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Original Article

Oligosacarinas stimulate the growth and yield on tomato (*Solanum lycopersicum* L) under protected conditions

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ABSTRACT

Biostimulant products exert various beneficial effects on plants, such as the induction of defensive mechanisms and stimulation of plant growth, in addition to having the advantage of not being harmful to the flora or to the environment. Oligosacarins, structural components of the cell wall of plants and microorganisms, exert effects on growth and development, among which are chitosan and its derivatives. The present work was developed between the period June to September 2017, on a typical Red Ferralitic soil, in a protected cultivation house, using the CADDO hybrid as plant material, with the general objective of evaluating the influence of the moments of application of Quitomax®, on growth, development, agricultural yield and presence of *Alternaria solani* Sor., on tomato crop under protected conditions. The results showed a positive effect of the product, where seeds imbibition of the in Quitomax® combined with the foliar spray of 300 mg ha^{-1} of the product at 7, 15 and 30 days after the transplant, positively influenced the height, diameter and number of leaves of the plants; as well as, increased agricultural yield by 18 % with respect to control treatment. The plants with Quitomax® application were more tolerant to the presence of *Alternaria solani* Sor.

Key words: vegetables, development, protected crop, biostimulant

INTRODUCTION

Tomato cultivation occupies an annual area of 4 803 680 ha and a worldwide yield of 33.68 t ha^{-1} [(1)]. In Cuba it is the main horticultural crop, with an open field area of 43,589 ha and an average annual yield of 11.34 t ha-1 year-1 [(2)], while for its production in the protected crop system 167 hectares are dedicated, with average yields between 100 and 140 t ha^{-1} $year^{-1}$ [(3)].

Globally, protected farming is recognized as an advanced agricultural technology that can effectively influence the production of fresh vegetables throughout the year. In Cuba, it is a promising technology to extend the harvesting schedules of vegetables and ensure their fresh supply to tourism, border market and population.

The Protected Cultivation system, as a modality of Cuban horticulture, has gained remarkable boom and diffusion since the 1990s. From that moment, it can be affirmed, the existing concern about forced (protected) crops, due to the abundant use of chemical products that deteriorate the ecosystem causing irreversible damage to the soil system; therefore, ecological alternatives are proposed for the management of agricultural crops under this productive system; since, it is known of the high consumption that is made of chemical products, both for nutrition and for the protection of plants, with a view to achieving the high yields that are achieved per unit area [(4-6)].

Governments, today, promote 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 that contain active ingredients, which act on the physiology of plants, increasing plant growth and development, as well as yields and crop quality [(7)].

In the current framework of agricultural biostimulants, the development of products that have chitosan as the main active ingredients has had a great boom in the last decade. These are glucosamine polymers and oligomers that are obtained by basic deacetylation of the chitin polymer, which, in turn, is extracted from the shellfish exoskeleton. Chitosan polymers and oligomers can have a wide agricultural application from the biological potentials that have been demonstrated to these compounds, such as promoting growth and improving yield and harvested fruits [(8)].

The Bioactive Products Group (GPB) of the National Institute of Agricultural Sciences (INCA) has developed a product based on chitosan polymers obtained from lobster exoskeleton chitin, whose trade name is Quitomax®. This product has been evaluated and extended in various crops of economic importance such as tomatoes, tobacco, potatoes, peppers, cucumbers, beans, soybeans, corn, and rice, among others, with positive and promising results [(9)].

Taking into account all of the above, it can be argued that there is a need to undertake studies where new biostimulant and biofertilizer products are introduced, as economically viable alternatives to increase production under protected crop conditions. All this will influence an economic, social and environmental benefit for agricultural production and therefore contribute to the country's food security. According to this background, the present work had as a general objective, to evaluate the influence of moments of application of Quitomax®, in the growth, development, agricultural yield and presence of *Alternaria solani* in tomato cultivation under protected conditions.

MATERIALS AND METHODS

To fulfill the proposed objective, the present study was carried out in the period from June to September 2017, at the National Institute of Agricultural Sciences (INCA), located in the Municipality of San José de las Lajas, Mayabeque province.

The experiment was carried out in a Tropical-A-12 model farmhouse with an “umbrella” effect, of 540 m^2. The soil is a Red Ferralitic lixiviate agrogenic, according to the new version of genetic classification of the soils of Cuba [(8)]. It was used, as a plant material, the CADDO hybrid characterized by being a strong and compact plant, with short internodes, excellent foliar cover and continuous moorings, fruit with extraordinary shelf life and a variety of early harvest cycle.

