



Use of QuitoMax[®] biostimulant for the growth and development of garlic (*Allium sativum* L.) plants

Efecto del bioestimulante QuitoMax[®] en el crecimiento y rendimiento del ajo (*Allium sativum* L.) clon ‘Criollo’

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ABSTRACT: The objective of this work was to evaluate the application effect of different concentrations of QuitoMax[®] in the growth and development of the garlic cultivar "Criollo Víctor". Prior to manual planting in beds, cloves were soaked for 24 hours in solutions with different concentrations of QuitoMax[®] (1, 5 and 10 mg L⁻¹) and a control that was soaked in water. At 50 days after planting (dap) a foliar application was made with these same concentrations. A randomized block experimental design with four treatments and three replications was used. The indicators of plant height, number of leaves and chlorophyll content were evaluated at 70; 90 and 110 dap. At 120 dap, the neck and equatorial diameters of the bulb, number of clove "seeds", fresh and dry mass of the bulb, as well as agricultural yield were determined. All the concentrations of QuitoMax[®] stimulated the different variables that were evaluated.

Key word: Alliaceae, agricultural productivity, chitosan, creole, garlic.

RESUMEN: El objetivo de este trabajo fue evaluar el efecto de la aplicación de diferentes concentraciones de QuitoMax[®] en el crecimiento, desarrollo y rendimiento del cultivo de ajo, clon ‘Criollo’. Previo a la plantación manual en canteros, los dientes “semillas” de ajo se embebieron durante 24 horas en soluciones con diferentes concentraciones de QuitoMax[®] (1; 5 y 10 mg L⁻¹) y un control que se embebió en agua. Se empleó un diseño experimental de bloques al azar con cuatro tratamientos y tres réplicas. Se evaluaron los indicadores altura de la planta, número de hojas y contenido de clorofilas, a los 70; 90 y 110 ddp. A los 120 ddp, se determinaron los diámetros del cuello y ecuatorial del bulbo, número de dientes “semillas”, masa fresca y seca del bulbo, así como el rendimiento agrícola. Todas las concentraciones de QuitoMax[®] estimularon las diferentes variables que se evaluaron.

Palabras clave: Alliaceae, productividad agrícola, quitosano, clon.

INTRODUCTION

It is known that garlic (*Allium sativum* L.), as well as onion (*Allium cepa* L.), are plants that originated in Central Asia. In Cuba, there are reports of areas dedicated to garlic cultivation since the beginning of the 19th century, constituting one of the crops most used as a condiment by the Cuban population. However, in our country, yields are very low, despite the fact that high volumes of mineral

fertilizers and pesticides are applied in its production system, which casts doubt on the sustainability of these productions (1). In the world, more than 1,250,000 ha are cultivated, with productions of 12 million tons and yields of 11.89 t ha⁻¹. The countries with the highest production of this Alliaceae are: China, India, South Korea and Spain; and in Latin America, Argentina, Chile and Mexico stand out (2).

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The use reduction of agrochemicals in a production system can be achieved with the introduction of growth biostimulators, which stimulate plant growth and development due to their capacity to improve plant efficiency in the absorption and assimilation of nutrients and tolerance to biotic and abiotic stress conditions (3). The Bioactive Products Group (GPB, according its acronyms in Spanish) of the National Institute of Agricultural Sciences has studied a liquid growth biostimulator based on chitosan polymers obtained from the chitin found in the exoskeleton of the locust (*Palunirus argus* Latrille), whose commercial name is QuitoMax® (4). The application of this growth biostimulator in production systems stimulates plant germination, growth and development, while activating plant defense mechanisms, which are closely related to phytopathogenic diseases (5).

This national product has been introduced and extended in several crops, mainly in grains and solanaceae of economic importance, with beneficial results in plant development, yields and anti-stress protection (6). Research related to the use of QuitoMax® in garlic cultivation is scarce. Therefore, the aim of this work is to evaluate the application effect of different QuitoMax® concentrations on the growth and development of the garlic crop, clone 'Criollo Victor'.

MATERIALS AND METHODS

The research was carried out at "La Jaula" farm, in San José de las Lajas municipality, Mayabeque province. Garlic "seeds" (*Allium sativum* L.), clone 'Criollo Victor', provided by a prominent producer of San Nicolas de Bari, Mayabeque province were used. Planting was carried out in the 2016-2017 and 2017-2018 planting seasons; on beds containing a Brown sialitic soil, according to the Cuban soil classification (7), with pH= 7.80 and organic matter (OM) 5.59 % content.

