured with a graduated ruler, from the root neck to the armpit of the youngest leaf.
- Stem diameter of the seedlings (cm): it was determined with a king's foot, from two centimeters above the neck of the root.
- Number of leaves per seedling: by visual count.
- Radical length (cm): The main root was measured with a graduated ruler.
- Fresh mass of seedlings (g): weighing in analytical balance (Sartorius).
- Dry mass of seedlings (g): stove drying (BrBOXUN) at 70 ^{0}C to constant mass and analytical balance weighing (Sartorius).

In the flowering - fruiting stage, 15 plants were selected for each randomized treatment, to which the following evaluations were performed (1):

- Number of clusters, flowers and fruits per plant: by visual count.
- Average mass of fruits (g): by dividing the total mass of the fruits between the number of fruits of the plot.
- Estimation of agricultural yield (t ha-1): by weighing the total production of the area of calculation, extrapolated to one hectare.

The obtained data were analyzed using a Simple Classification (seed) and double (field) ANOVA. The resulting means were compared with the Duncan Multiple Rank Test (15) for p≤0.05 when there were significant differences among the treatments, processed with the Centurion Statgraphics program (16).

RESULTS AND DISCUSSION

Concentration effects of QuitoMax® in the tomato seedlings

The results obtained when evaluating the height of the tomato seedlings (Figure 1A), show that the concentrations of QuitoMax® used had a positive effect on this variable, specifically in the treatments where the seeds were soaked with the concentrations of 0,5 and 1,0 g L^{-1}.

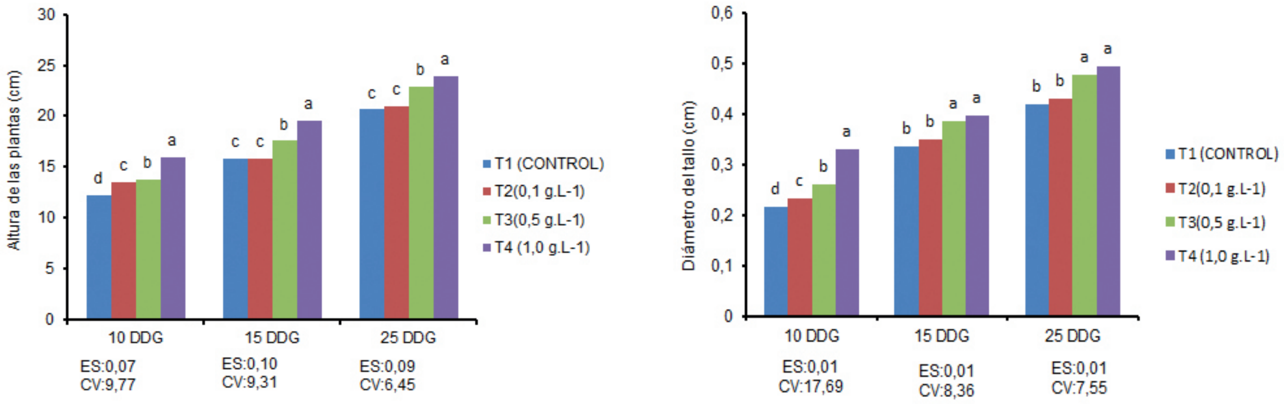
At 15 DAG, the seedlings of both treatments reached the optimal size required for the transplant, which is 15-18 cm (9). However, the treatment with the lowest concentration (0,1 g L^{-1}) exceeded the control at 10 DAG, whereas at 15 and 25 DAG, behaved in the same way, so it does not denote an effect of this dose in this growth variable.

The height of the plants is the first visible indicator that indicates the moment of the transplant, shortening this period involves economic savings throughout the crop cycle. Similar results of increase in height of the plants in a shorter time than indicated by the instruction of the crop have been obtained for example, with the application of rhizobacteria stimulating the plant growth in which is achieved with seven days of difference, a posture of quality tomato (17).

