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Original article

# Oligosaccharines promote growth and development of elefante garlic (*Allium ampeloprassum* L.) plants

## Las Oligosacarinas estimulan el crecimiento y desarrollo de plantas de ajo elefante (*Allium ampeloprassum* L.)

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**ABSTRACT:** The use of biostimulants in plant production is an agricultural practice friendly to environment and one of the tools employed in the sustainable agriculture. The present paper was to determine the influence that the application of products based in oligosaccharines exerted on plant growth and development of garlic (*Allium ampeloprasum* L.) cv. 'Fredy'. The present work was performed, under field conditions, during the period October 2020 - February 2021 with the objective of determining the influence of the application of products based in oligosaccharines on the growth and development of elephant garlic plants (*Allium ampeloprasum* L.) cv. 'Fredy'. The effect of determined doses of QuitoMax® and PectiMorf® products, in different ways of application ("seed" imbibition, foliar spray at 80 DAP and the combination of both with the particularity was evaluated of also, combining the products) on the behavior of emergence percentage at 7 and 14 DAP. Besides, plant height (cm) and leaf number at 70, 90 and 110 DAP; as well as, the bulb characteristics and the survival percentage at harvest time was evaluated too. In addition, plant yield was estimated. Results shown that, in general, the application of QuitoMax® was more efficient than that of PectiMorf® in stimulating all the indicators evaluated, although the best results were obtained when both products were used in combination, that is, the "seed" imbibition with QuitoMax® and the foliar spray of the plants with PectiMorf® or viceversa.

**Key words:** chitosan, oligosaccharides, height, yield.

**RESUMEN:** El uso de bioestimulantes en la producción de plantas es una práctica agrícola amigable con el medio ambiente y una de las herramientas empleadas en la agricultura sostenible. El presente trabajo se realizó, en condiciones de campo, durante el período octubre 2020 -febrero 2021, con el objetivo de determinar la influencia de la aplicación de productos a base de oligosacarinas en el crecimiento y desarrollo de plantas de ajo elefante (*Allium ampeloprasum* L.) cv. 'Fredy'. Se evaluó el efecto de dosis de los productos QuitoMax® y PectiMorf®, en diferentes formas de aplicación (imbibición de las "semillas", aspersión foliar a los 80 DDP y la combinación de ambas; con la particularidad, también, de combinar los productos) sobre el comportamiento del porcentaje de emergencia a los 7 y 14 DDP, la altura (cm) y el número de hojas a los 70, 90 y 110 DDP; así como, las características de los bulbos y el porcentaje de sobrevivencia en el

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momento de la cosecha. Además, se estimó el rendimiento de las plantas. Los resultados demostraron que, de forma general, la aplicación de QuitoMax® fue más eficiente que la del PectiMorf® en estimular todos los indicadores evaluados, aunque los mejores resultados se obtuvieron cuando ambos productos se utilizaron de forma combinada, es decir, la imbibición de las "semillas" con QuitoMax® y la aspersión foliar de las plantas con PectiMorf® o viceversa.

**Palabras clave:** quitosano, oligosacáridos, altura, rendimiento.

## INTRODUCTION

Garlic (*Allium sativum* L.), native to Central Asia and the Mediterranean, is one of the oldest vegetables cultivated by man (1). It is the natural condiment par excellence and is part of the dietary and therapeutic habits of many cultures. According to FAO (2019), world garlic production is approximately 30.7 million tons, with an average yield of around 19 t $ha^{-1}$ (2).

In Cuba, the production of this crop rests, fundamentally, on the "Criollo" and "Vietnamese" clones with yields ranging between 4 and 9 t $ha^{-1}$ (3), which are low compared to those obtained by other countries that report yields higher than 10 t $ha^{-1}$ (4). Hence, the need for the country to increase the production and yield of this crop; however, this requires the introduction or obtaining of new clones and the use of sustainable agricultural practices that do not contribute to environmental contamination or soil degradation.

The use of biostimulants in plant production has been widely considered as an environmentally friendly agricultural practice and, therefore, among the tools used in sustainable agriculture. In particular, in horticultural production, various biostimulants have been applied with the objective of increasing yields, both under normal and stress conditions (5, 6).