Three beds were formed with cow dung, homogeneously mixed with the soil, before raising them, with a dimension of 20 m long; 1.4 m wide and 0.3 m high. The "seed" tines were planted manually with a planting distance of seven rows at 20 cm x 10 cm between plants (8).

The chitosan formulation, known as QuitoMax® (RCF 010/17, Central Fertilizer Registry of Cuba), which is a liquid biostimulator of growth based on chitosan polymers and chemical salts, obtained by the Bioactive Products Group of INCA (4), was used.

The "seed" clove were soaked for 24 hours in QuitoMax® solutions at concentrations of 1, 5 and 10 mg L⁻¹, and the control treatment in water, before planting.

Foliar applications were made at concentrations of 1, 5 and 10 mg L⁻¹ of the growth biostimulator and with water (control treatment), at 50 days after planting (dap), for a total of four treatments. Throughout the experiment, irrigation with a 10 L watering can and the elimination of weeds by manual weeding were carried out every other day.

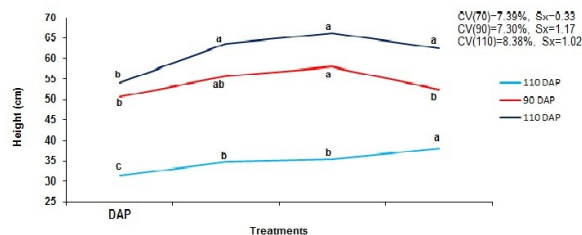
At 70, 90 and 110 days after planting (dap), the indicators plant height (cm), number of leaves and chlorophyll content

[SPAD units] (9) were evaluated with a portable meter model SPAD-502, Minolta. At 120 dap, harvest was performed and the diameters of the neck (cm) and equatorial bulb (cm), number of "seeds", fresh (g) and dry mass of the bulb (g) were determined in 10 plants selected at random for each plot, as well as the agricultural yield (t ha⁻¹) in all plants of each treatment.

The experiment was repeated twice under a randomized block design with three replications and four treatments. Data were tabulated and plotted using Microsoft Office (2019) Excel tool and the means of the two years were used, these data were processed using a Simple Classification Analysis of Variance (ANOVA) and the comparison of means was performed using Tukey's test at 95 % confidence, when there were significant differences between treatments; the statistical package STATGRAPH Version 5.1 was used.

RESULTS AND DISCUSSION

The results presented in Figure 1 showed that, for plant height, at 70 dap, T4 was the best performing, followed by T3, T2 and T1; at 90 dap, the best performing treatments were T3 and T2, followed by T4 and T1; at 110 dap, T2, T3 and T4 were the treatments that determined the greatest plant height, followed by T1.



T1: Control, T2: 1 mg L⁻¹, T3: 5 mg L⁻¹ y T4: 10 mg L⁻¹. CV (70) =7,39%, SEx=0,33; CV (90) =7,30%, SEx=1,17; CV (110) =8,38%, ESx=1,02. Different letters show significant differences between the treatments according to the Tukey 95 % test

Figure 1. Height of garlic plants (*Allium sativum* L.) clone 'Criollo Victor' treated with different concentrations of the growth biostimulator QuitoMax®, evaluated at 70, 90 and 110 days after planting (dap)

The application of this growth biostimulator in productive systems allows the stimulation of germination, growth and development of plants, while activating defense mechanisms in plants, which are closely related to the attack of phytopathogenic diseases (5).

Plant height values were found to be within the range reported in the literature for other garlic clones (10,11). However, in research that preceded this one (12), they reported a range between 28.5-29.5 cm of foliage height in the clone 'Criollo-9'. According to these authors (11,13,14), in Cuba, different clones are known with the term "Criollo", which were mixed through time, which could explain the results of this work, since the research worked with the clone 'Criollo Victor'.

It is known that QuitoMax is a complex carbohydrate capable of shaping the growth and development of plants at low concentrations, so the use of these biostimulants allows a more rational use of resources, reducing production costs without affecting the quality and yields of crops (15); these last aspects should be considered, due to their importance in our production conditions.