As regards stem diameter (Figure 1B), the results obtained demonstrate the positive action of QuitoMax® in this growth variable, where the highest values corresponded to the treatments where the seeds were soaked with the concentrations of 0,5 and 1,0 g L^{-1} of QuitoMax® with respect to the treatment with the lowest evaluated concentration of the product (0,1 g L^{-1}) and to the control.

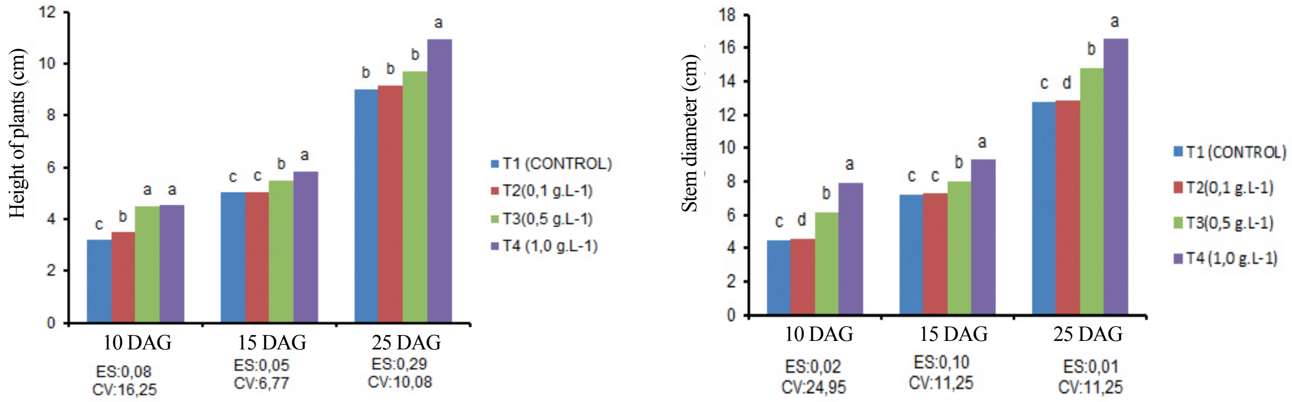
The results obtained in the radical length (Figure 2B), showed significant differences $p < 0.05$ in all concentrations evaluated, so that the different doses of QuitoMax® had a positive effect on this growth variable. The highest values were obtained in the treatment with the 1,0 g L^{-1} concentration, followed by the treatment with the concentration of 0,5 g L^{-1}, while the lowest values corresponded to the control and the treatment with the concentration of 0,1 g L^{-1} of the product.

[A]MINAGRI. *Instructivo técnico del cultivo del tomate*, Cuba, 1992, p. 52.



Different letters show significant differences between treatments for p≤0.05; According to the Duncan Multiple Rank Test

Figure 1. Effect of QuitoMax® concentrations on height (A) and diameter (B) of tomato seedlings in the seedling stage



Different letters show significant differences between treatments for p≤0.05; According to the Duncan Multiple Rank Test

Figure 2. Effect of QuitoMax® concentrations on the number of leaves (A) and root length (B) on tomato seedlings in the seedling stage

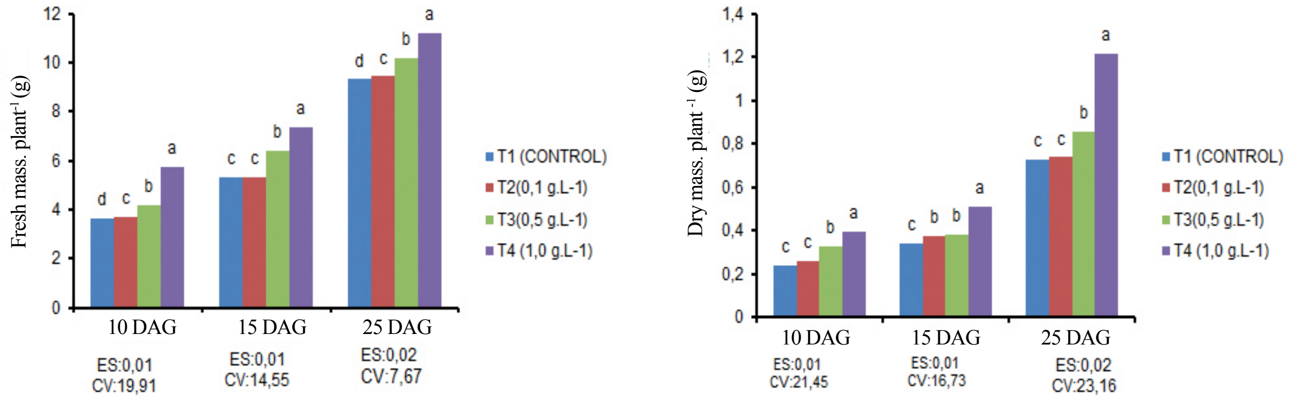
The latter had a negative effect on the root length of the seedlings, mainly in the initial and final stages of the seedlings.