Particularly, in garlic cultivation, various biostimulants based on amino acids (7,8), algae extracts (9, 10), humic acids (7, 11) and chitosan (1, 12, 13), among others, have been used to increase yield and improve postharvest quality. It should be noted that most of these results have been obtained using foliar spraying as a form of application. However, there is hardly any information about the influence that biostimulants can exert when applied to "seeds", prior to planting, nor has been found information on the use of oligogalacturonides in this crop.

Recently (3), the introduction of a new genotype of elephant garlic ('Fredy') with higher yield potential than the clones currently in use was reported in the country. Therefore, it was decided to undertake the following work whose main objective was to evaluate the influence that the biostimulants QuitoMax® and PectiMorf®, applied in different forms, exert on the growth and development of this new garlic genotype.

## MATERIALS AND METHODS

The experiment was carried out at the "La Rebeca" farm, belonging to the "Niceto Pérez" Credit and Service Cooperative (CCS), located in La Cachimba town, Cajío Road #4, Güira de Melena, Artemisa province. The new genotype of elephant garlic (*Allium ampeloprasum* L.) named 'Fredy' (3) was used, whose "seeds" were planted on October 26, 2020 in Ferrallitic Red Leached Soil (humic FRRL) (14), in a mechanized way, at a distance of 0.90 x 0.05 m. The oligosaccharins used as biostimulants were the registered products known as QuitoMax® (based on a chitosan polymer and chemical salts) and PectiMorf® (based on a mixture of bioactive oligogalacturonides), both produced by the Bioactive Products Group of the National Institute of Agricultural Sciences. The forms of application used were treatment to the "seeds" prior to planting, foliar spraying at 80 days after planting (DAP) and the combination of both forms and products.

The treatments used were:

1. Control.
2. Immersion of the "seeds" for 12 hours in PectiMorf® 1 mg $L^{-1}$.
3. Immersion of the "seeds" for 4 hours in QuitoMax® 5 mg $L^{-1}$.
4. Foliar spray with PectiMorf® 400 mg $ha^{-1}$.
5. Foliar spray with QuitoMax® 300 mg $ha^{-1}$.
6. Immersion of the "seeds" for 12 hours with PectiMorf® 1 mg $L^{-1}$ + Foliar spray with QuitoMax® 300 mg $ha^{-1}$.
7. Immersion of the "seeds" for 4 hours with QuitoMax® 5 mg $L^{-1}$ + Foliar spray with PectiMorf® 400 mg $ha^{-1}$.

A randomized block design with seven treatments and three replications was used. The plots were composed of 28 rows 84 m long, i.e., four rows per treatment and per replicate. A background fertilization with NPK complete formula (9:13:17) was carried out at a rate of 500 kg $ha^{-1}$ and, in addition, lime hydrate (4 kg $ha^{-1}$) was applied at 90 DAP. Irrigation was carried out by watering every five days until 15 days before harvest and weed control was done manually. There were no pests.

The evaluations made were percentage of emergence at 7 and 14 days after planting (DAP), 30 plants per treatment (10 per plot) were evaluated for height (cm), and number of leaves at 70, 90 and 110 DAP. At harvest, i.e. at 120 DAP, the percentage of survival was evaluated, 30 bulbs per treatment were selected, and their mass (g), equatorial and polar diameters (mm), as well as the number and average mass of a "tooth" (g) were determined. The following formula was used to estimate yield:

$$Yield\left(t\ ha^{-1}\right) = \frac{[Bulb\ mass\ (g) \times]}{1\,000\,000} \frac{\left[\left(no.\ plants\ ha^{-1} \times \%\ suvival\right)/100\right]}{1\,000\,000}$$

Data were processed by double ranked analysis of variance and, in the case of significant differences, means were compared using Tukey's multiple range test at

p<0.05 and IBM SPSS Statistics Program, version 22 was used for this purpose.

## RESULTS AND DISCUSSION

The influence that seed treatment with QuitoMax® or PectiMorf®, prior to planting, exerted on seedling emergence at 7 and 14 DAP, as well as on the height and number of leaves at 70 DAP, is presented in Table 1. The significant increase caused by immersion of the "teeth" in either of the two products in the indicators evaluated can be seen, with QuitoMax® standing out, which induced a significantly higher percentage of emergence than PectiMorf®, both at 7 and 14 days after planting.