The results for the indicator number of leaves at 70; 90 and 110 days after planting the garlic crop are shown in Figure 2). There was no significant difference between treatments T2, T3 and T4, but there was a significant difference with respect to the control (T1). All QuitoMax® concentrations tested were higher than the control at the three evaluation times, except for the concentration of 1 mg L⁻¹ (T2) at 70 dap.

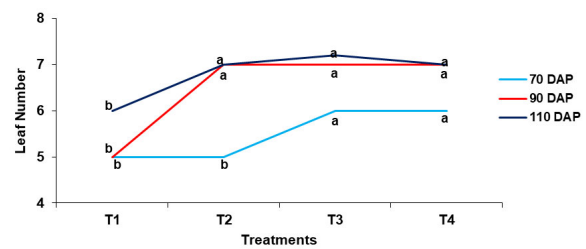
For the treatments with QuitoMax® at 70, 90 and 110 ddp, no significant differences were observed between them, but significant differences were observed with respect to the control treatment, due to the fact that at these moments the plants are in the vegetative phase and, specifically, in the phase of formation and growth of the bulbs; therefore, it is expected that the vegetative phase has already finished (16,17).

Plants at 90 and 110 dap had seven leaves in the QuitoMax® treatments, which are lower than those reported by other authors (18), who stated that with 'seed' clove of the 'Chinese' variety treated with Chito-Care®, a commercial product based on chitosan, from Egypt, they obtained between 7.50-7.75 leaves at 120 dap of cultivation. The number of leaves can increase or decrease in cultivars due to environmental conditions and inadequate cultural attentions, such as planting date (16).

The increase in the number of leaves with the use of the chitosan-based growth biostimulator could cause an increase in leaf area in these plants, while allowing them to make efficient use of solar radiation and, consequently, increase those processes dependent on light, for example, photosynthesis.

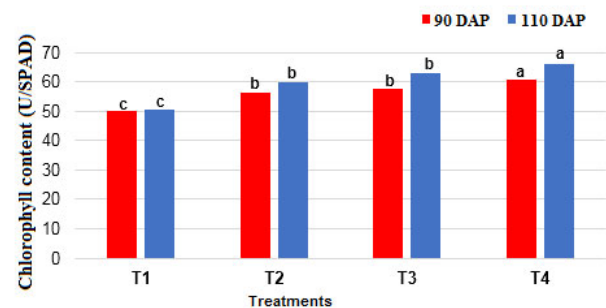
The results related to chlorophyll content in the leaves of garlic plants evaluated at 90 and 110 days after planting are shown in Figure 3.

At 90 and 110 ddp, the highest results were for the plants of treatment T4, which significantly outperformed the rest of the plants of treatment T2 and T3, followed by T1. The values of SPAD units regidrated correspond to the values reported for other garlic cultivars (17), although they are lower than those reported by other authors, who report values between 69.4-75.2 SPAD units in leaves of garlic plants, variety 'Sids 40', treated with three biostimulants (hydroalcoholic extract of moringa, cysteine and glutamine) by foliar spraying. These authors made three foliar applications of the biostimulants, at 30, 60 and 90 days after garlic planting; and in the first year of application (2013-2014), they found that chlorophyll content had been stimulated by the use of the biostimulants. However, in the second year (2014-2015), no differences between treatments were found. It is possible that the lower SPAD



T1: Control, T2: 1 mg L⁻¹, T3: 5 mg L⁻¹ and T4: 10 mg L⁻¹. CV (70) =9.32%, SE x=0.11; CV (90) =13.67%, SE x=0.20; CV (110) =10.23%, SE x=0.15. Different letters show significant differences between treatments, according to Tukey's test 95%

Figure 2. Leaf number of garlic plants, clone 'Criollo Victor', treated with different concentrations of the growth biostimulator QuitoMax®, evaluated at 70, 90 and 110 days after planting (dap)



T1: Control, T2: 1 mg L⁻¹, T3: 5 mg L⁻¹ and T4: 10 mg L⁻¹. CV=6.62%, SE x=5.80; CV=7.21%, SE x=4.75. Different letters show significant differences between treatments according to Tukey's test 95 %

Figure 3. Chlorophyll content in leaves of garlic plants, clone 'Criollo Victor', treated with different concentrations of the growth biostimulator QuitoMax®, at 90 and 110 days after planting (dap)

unit values found in this article may be due to other factors such as mineral nutrition and clone (18).