Quitomax® was used, whose active ingredient is a chitosan polymer, obtained by the INCA Bioactive Products Group, from the basic deacetylation (NaOH) of the chitin present in the lobster shell [(10)]. A stock solution of 1 % Quitomax® was prepared, which was diluted in sterile distilled water until the desired concentrations were obtained for the experiments.

The production of the seedlings was carried out in Cuban trays of expanded polystyrene with 247 alveoli of 32.50 cm^3 in volume. A mixture of 90 % earthworm humus + 10 % rice husk was used as substrate. Irrigation was carried out twice a day for five minutes, with an aerial micro sprinkler system.

Under a completely randomized design with four repetitions, two treatments were studied: with Quitomax® and a control treatment. The imbibition of the seeds in Quitomax® was carried out for 1 hour before sowing, using the dose of 1 g L^{-1}. At 15 and 30 days after germination (DAG) in seed conditions, 15 plants were selected for each random treatment, to which the following evaluations were performed:

- Seedling height (cm): measured with a graduated ruler, from the root neck to the youngest leaf's armpit
- Diameter of the stem of the seedlings (cm): it was determined with a king's foot, from two centimeters above the root neck
- Number of leaves per seedling: per visual count

The transplant was performed 30 days after sowing, in flat beds 180 cm wide and 15 cm high. The planting scheme used was that of a row, with a distance between plants of 40 cm, under a completely randomized design with four repetitions. For the foliar spray the dose of 300 mg ha^{-1} was used, applied three times after the transplant, these sprays were made in the early hours of the morning (8:00-9:00 am), to take advantage of the stomatic opening of the leaves, and were made manually using a backpack of 16 L capacity, with constant pressure cone nozzle. The following treatments were studied (Table 1).

Table 1. Treatments studied

Nu.	Treatments	
	Seedbed	**Transplant**
1	Imbibition of the seeds in Quitomax®	Imbibition
2		Imbibition + Foliar spray to 7 DAT
3		Imbibition + Foliar spray to 7 and 15 DAT
4		Imbibition + Foliar spray to 7, 15 and y 30 DAT
5		Foliar spray to 7, 15 and 30 DAT
6	Control (seeds embedded in water)	Control (without Quitomax®)

DAT: Days After Transplant

At 45 and 60 days after transplantation (DAT) at 15 plants per treatment, the variables were evaluated: plant height (cm), stem diameter (cm) and number of leaves per seedling, under the same methodology described previously.

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

Number of clusters, flowers and fruits per plant: by visual count

Average mass of the fruits (g): by division of the total weight of the fruits by the amount of fruits of the plot. It was performed in analytical balance

Agricultural yield (t ha^{-1}): by weighing the total production of the calculation area, extrapolated to one hectare

To evaluate the presence of *Alternaria solani*, the following evaluations were performed at 7 (1st evaluation) and 15 days (2nd evaluation) after the transplant:

The following formula was applied for the percentage of infection [11].

$$\% I = \sum (a.b) / n.k$$

The resulting value will be multiplied by 100. where:

a-degree of the scale

b- # of organs corresponding to each grade. n- sample size

k- higher scale value

The 6-grade scale was used at 15 plants per Silva treatment, 2009 [11] where:

0 - No symptoms (highly tolerant)

1 - Damage to the main root coping pint (tolerant)

2 - Damage of the coot of the main root, chlorosis old leaves (tolerant)

3 - Damage to the hypocotyl axis of the root and poor development of secondary roots, chlorosis of the leaves (susceptible)

4 - Damage to the hypocotyl axis of the root, which extends to the stem and poor development of secondary roots, leaf chlorosis (susceptible)

5 - Effects that led to the death of plants (highly susceptible)

To determine the level of involvement, the affected plants were quantified by treatments and the incidence was calculated by the following formula:

$$\%\ \text{Incidence} = \frac{\text{Affected plants}}{\text{Observed plants}} * 100$$

Criteria for estimating the infection level according to Silva, 2009 [9]:

-10 % Light

10-30 % Medium

+ 30 % Intense

The data obtained were analyzed using a Simple Classification ANOVA. The resulting means were compared with the Duncan Multiple Range Test for p≤ 0.05 when there were significant differences between treatments, processed with the Statgraphics Centurion program (2013) under the Windows7 operating system.