The effectiveness of PectiMorf® in accelerating seed germination has been demonstrated in several crops. Thus, in sorghum (15), soybean (16) and leucaena (17), seed treatment with a concentration of 10 mg $L^{-1}$ accelerated germination in the first two crops and increased the percentage of emergence in the third. In vegetables, it has been found that treatment of bean seeds with the same concentration of the product for four hours stimulated both the height and the number of leaves (18).

Chitosan, on the other hand, alters the permeability of the plasma membrane of seeds, increasing the concentrations of sugars and proline, as well as accelerating the activities of some enzymes such as peroxidase, catalase, phenylalanine ammonium lyase and tyrosine ammonium lyase. Thus, it has been shown that immersion of peanut seeds in chitosan increased energy and germination percentage, lipase activity, and levels of gibberellic acid and indolacetic acid (19). In wheat (20) and tomato (21) it was found that seed treatment with chitosan or chitosan hydrolysate stimulated germination and seedling growth.

In the case of QuitoMax®, it has been reported that treatment of cucumber seeds with certain concentrations stimulated germination and initial plant growth (22); while, in two tomato cultivars, it was found that immersion of seeds for four hours in a solution of 1 g $L^{-1}$ improved the quality of seedlings at the time of transplanting (23).

The results of this work confirmed the effectiveness of seed treatment with PectiMorf® or Quitomax® to stimulate germination and plant growth of various crops, even though the treatment times and concentrations of the solutions used were different.

Table 2 shows the results of the influence of "seed" treatment, foliar spraying and the combination of both products and forms of application on the behavior of plant height and number of leaves at 90 and 110 DAP.

As can be seen, the response of plant height to treatment of the "seeds" with PectiMorf® or QuitoMax® extended until the end of the crop cycle, and no significant differences were found with the other forms of application used. The behavior of the number of leaves was different, since the imbibition of the "seeds" with QuitoMax® differed significantly from the control, both at 90 and 110 DAP. However, the imbibition with PectiMorf® did not differ from the control at 90 DAP; in addition, the best results were obtained, at both times, with the imbibition of the "seeds" with QuitoMax® + foliar spraying with PectiMorf®, that is, with the combination of both products and application forms.

These results showed that the treatment of "seeds" with oligosaccharins, prior to planting, was better than foliar

**Table 1.** Effect of treatment of 'seeds' of elephant garlic cv. 'Fredy' with PectiMorf® or QuitoMax® on emergence and some plant growth indicators

| Treatments | Emergence percentage | | Height of plants (cm) | Leaf number |
|---|---|---|---|---|
| | 7 DAP | 14 DAP | 70 DAP | 70 DAP |
| Control | 37 c | 57 c | 47.27 b | 8.07 b |
| PectiMorf® 1 mg $L^{-1}$ | 50 b | 63 b | 60.70 a | 9.67 a |
| QuitoMax® 5 mg $L^{-1}$ | 70 a | 83 a | 64.83 a | 10.30 a |
| S.E.x | 0.20 | 0.16 | 1.45 | 0.19 |

Means with equal letters do not differ significantly according to Tukey's multiple range test at p<0.05

**Table 2.** Influence of PectiMorf® and QuitoMax®, applied in different forms, on the height and number of leaves of elephant garlic plants cv. 'Fredy'

| Treatments | Height of plants (cm) | | Leaf number | |
|---|---|---|---|---|
| | 90 DAP | 110 DAP | 90 DAP | 110 DAP |
| Control | 55.40 b | 62.03 b | 9.8 c | 10.0 d |
| Imb. PectiMorf® | 66.96 a | 70.40 a | 10.6 bc | 11.4 bc |
| Imb. QuitoMax® | 65.40 a | 70.20 a | 11.6 a | 11.6 b |
| A.F. PectiMorf® | 69.03 a | 70.33 a | 10.2 bc | 10.5 d |
| A.F. QuitoMax® | 64.90 a | 69.60 a | 10.7 b | 10.7 cd |
| Imb. PectiMorf® + A.F. QuitoMax® | 69.16 a | 70.00 a | 11.6 a | 11.6 b |
| Imb. QuitoMax® + A.F. PectiMorf® | 70.40 a | 71.00 a | 12.1 a | 12.6 a |
| S. E. x | 2.58 | 2.40 | 0.26 | 0.22 |

Imb: Imbibition A.F. Foliar spray DAP: Days after planting.