On the other hand, the bulb filling phase and the differentiation of the "seed" cloves, which occur at the same time, depend on the growth of the aerial part (13,11), an aspect that was confirmed in the results in this work for the bulb neck diameter and bulb equatorial diameter indicators (Table 1), in which an increase in these indicators, such as the bulb equatorial diameter and the number of "seed" cloves, was observed with the use of the QuitoMax® chitosan polymer. There were no significant differences between treatments in bulb neck diameter values.

The values of bulb neck diameter are within the range reported in the literature (13) and they are higher than those obtained with the application of FitoMas-E® and Ecomic® products, the former through foliar sprays and the latter through the coating of the "seeds", during 2013-2014 (1).

The increase in garlic bulb diameter in plants grown with biostimulants has been reported by several authors (18,19),

Table 1. Bulb neck and bulb equatorial diameter and number of cloves "seeds" of garlic plants (*Allium sativum* L.) clone 'Criollo Victor' treated with different concentrations of the growth biostimulator QuitoMax®

Treatments	Bulb neck diameter (cm)	Bulb equatorial diameter (cm)	Number of cloves "seeds"
T1	0.642	2.768 c	16.8 d
T2	0.760	3.454 ab	19.8 c
T3	0.776	3.684 a	23.2 a
T4	0.702	3.100 bc	20.6 bc
CV (%)	0.14	0.35	1.22
SE x	0.03 n.s	0.09	1.406

T1: Control, T2: 1 mg L⁻¹, T3: 5 mg L⁻¹ and T4: 10 mg L⁻¹

Different letters show significant differences between the means of the treatments according to Tukey's test 95 %

therefore, the QuitoMax® application could have an important commercial and economic implication, once the bulb is the commercial attribute of this crop.

The action mode of the different biostimulants is not well defined and can be considered multifaceted. In the case of chitosans (QuitoMax®), the increases in growth indicators are related to the stimulation of the photosynthetic process, of enzymes related to N metabolism, as well as an antitranspirant effect through stomatal closure by direct action on the abscisic acid levels of cells accompanying the stomata, which enhances the water status of plant, as well as other physiological processes (20).

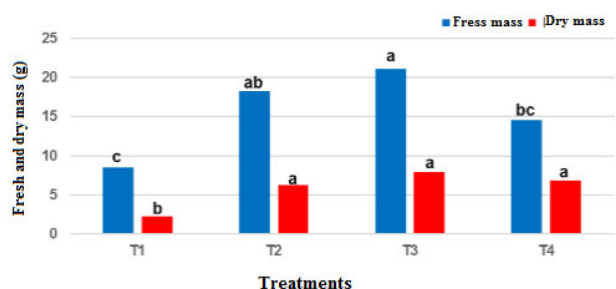
Regarding the number of "seed" cloves, the best results were achieved by plants that emitted bulbs with treatment 3 (5 mg L⁻¹ of QuitoMax®), which differed statistically from the plants that emitted bulbs with the rest of the QuitoMax® treatments, and all of these were superior to the control treatment.

Regarding the number of "seed" cloves, the results of this work correspond to the range of values reported in the literature (15-30 seed "cloves") for the 'Criollo' clone (21).

For the indicators fresh and dry mass of garlic bulbs treated with different concentrations of QuitoMax®, the highest values of fresh mass were obtained with treatments T3 and T2, followed by T4 and T1. As for dry mass, the treatments with QuitoMax® concentrations showed significantly higher values than the control treatment (Figure 4).

The values of fresh bulb mass (9-21 g) were below the reported values of 28.4-37 g for this type of clone (22). They were also below those obtained by other authors (20) between 2009-2010 (49.22-51.23 g) and 2010-2011 (47.34-48.42 g) with garlic of the 'Chinese' variety treated with a commercial product based on chitosan, called Chito-Care®. However, they are within the range of values reported during 2011-2012 (18-23 g) and 2013-2014 (8-11 g), where the lower mass in the second year is attributed to the prevailing climatic conditions and the incidence of *Thrips tabaci* L., with an infection rate of 25 % (1).