The fresh mass of the aerial part of the seedlings (Figure 3A) showed significant differences p <0.005 in all tested concentrations, with the highest values being the treatment with 1,0 g L^{-1} concentration, followed by treatments with concentrations of 0,5 and 0,1 g L^{-1}, although the latter did not significantly differ from the control at 15 DAG.

Results similar to the fresh mass were found in the dry mass of the aerial part of the seedlings with the application of QuitoMax® by imbibition of the seeds (Figure 3A), where the treatments with the highest concentrations of the product (0,5 and 1,0 g L^{-1}) benefited this growth variable. In the treatment where the seeds were soaked with the lowest concentration of QuitoMax® (0,1 g L^{-1}), it did not show significant differences with the control during the whole growth period evaluated, except for 15 DAG.

Similar results to the fresh mass were found in the dry mass of the aerial part of the seedlings with the QuitoMax® application by imbibition of the seeds (Figure 3 A), where the treatments with the highest concentrations of the product (0.5 and 1.0 g L^{-1}) benefited this growth variable. In the treatment where the seeds were imbibed with the lowest concentration of QuitoMax® (0.1 g L^{-1}), it did not show significant differences with the control in the whole period of growth evaluated, except at 15 DAG.

In general, in all the growth variables evaluated, the application of QuitoMax® by imbibition of seeds was beneficial for the growth of tomato seedlings with respect to seedlings from untreated seeds. The best results were obtained in the treatments with the highest concentrations, specifically with the concentration of 1,0 g L^{-1}.



Different letters show significant differences between treatments for p≤0.05; According to the Duncan Test

Figure 3. Effect of QuitoMax® concentrations on fresh (A) and dry (B) mass of tomato seedlings at the seed stage

Similar results were obtained in Solanaceae but in potato minitubers whose growth was favored with the application of different concentrations of chitosan by imbibition of seeds. This provided desirable effects in the agricultural context, such as promoting vegetative growth and inducing germination in addition to improving tolerance to abiotic stress (18).

In a study of tomato cultivar var. Amalia, the seed imbibition was performed at different concentrations for four and eight hours, only the 1,0 g L^{-1} concentration exerted a positive effect on the dry mass of the seedlings of the crop; however, did not present modifications in the other variables evaluated (19). These results do not agree with those presented in this study, where the concentrations used stimulated the growth variables even though the imbibition time was considerably lower than that used by these authors.

The response shown by the different growth variables could be explained by the ability of QuitoMax® to stimulate the growth of the seedlings, which also maintains a close relation with the concentrations used, the molecular size and the application form of the product To the crop, which includes the time of contact with the organ perceiving the application, in this case the seed, the germination rate being stimulated and accelerating the growth. Chitosan has been shown to stimulate leaf protein levels as well as enzyme levels, increasing the basal resistance of plants (4, 19).

Effect of doses and QuitoMax forms® application on the development and agricultural yield of tomato plants, under field conditions.

With the application of the QuitoMax® product, a significant increase was achieved in all performance components evaluated with respect to control, demonstrating the positive effect of this product on the development of tomato plants under field conditions.