RESULTS AND DISCUSSION

Seedling growth effect

The results obtained when evaluating the height, diameter and number of leaves per plant (Table 2) showed that in the first evaluation made 15 days after sowing, no significant differences were obtained with respect to the control treatment. However, in the evaluation performed 30 days after planting, the two treatments are differentiated from each other for each of the variables evaluated.

Table 2. Effect of Quitomax® on tomato (CADDO hybrid) plants growth

Treatments	Plant height (cm)		Stem diameter (cm)		Number of leaves/plant	
	15 DAS	30 DAS	15 DAS	30 DAS	15 DAS	30 DAS
With Quitomax®	11.28	18.26 a	0.23	0.35 a	2.55	4.75 a
Control (sin Quitomax®	10.09	16.14 b	0.22	0.31 b	2.40	3.18 b
ES x	0.54 n.s	0.61***	0.008 n.s	0.05***	0.16 n.s	0.04***

Different letters show significant differences between treatments according to the Duncan Test for p≤0.05. (n = 15)

In the case of height, the control treatment was exceeded by 13 %, the diameter is greater by 12 % and the number of leaves increases to the control by 49 %. However, in both treatments a quality posture was achieved for the transplant where a tomato plant is suitable for transplantation when it has in a cycle of 28-30 days, a height of 15-18 cm, 5 leaves and a diameter of stem greater than 3 mm [3].

Similar results were obtained in the potato crop (*Solanum tuberosum* L.) whose growth is favored with the application of two oligosaccharins (Pectimorf® and Quitomax®), providing desirable effects in the agricultural context, such as promoting vegetative growth, production of tubers and agricultural yield [12].

In the same tomato crop (*Solanum lycopersicum* L), studies of different concentrations of chitosan by imbibition of seeds (var. Amalia) for four and eight hours, only the concentration of 1.0 g L^{-1} exerted a positive effect on dry mass of crop seedlings, although it did not change the rest of the growth indicators evaluated [13]; however, in this work the dose used stimulated the growth variables even though the imbibition time was considerably shorter than that used by these authors.

Table 3 shows that the growth variables evaluated at 45 days after transplantation showed significant differences for each of them. With respect to the height of the plants, this was superior in the treatment where it was split from seeds embedded in Quitomax® and subsequently foliar spraying was performed at seven and fifteen days after transplantation; the rest of the treatments did not differ from this, but not from the control.

In the case of stem diameter, also the treatment of Quitomax® Imb. + A.F. (7.15 and 30 DAT), it was statistically different from the other treatments. For this growth variable, all treatments with Quitomax® outperformed the control. A result similar to that of height was obtained in the number of leaves per plant, where the same treatment is maintained as the one with the best result.

Table 3. Effect of Quitomax® on the growth of CADDO hybrid tomato plants) 45 days after transplantation (DDT)

Treatments	Plant height (cm)	Stem diameter (cm)	Number of Leaves/ plant
Control (without Quitomax®)	40.1 b	0.403 c	7.25 c
Quitomax® (Foliar spray 7-15-30 DAT)	42.1 ab	0.584 b	8.05 abc
Quitomax® (1 hour imbibition)	42.4 ab	0.585 b	7.35 c
Quitomax® (Imbibition. + Foliar spray to 7DAT)	43.75 ab	0.542 b	7.85 bc
Quitomax® (Imbibition + Foliar spray to 7 and 15 DAT)	41.65 ab	0.539 b	8.60 ab
Quitomax® (Imbibition + Foliar spray to 7, 15 and 30 DAT)	45.3 a	0.656 a	8.90 a
ESx	1.41*	1.64*	0.33*

Different letters show significant differences between treatments according to the Duncan Test for $p \leq 0.05$. (n = 15)

The response shown by the different growth variables could be explained based on the ability of Quitomax® to stimulate seedling growth, which also maintains a close relationship with the concentration used, the molecular size and the method of application of the product to the crop, which includes the contact time with the organ that perceives the application, in this case the seed, the germination speed being stimulated and the growth accelerated. It has been shown that chitosans stimulate protein levels in the leaves, as well as enzyme levels, increasing the basal resistance of plants [14,15].

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

Table 4 shows that the highest values in the number of fruits per plant, as well as in the fresh mass of the fruits, were achieved with the treatment in which the seeds were embedded in 1.0 g L^{-1} of Quitomax® combined with the foliar spray of 300 mg ha^{-1} of the product sprayed to the plants at 7, 15 and 30 after transplantation.