Means with equal letters do not differ significantly according to Tukey's multiple range test at p<0.05

spraying at 80 DAP, in stimulating the number of leaves of the plants at the end of the crop cycle (110 DAP), but not in height. It may be related to the physiological stage at which the spraying was performed and the dose of the product used.

The influence of foliar spraying alone and imbibition of "seeds" + foliar spraying with QuitoMax® on plant growth has been reported previously. Thus, in tomato, it was shown that foliar spraying, at 12 DAT, with doses of 300 mg $ha^{-1}$ enhanced plant height and root length under salinity conditions (24). Recently, it was reported that treatment of garlic 'Criollo Victor' clone "seeds" for 24 hours with QuitoMax® solutions (1, 5 and 10 mg $L^{-1}$) and foliar spraying at 50 DAT with the same concentrations increased the height and number of leaves at 70, 90 and 110 DAT (13).

The results of the present work confirm the previous ones, despite the fact that a much shorter "seed" treatment time (4 hours) was used and the foliar spraying was carried out at a later stage of the crop cycle (80 DAP).

Given the influence exerted by the treatments studied on the percentage of emergence and on the growth indicators of the plants evaluated, it is expected to have a favorable effect on the crop yield, so Table 3 shows the characteristics of the bulbs at the time of harvest, i.e., the mass, size, number and average mass of the "teeth".

The application of PectiMorf® or QuitoMax® significantly increased all the bulb characteristics evaluated, compared to the control treatment, except the number of "teeth", highlighting the treatments where the products and forms of application were combined, followed by the imbibition treatment of the "seeds" with QuitoMax®.

In this work, QuitoMax® application, either by treatment to the "seeds" or by foliar spraying, produced a significantly higher average mass of "teeth" than that obtained with PectiMorf®; however, in the combined application form, no significant differences were obtained, which may be because in this case the products were combined.

Internationally, the influence of different biostimulants on the characteristics of garlic bulbs has been reported. Thus, biostimulants based on amino acids, applied foliarly, increased bulb mass (25) and the number of cloves (26); as well as satisfactory results in both indicators were obtained when the seeds of two garlic cultivars were immersed for 12 hours in a solution based on a macroalgae extract (10).

The behavior of the estimated yield is presented in Figure 1. The treatment to the "seeds" and the foliar spray with QuitoMax®, as well as the combination of the products and both forms of application significantly increased the yield, with the treatment of imbibing the "seeds" with QuitoMax® + foliar spray with PectiMorf® standing out, which differed significantly from the rest of the treatments and increased the yield by 119 %. Note that the application of Pectimorf® alone, both by "seed" treatment and by foliar spray, stimulated yield, but not statistically significantly.

Several authors have reported the stimulation that QuitoMax® application exerts on crop yield. For example, in corn, seed treatment with a solution of 1 g $L^{-1}$ increased the yield of two white corn cultivars (27). Recently, it was found that in the corn cultivar Francisco Mejorado, seed imbibition with QuitoMax® 0.5 and 1 g $L^{-1}$ for one hour did not stimulate crop yield, but it was necessary to add two foliar sprays (15 and 30 days after emergence) to obtain this effect (28). In tomato grown under saline conditions, foliar spraying with doses of 300 and 400 mg $ha^{-1}$ caused yield increases of 43 and 76 %, respectively (24).