Table III shows that the highest values in the number of clusters, flowers and fruits were for the concentration of 1,0 g L^{-1} of QuitoMax® combined with the foliar spray of 0,3 g ha^{-1} of the product (T7); This treatment did not differ only from the one where the seeds were soaked with the 0,5 g L^{-1} concentration combined with the foliar spray of 0,3 g ha^{-1} of the product for the variable number of flowers per plant.

Table III. Effect of concentrations, dosage and form of application of QuitoMax® on some components of crop yield. (AF) = 0,3 g ha^{-1}

Treatments	Nu. Bunches, plant^{-1}	Nu. Flowers, plant^{-1}	Nu, Fruits, plant^{-1}	Mass, fruit^{-1} (g)
1,Control (without QuitoMax®)	4,16 f	10,26 d	9,19 g	70,11 g
2,0,1 g L^{-1} (Imb)	4,17 g	10,41 d	9,27 f	70,23 f
3,0,1 g L^{-1} (Imb) + (AF)	6,42 c	12,28 b	11,19 c	80,18 c
4,0,5 g L^{-1} (Imb)	5,39 e	11,08 cd	9,75 e	70,43 e
5,0,5 g L^{-1} (imb) + (AF)	7,39 b	13,45 ab	11,47 b	85,26 b
6,1,0 g L^{-1} (Emb)	6,02 d	11,19 c	9,89 d	75,41 d
7,1,0 g L^{-1} (imb) + (AF)	7,48 a	13,77 a	12,83 a	88,32 a
ESx	0,01*	0,51*	0,01*	0,02*
CV (%)	18,62	13,06	11,02	7,93

Stockings with common letters do not differ significantly according to Duncan (p <0.05)

On the other hand, the lowest values of the evaluated components corresponded to the plants that did not receive any treatment with the product (control), although the latter did not present significant differences in the number of clusters and flowers with the treatment where the seeds were embedded In the concentration of 0,1 g L^{-1}, does not differ in the number of flowers from the treatment with the concentration of 0,5 g L^{-1} applied by imbibition of seeds, which shows that the seeds imbibed in these doses do not respond positively for these components of plant yield. Therefore, low doses applied only to the seed, do not exert an effect on the productivity of the plants, demonstrating that for this to happen it is necessary then the combination with the leaf spray at the initial moment of the development of the plants.

Regarding agricultural yield, the applications of the product in its different forms had a positive influence on this productive indicator (Table IV). All the treatments with the QuitoMax® surpassed the control and in turn there were significant differences among them in dependence of the concentrations and their combinations with without the foliar spray of the product.

The highest yield (25,26 t ha^{-1}) corresponds to the treatment where the seeds were soaked with the 1,0 g L^{-1} concentration combined with the foliar spray of 0,3 g ha^{-1} of the product, after the transplantation, with an increase of 55 %, with respect to the control.

Table IV. Effect of concentrations, doses and form of application of QuitoMax®on the agricultural yield of cv Mara. (AF) = 0,3 g ha^{-1}

Treatments	Yield (t ha^{-1})	Increase (%)
1,Control (without QuitoMax®)	16,28 g	----
2,0,1 g $L^{-1}_{(Imb)}$	17,38 f	6
3,0,1 g $L^{-1}_{(Imb)}$ + $_{(AF)}$	23,13 c	48
4,0,5 g $L^{-1}_{(Imb)}$	18,56 e	14
5,0,5 g $L^{-1}_{(Imb)}$ + $_{(AF)}$	24,46 b	50
6,1,0 g $L^{-1}_{(Imb)}$	19,24 d	18
7,1,0 g $L^{-1}_{(Imb)}$ + $_{(AF)}$	25,26 a	55
ESx	0,06	----
CV (%)	14,74	----

Stockings with common letters do not differ significantly according to Duncan ($p < 0.05$)

Other yield-favorable treatments were where the seeds were soaked in the concentrations of 0,1 and 0,5 g L^{-1} and also added a foliar spray of 0,3 g ha^{-1} of the product, with values of 23,13 and 24,46 t ha^{-1} respectively for a 42 and 50 % increase. On the other hand, the other treatments in which the product was applied on a single occasion, showed a range of values ranging from 17 to 19 t ha^{-1} which were superior to the control with a superior yield of 16,28 t ha^{-1}.