It is noteworthy the increase that was achieved in the amount of fruits per plants with respect to the control treatment where only the soil was fertilized, so it can be inferred that the biostimulant product allows plants to more efficiently absorb the nutrients present in the solution of the soil and with it a greater production.

Table 4. Effect of Quitomax® on tomato (CADDO hybrid) yield components under protected culture conditions

Treatments	Number of fruits / plant	Fresh mass of fruits/plant (kg)
Control (without Quitomax®)	20.9 d	3.52 c
Quitomax® (Foliar spray to 7-15-30 DAT)	25.2 bc	4.25 bc
Quitomax® (Imbibition 1 hour)	21.7 cd	3.66 c
Quitomax® (Imbibition. + Foliar spray to 7 DAT)	26.3 ab	4.43 b
Quitomax® (Imbibition. + Foliar spray to 7 and 15 DAT)	27.6 a	4.60 b
Quitomax® (Imbibition. + Foliar spray to 7, 15 and 30 DAT)	27.3 a	4.65 a
ESx	0.64*	7.41*

Different letters show significant differences between treatments according to the Duncan Test for $p \leq 0.05$. (n = 15)

Regarding agricultural yield, the applications of the product in its different forms (imbibition or foliar spray) positively influenced this productive indicator (Figure 1). All treatments with Quitomax® outperformed the control (except in the treatment where only the seeds were embedded in the product) and in turn there were significant differences between them, depending on the amount of foliar sprays made of the product after transplantation.

Corresponding to the performance components, this was superior in the treatment of Quitomax® Imb. + A.F. (7, 15 and 30 DAT) with a yield of 41.25 t ha$^{-1,}$ which exceeded the control treatment by 18 %.

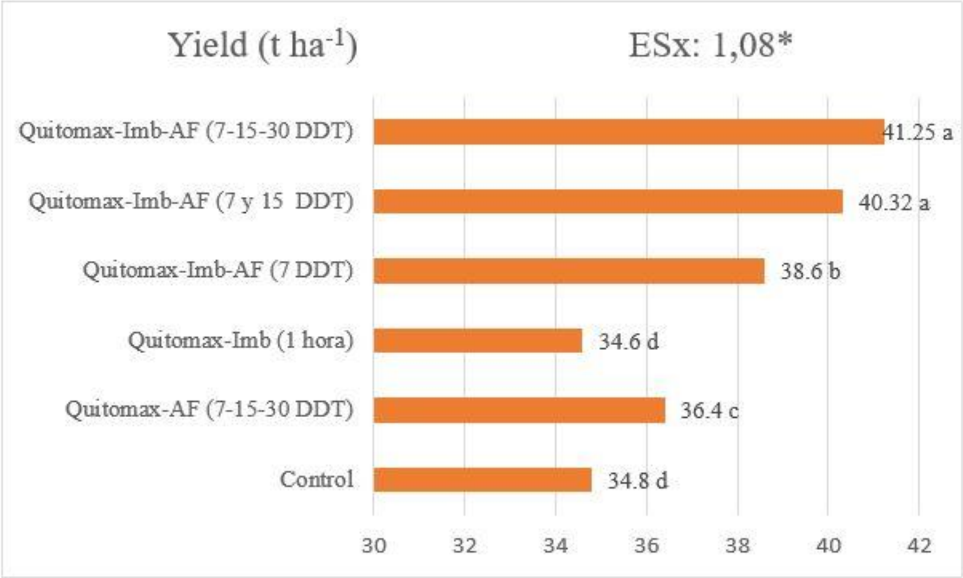

Figure 1. Effect of Quitomax® on tomato yield (CADDO) under protected cultivation conditions. Means with different letters show significant differences between treatments according to the Duncan test for $p \leq 0.05$. (n = 15)

The aforementioned results are in correspondence with those obtained in mini potato tubers and in tomato cultivation by achieving the advance of the flowering and fruiting period, the increase in the size and mass of the fruits, as well as the number of flowers and fruits with chitosan applications [16]. A similar result has been obtained in the germination of the seeds of these crops [17].

On the other hand, results similar to those found in this work have been published from studies carried out in different crops (tomato, safflower and sunflower), in order to determine the potential of chitosan polymers, in which they managed to stimulate growth by imbibition of seeds in seedling stages, as well as, the yields by foliar sprinkling in the field with promising results [17,18].