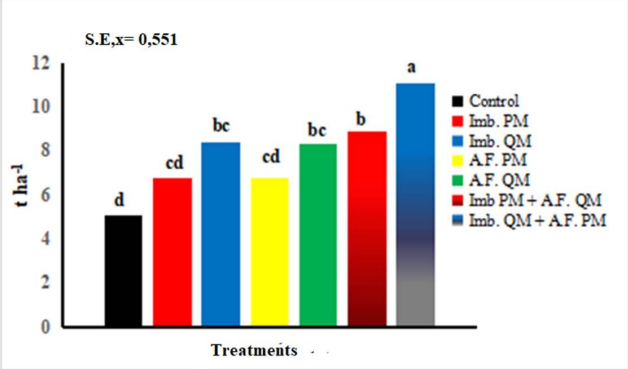


**Figure 1.** Effect of different application modes of PectiMorf® and QuitoMax® on the estimated yield of elephant garlic plants cv. 'Fredy'

In contrast to the above, in 'Chilean' garlic it was found that foliar spraying of QuitoMax® at doses of 100, 150 and

**Table 3.** Influence of different application modes of PectiMorf® and QuitoMax® on bulb characteristics of elephant garlic cv. 'Fredy', at harvest time (120 DAP)

| Treatments | Mass (g) | Equatorial diameter (mm) | Polar diameter (mm) | Number of cloves "teeth" | Average mass of cloves "teeth" (g) |
|---|---|---|---|---|---|
| Control | 33.8 e | 36.2 e | 22.3 e | 26.4 | 2.85 e |
| Imb. PectiMorf® | 42.0 cd | 46.3 cd | 33.0 cd | 27.5 | 4.43 cd |
| Imb. QuitoMax® | 45.5 abc | 49.3 bc | 36.0 bc | 27.2 | 4.76 b |
| A.F. PectiMorf® | 39.6 d | 44.3 d | 31.0 d | 27.6 | 4.23 d |
| A.F. QuitoMax® | 43.3 bcd | 47.2 cd | 34.0 cd | 27.5 | 4.55 bc |
| Imb. PectiMorf® + A.F. QuitoMax® | 48.0 ab | 51.9 ab | 38.6 ab | 26.7 | 5.05 a |
| Imb. QuitoMax® + A.F. PectiMorf® | 50.0 a | 54.0 a | 40.6 a | 27.0 | 5.28 a |
| S.E. x | 1.69 | 1.26 | 0.93 | 2.73 NS | 0.084 |

Imb: Imbibition. A.F. Foliar spray. DAP: Days after planting. Means with equal letters do not differ significantly according to Tukey's multiple range test at $p<0.05$

200 mg $ha^{-1}$ at 30 and 60 DAP did not modify bulb mass and, therefore, did not increase crop yield (1). It should be noted that in the present work a higher dose (300 mg $ha^{-1}$) and a single application later (80 DAP) were used, which may have influenced the response obtained.

In the case of Pectimorf®, foliar spraying with 10 mg $L^{-1}$ to tomato plants on eight occasions starting from transplanting, significantly favored crop yield (29). However, later, it was shown that foliar spraying at flowering with a similar concentration stimulated crop yield in beans, although the best response was obtained when seed imbibition + foliar spraying was used (18). These results showed that both single and multiple foliar applications significantly increased crop yield; however, in the present work, crop yield did not respond to a single foliar spray, which may be related to the dose and timing of the application.

An integral analysis of the above results shows that QuitoMax® or PectiMorf® applied to the garlic crop, at the selected doses, both by imbibing "seeds" and by foliar spraying plants, as well as by the combination of both products and modes of application, significantly increased the height of the plants, as well as the mass and size of the bulbs. However, the behavior of the survival percentage, used for yield estimation, influenced that only the QuitoMax® application or the combination of both products and modes of application were able to increase significantly crop yield.

These results are the first obtained under field conditions using this garlic cultivar and the national products QuitoMax® and PectiMorf®, and if confirmed, they would be an ecological and sustainable alternative to increase garlic yield in the country.

## CONCLUSIONS

With the doses and moments of application used in this work, the application of the product QuitoMax® was more efficient than PectiMorf® in stimulating the growth and development of the garlic crop. The best results were obtained when both products were used in combination, that is, imbibing the "seeds" with QuitoMax® and foliar spraying the plants with PectiMorf® or vice versa.

## RECOMMENDATIONS

Validate the results obtained with other garlic clones under different soil and climatic conditions and in a larger area, in order to be able to make an economic assessment of them and, in the future, generalize their use in production practice.

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