The above results are in correspondence with results obtained in mini potato tubers and in the cultivation of tomato to achieve the promotion of the aerial and radical growth of the plants, the advancement of the period of flowering and fruiting, the increase of size and mass of fruits, as well as the number of flowers and fruits with chitosan applications (18, 20).

On the other hand, results similar to those found in this work have been published from studies in different crops such as tomato (*Solanum lycopersicum* L.), safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) to determine the potentialities of the polymers of chitosan, in which they succeeded in stimulating growth by imbibition of seeds in seedlings, as well as yields by leaf spray in the field with promising results (21, 22).

Also, it is pointed out that the application of biostimulants enhances the auxins involved in the plant reproduction process, resulting in a synergism between the applied substances and the natural hormones of the plants (23), which suggests that similar behavior happens when applies the QuitoMax® to tomato cultivation, managing to stimulate from growth to yield.

Similarly, these results confirm studies demonstrating that the combination of application forms such as seed treatment and application to the soil or plant of chitosan solutions in potato minitubers increases crop yield (18).

The favorable response of the productive indicators may be due to the fact that the leaf spray of QuitoMax® stimulated the physiological processes of the plants, increasing the size of the cells, which makes them more assimilable by the nutrients (24, 25). On the other hand, this effect could also be related to the ability of the product to act as antiperspirant by causing a partial or total closure of the stomata, favoring the water status of the plant and other physiological processes that contribute to increase biomass production and the agricultural yield, while reducing the losses of water in the plants (5).

CONCLUSIONS

According to the results obtained it can be concluded that the QuitoMax® applied by imbibition of the seeds, by foliar spraying or in their combination, stimulates the variables of growth, development and yield. The imbibition of the seeds with the highest concentration (1,0 g L^{-1}) produced the highest growth increases, at the seedbed level; while its combination with the subsequent foliar spraying of 300 mg ha^{-1} also led to increases in crop yield by 55 % with respect to the production control.