Also, it is noted that the application of biostimulants, enhances the auxins involved in the process of plant reproduction, occurring a synergism between the substances applied and the natural hormones of plants [19], which suggests that similar behavior happens when apply Quitomax® to tomato cultivation, stimulating growth to yield.

These results are related to studies that show that the combination of forms of application such as treatment to seeds and application to the soil or on the plant of chitosan solutions, can contribute to increasing crop yields [20].

The favorable response of the productive indicators may be due to the fact that the foliar sprinkling of Quitomax® stimulated the physiological processes of the plants, increasing the size of the cells, which makes the nutrients more assimilable by them and, on the other hand, this effect It could also be related to the ability of the product to act as an antiperspirant and cause a partial or total closure of the stomata, favoring the water status of the plant and other physiological processes that contribute to increasing biomass production and agricultural performance, which in turn reduces water losses in plants (21).

Effect of Quitomax® on the degree of incidence and percentage of infection by *Alternaria solani*

The use of microbial antagonists for the control of phytopathogens has been cataloged as an important complement in the integrated management of pests, considering this aspect among the various proposed mechanisms that explain the promotion of plant growth.

Therefore, when evaluating the incidence of Quitomax®, in its influence on the *Alternaria solani* fungus, statistical differences were obtained between the three treatments studied (Table 5), the degree of incidence of the fungus in the control treatment being higher with a degree of susceptibility to the pest, corroborating the "Theory of Trophobiosis", whose scientific argument is based on the fact that plants with inadequate nutritional status will be more susceptible to attack by pathogens than those that have been well fed, and there is a proportional response to the use of Ecological alternatives and inversely proportional to the use of pesticides (22).

In the case of treatment, where Quitomax® was applied via imbibition of the seeds plus two foliar sprays, the leaf effects caused by *A. solani* were minor, obtaining in these plants, slight indexes of affectation in the first evaluation (15 days later of the transplant), with a 7 % infection which is increased in the second evaluation with an index of 13 % (45 days after the transplant) with respect to the control.

Table 5. Influence of Quitomax® in the presence of *Alternaria solani* in tomato cultivation under protected conditions

Treatments	**1rst evaluation (7 DAT)**	
	G. incidence	**% infection**
Quitomax® (Aspersión foliar)	2º	9 b
Quitomax® (Imbibition + Foliar spray)	2º	7 c
Control (without Quitomax®)	3º	12 a
ESx	-	0.16**
	2nd evaluation (15 DAT)	
Quitomax® (Foliar spray)	3º	20 b
Quitomax® (Imbibition + Foliar spray)	3º	13 c
Control (without Quitomax®	4º	33 a
ES x	-	0.21*

Different letters show significant differences between treatments according to the Duncan Test for $p \leq 0.05$. (n = 15)

For the control treatment, in the first evaluation the levels were light; however, an intense level of infection was denoted in the following evaluation; therefore, this result allows to corroborate the effect of Quitomax® as a biocontrol product of pathogens since in the two treatments with this bioproduct the plants were less affected. This pathogen is present throughout the country causing significant damage to the crop, preferably when there are nutritional and water deficits in the plants, which increases the susceptibility to the pest.

The ecological management of agroecosystems favors the diversity of natural enemies, reducing the economic damage caused by insect pests. The levels of phenols in the plants can constitute a way for the partial or total control of fungal pathogens in some species of plants, in this way, these were determined to know to what extent, and the products stimulated the contents of phenols in the plants by be one of the possible pathways for greater tolerance to pathogen attack (9).

One of the greatest agricultural needs is the protection of seedlings against diseases caused by pathogens. In this sense, both the chitosan polymer and its oligomers are potent inducers of defensive responses and resistance in the plant against pathogens (9). This protection may be due to the antimicrobial activity that these polymers and oligomers exert on the microorganisms or it may be the result of the elevation of the basal resistance of the plant, caused by the activation of induced resistance exerted by these compounds in the plant [23,24].

CONCLUSIONS

The positive effect of the Quitomax® biostimulant in the stimulation of growth, development and yield in plants from embedded seeds was combined, combined with foliar sprays at 7, 15 and 30 days after transplantation, 18 % higher than control. On the other hand, plants treated with Quitomax® have a low incidence and percentage of infection with Alternaria solani demonstrating the effect of the product as biocontrol.

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