It is often difficult to make comparisons when the time at which the determinations were made is not fully described. In this sense, the literature consulted does not specify whether the fresh mass is immediately after harvesting the garlic or after curing, nor does it specify the



T1: Control, T2: 1 mg L⁻¹, T3: 5 mg L⁻¹ and T4: 10 mg L⁻¹. CV=11.14%, SE x=1.44; CV=12.36%, SE x=0.41. Different letters show significant differences between treatments according to the Tukey 95 % test

Figure 4. Fresh and dry mass of bulbs of garlic plants, clone 'Criollo Victor', treated with different concentrations of QuitoMax® biostimulator

curing time. There is a loss of bulb mass after harvesting and/or curing, which varies depending on the garlic clone (21).

The bulb is the organ where nutritive substances accumulate, which come from the photosynthetic process, therefore, the dry mass of the bulb will depend on the development of the aerial part (13), which in this work was benefited with the use of the different concentrations of the growth biostimulator (Figures 1 and 2), and which corresponds with the results obtained for this dry mass indicator (Figure 4).

The use of different QuitoMax® concentrations caused an increase in plant height, number of leaves, chlorophyll content, bulb diameter and bulb dry mass; the last two attributes are of great importance for the commercialization and consumption of this agricultural product.

The application of QuitoMax® different concentrations caused yield increases (Figure 5), the best results were obtained with the treatments in which QuitoMax® was applied at a concentration of 5 mg L⁻¹ (T3), followed by T4 and T2, with respect to the Control treatment (T1), respectively.

Except for the control treatment, with which a low yield was obtained, with a value lower than the mean reported in the country, which is 2 t ha⁻¹ (1), the rest of the treatments exhibited yields between 4 and 9 t ha⁻¹, range reported for

the most used clones in the country ('Criollo' and 'Vietnamese') in the edaphoclimatic conditions of Cuba (14).

Yields obtained in this work are below the potential reported for the clone 'Criollo-9' of 16.8 t ha⁻¹ (13) and for the cultivar 'HOV-1', from Viet Nam, of 11.9 t ha⁻¹ (18). However, they are higher than those obtained by other authors (1), who evaluated the influence of a biofertilizer and a biostimulant, with which they obtained yields between 3.65-4.54 t ha⁻¹ in 2011-2012 and between 1.71-2.10 t ha⁻¹ in 2013-2014. It is possible that the yields obtained in this work are conditioned by clone, stocking density, as well as crop conditions.

It is known that the application of chemical fertilizers increases crop growth and productivity; however, the efficiency in the absorption and use of nutrients is sometimes limited and this situation could be reversed with the use of growth biostimulators, alone or combined with biofertilizers. QuitoMax® could be an alternative for the sustainability of garlic production in our country, where high volumes of inputs are applied for the management of phytopathogenic diseases and fertilization in this crop (23).

CONCLUSIONS

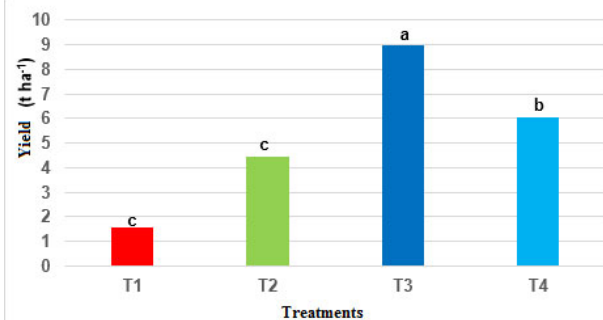
- Plant height and number of leaves indicators of 'Criollo Victor' clone garlic plants grown in beds increased with the use of different doses of the biostimulant QuitoMax®.
- Yield indicators, bulb equatorial diameter and number of "seed" cloves of 'Criollo Victor' clone grown in beds were favored with the use of QuitoMax®. The dose of 5 mg L⁻¹ was the promising.
- The use of QuitoMax® increases crop production without affecting crop quality, making this biostimulant an agroecological alternative for garlic production.

RECOMMENDATIONS

- To study the combined action of QuitoMax® with fertilizer doses, complete NPK formula, on garlic crop.
- Evaluate the effect of QuitoMax® on other garlic clones or other alliaceae.

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T1: Control, T2: 1 mg L⁻¹, T3: 5 mg L⁻¹ and T4: 10 mg L⁻¹. CV=1.50 %, SE x=1.05. Different letters show significant differences between treatments according to Tukey's test 95 %

Figure 5. Yield of garlic production, clone 'Criollo Victor' and use of QuitoMax® concentrations

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