BIBLIOGRAPHY

1. Rodríguez, R. C. R.; Figueredo, J. V. y González, P. O. S. "Influencia de la quitosana en tomate (*Solanum lycopersicum*, Mill) var. «Amalia»". *Centro Agrícola*, vol. 40, no. 2, 2013, pp. 79-84, ISSN 2072-2001.
2. Ordookhani, K.; Khavazi, K.; Moezzi, A. y Rejali, F. "Influence of PGPR and AMF on antioxidant activity, lycopene and potassium contents in tomato". *African Journal of Agricultural Research*, vol. 5, no. 10, 31 de mayo de 2010, pp. 1108-1116, ISSN 1991-637X.
3. Fernández-Larrea, V. "Programa para la recuperación de bioplaguicidas, biofertilizantes y bioestimulantes en Cuba". *Agricultura Orgánica*, no. 2, 2013, pp. 2-5, ISSN 1028-2130.
4. Falcón-Rodríguez, A.; Costales, D.; Cabrera, J. C. y Martínez-Téllez, M. A. "Chitosan physico–chemical properties modulate defense responses and resistance in tobacco plants against the oomycete *Phytophthora nicotianae*". *Pesticide Biochemistry and Physiology*, vol. 100, no. 3, 2011, pp. 221-228, ISSN 0048-3575, DOI 10.1016/j.pestbp.2011.04.005.
5. Mansilla, A. Y.; Albertengo, L.; Rodríguez, M. S.; Debbaudt, A.; Zúñiga, A. y Casalongué, C. A. "Evidence on antimicrobial properties and mode of action of a chitosan obtained from crustacean exoskeletons on *Pseudomonas syringae* pv. tomato DC3000". *Applied Microbiology and Biotechnology*, vol. 97, no. 15, 2013, pp. 6957-6966, ISSN 0175-7598, 1432-0614, DOI 10.1007/s00253-013-4993-8.
6. Badawy, M. E. I. y Rabea, E. I. "A Biopolymer Chitosan and Its Derivatives as Promising Antimicrobial Agents against Plant Pathogens and Their Applications in Crop Protection". *International Journal of Carbohydrate Chemistry*, vol. 2011, 2011, pp. 1-29, ISSN 1687-9341, 1687-935X, DOI 10.1155/2011/460381.
7. Shehata, S. A.; Fawzy, Z. F. y El-Ramady, H. R. "Response of cucumber plants to foliar application of chitosan and yeast under greenhouse conditions". *Australian Journal of Basic and Applied Sciences*, vol. 6, no. 4, 2012, pp. 63–71, ISSN 1991-8178.
8. Lizárraga, P. E. G.; Miranda, C. S. P.; Moreno, M. E.; Lara, S. A. V. y Torres, P. I. "Maize seed coatings and seedling sprayings with chitosan and hydrogen peroxide: their influence on some phenological and biochemical behaviors". *Journal of Zhejiang University SCIENCE B*, vol. 14, no. 2, 2013, pp. 87-96, ISSN 1673-1581, 1862-1783, DOI 10.1631/jzus.B1200270.
9. Casanova, A.; Gómez, O.; Cardoza, H.; Piñeiro, F.; Hernández, C.; Murguido, A.; Fundora, L. y Hernández, A. *Guía técnica para la producción del cultivo del tomate*. 2.ª ed., Ed. Instituto de Investigaciones Hortícolas Liliana Dimitrova, 2010, La Habana, Cuba, 57 p., ISBN 978-959-7210-07-8, Biblioteca ACTAF.
10. Hernández, J. A.; Pérez, J. M.; Bosch, D.; Rivero, L.; Camacho, E.; Ruíz, J.; Salgado, E. J.; Marsán, R.; Obregón, A.; Torres, J. M.; Gonzáles, J. E.; Orellana, R.; Paneque, J.; Ruiz, J. M.; Mesa, A.; Fuentes, E.; Durán, J. L.; Pena, J.; Cid, G.; Ponce de León, D.; Hernández, M.; Frómeta, E.; Fernández, L.; Garcés, N.; Morales, M.; Suárez, E. y Martínez, E. *Nueva versión de clasificación genética de los suelos de Cuba*. Ed. AGROINFOR, 1999, La Habana, Cuba, 64 p., ISBN 959-246-022-1.
11. Paneque, P. V. M.; Calaña, N. J. M.; Calderón, V. M.; Borges, B. Y.; Hernández, G. T. C. y Caruncho, C. M. *Manual de técnicas analíticas para análisis de suelo, foliar, abonos orgánicos y fertilizantes químicos* [en línea]. Ed. Ediciones INCA, 2010, La Habana, Cuba, 157 p., ISBN 978-959-7023-51-7, [Consultado: 27 de enero de 2016], Disponible en: <http://mst.ama.cu/578/>.
12. Echevarría, H. A.; Cruz, T. A.; Rivero, G. D.; Rodríguez, P. A. T.; Ramírez, A. M. A. y Cárdenas, T. R. M. "Actividad antifúngica de la quitosana en el crecimiento micelial y esporulación del hongo *Pyricularia grisea* Sacc". *Cultivos Tropicales*, vol. 33, no. 3, 2012, pp. 80-84, ISSN 0258-5936, 1819-4087.
13. González, P. D.; Costales, D. y Falcón, A. B. "Influencia de un polímero de quitosana en el crecimiento y la actividad de enzimas defensivas en tomate (*Solanum lycopersicum* L.)". *Cultivos Tropicales*, vol. 35, no. 1, 2014, pp. 35-42, ISSN 0258-5936.
14. Badii, M. H. J.; Castillo, A. G. y Guillén, A. "Tamaño óptimo de la muestra". *Innovaciones de Negocios*, vol. 5, no. 1, 2008, pp. 53–65, ISSN 1665-9627.
15. Duncan, D. B. "Multiple Range and Multiple F Tests". *Biometrics*, vol. 11, no. 1, 1 de marzo de 1955, pp. 1-42, ISSN 0006-341X, DOI 10.2307/3001478.
16. StatPoint Technologies. *Statgraphics Centurion* [en línea]. (ser. Centurion), versión 16.1 (XV), [Windows], 21 de mayo de 2010, Disponible en: <http://statgraphics-centurion.software.informer.com/download/>.
17. Terry, E. A.; Terán, R.; Martínez-Viera, R. y Pino, M. A. "Biofertilizantes, una alternativa promisoria para la producción hortícola en organopónicos". *Cultivos Tropicales*, vol. 23, no. 3, 2002, pp. 43-47, ISSN 0258-5936, 1819-4087.
18. Kowalski, B.; Terry, F. J.; Herrera, L. y Agramonte, P. D. "Application of Soluble Chitosan *in vitro* and in the Greenhouse to Increase Yield and Seed Quality of Potato Minitubers". *Potato Research*, vol. 49, no. 3, 2006, pp. 167-176, ISSN 0014-3065, 1871-4528, DOI 10.1007/s11540-006-9015-0.

19. González, F.; Casanova, A.; Hernández, A.; Méndez, M.; González, R. y Delgado, A. “Efecto de la aplicación de Biobras-16 en la producción de plántulas injertadas de tomate (*Lycopersicon esculentum* Mill)”. *Temas de Ciencia y Tecnología*, vol. 10, no. 30, 2006, pp. 53–63, ISSN 2007-0977.
20. El-Mohamedy, R. S.; Abdel-Kader, M. M.; Abd-El-Kareem, F. y El-Mougy, N. S. “Inhibitory effect of antagonistic bio-agents and chitosan on the growth of tomato root rot pathogens *in vitro*”. *Journal of Agricultural Technology*, vol. 9, no. 6, 2013, pp. 1521–1533, ISSN 1686 - 9141.
21. Jabeen, N. y Ahmad, R. “The activity of antioxidant enzymes in response to salt stress in safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) seedlings raised from seed treated with chitosan: Enzyme activity in seedlings from chitosan-treated seed”. *Journal of the Science of Food and Agriculture*, vol. 93, no. 7, 2013, pp. 1699-1705, ISSN 00225142, DOI 10.1002/jsfa.5953.
22. Rodríguez, A.; Acosta, A. y Rodríguez, C. “Fungicide resistance of *Botrytis cinerea* in tomato greenhouses in the Canary Islands and effectiveness of non-chemical treatments against gray mold”. *World Journal of Microbiology and Biotechnology*, vol. 30, no. 9, 2014, pp. 2397-2406, ISSN 0959-3993, 1573-0972, DOI 10.1007/s11274-014-1665-5.
23. Sathiyabama, M.; Akila, G. y Charles, R. E. “Chitosan-induced defence responses in tomato plants against early blight disease caused by *Alternaria solani* (Ellis and Martin) Sorauer”. *Archives Of Phytopathology And Plant Protection*, vol. 47, no. 16, 2014, pp. 1963-1973, ISSN 0323-5408, 1477-2906, DOI 10.1080/03235408.2013.863497.
24. Lizárraga-Paulín, E. G.; Torres-Pacheco, I.; Moreno-Martínez, E. y Miranda-Castro, S. P. “Chitosan application in maize (*Zea mays*) to counteract the effects of abiotic stress at seedling level”. *African Journal of Biotechnology*, vol. 10, no. 34, 2011, pp. 6439-6446, ISSN 1684-5315.
25. Hadwiger, L. A. “Multiple effects of chitosan on plant systems: Solid science or hype”. *Plant Science*, vol. 208, 2013, pp. 42-49, ISSN 0168-9452, DOI 10.1016/j.plantsci.2013.03.007.

Received: March1rst, 2016
Accepted: May 24th, 2016

Revista Cultivos Tropicales
Instituto Nacional de Ciencias Agrícolas