Cultivos Tropicales, Vol. 43, No. 2, April-June 2022, e-ISSN: 1819-4087, p-ISSN: 0258-5936, https://ediciones.inca.edu.cu

Cu-ID: https://cu-id.com/2050/v43n2e05

Original article

Use of QuitoMax® growth biostimulator in the acclimatization of pepper plantlets

Utilización de bioestimulador del crecimiento QuitoMax® en la aclimatización de plántulas de pimiento

Humberto Izquierdo-Oviedo[1], Victor M. Calaña-Janeiro[2], Lluvia de Abril A. Soriano-Melgar[3], Marian Rodríguez-Hernández[1], Yaritza Rodríguez-Llanes[4*], Rodolfo Guillama-Alonso[1], Idalmis de la C. Hernández-Escobar[5], Irelio Urra-Zayas[5]

[1]Instituto Nacional de Ciencias Agrícolas (INCA), carretera San José-Tapaste, km 3½, Gaveta Postal 1, San José de las Lajas, Mayabeque, Cuba. CP 32 700.

[2]Agencia Internacional de Inspección, Ajuste de Averías y Otros Servicios Conexos (Intermar S. A.), calle F N0. 560 e/n 23 y 26 Vedado. Plaza de la Revolución, La Habana, Cuba, CP 10400.

[3]Centro de Investigación en Química Aplicada (CIQA), Blvd. Enrique Reyna Hermosillo No. 140 Col. San José de los Cerritos. Saltillo, Coahuila de Zaragoza, México, CP 25294.

[4]Centro de Investigaciones Científicas de Yucatán. Mérida (CICY), México.

[5]Facultad de Agronomía. Universidad Agraria de La Habana (UNAH), Autopista Nacional km 2½, San José de las Lajas, Mayabeque, Cuba, CP 32700.

ABSTRACT: QuitoMax® is a Cuban biostimulator of growth that could be used to reduce rooting and survival problems in pepper. The aim of this work was to determine the effect of QuitoMax® on the acclimatization of pepper plantlets (*Capsicum annuum* L.) cultivar 'YAMIL'. Plantlets from the *in vitro* multiplication stage, without roots, were used. Plantlets were soaked in QuitoMax® (1-10 mg L^{-1}) or AIA (2 mg L^{-1}) and sprayed 15 days later with the same concentrations; a control treatment was used in which plantlets were neither soaked nor sprayed. Planting was carried out in polyethurane trays containing a substrate composed of organic matter (cachaça) and eutrophic compacted Red Ferrallitic soil. A completely randomized design was used and the experiment was repeated three times over time. Data were processed using a Simple Ranked Analysis of Variance (ANOVA) and the comparison between means was performed according to Tukey's test ($p>0.05$). Treatments 2 [IAA (2 mg L^{-1})], 3 [QuitoMax® (1 mg L^{-1})], 4 [QuitoMax® (5 mg L^{-1})] and 5 [QuitoMax® (10 mg L^{-1})] achieved survival ranging from 90-100 %. All treatments with QuitoMax® showed the most vigorous plants and those with the greatest height were those with the lowest concentration of the product. The number and length of roots were similar in the treatments with AIA (2 mg L^{-1}) and QuitoMax (1 mg L^{-1}).

Key words: *Capsicum annuum*, tissue culture, chitosan, survival.

RESUMEN: El QuitoMax® es un bioestimulador del crecimiento cubano que se pudiera emplear para disminuir los problemas de enraizamiento y supervivencia en el pimiento. El objetivo de este trabajo fue determinar el efecto del QuitoMax® en la aclimatización de plántulas de pimiento (*Capsicum annuum* L.) cultivar 'YAMIL'. Se emplearon plántulas provenientes de la fase de multiplicación *in vitro*, sin raíces. Las mismas se embebieron en QuitoMax® (1-10 mg L^{-1}) o AIA (2 mg L^{-1}) y 15 días después se asperjaron con las mismas concentraciones; se empleó un tratamiento control en el que no se embebieron ni asperjaron las plántulas. La plantación se realizó en bandejas de polieturano, que contenían un sustrato compuesto por materia orgánica (cachaza) y suelo Ferralítico Rojo compactado eútrico. Se empleó un diseño completamente aleatorizado y el experimento se repitió tres veces en el tiempo. Los datos se procesaron mediante un Análisis de Varianza de Clasificación Simple (ANOVA) y la comparación entre las medias se realizó según la prueba de Tukey ($p\leq0,05$). Los tratamientos 2 [AIA (2 mg L^{-1})], 3 [QuitoMax® (1 mg L^{-1})], 4 [QuitoMax® (5 mg L^{-1})] y 5 [QuitoMax® (10 mg L^{-1})] alcanzaron una supervivencia que osciló entre 90-100%. Todos los tratamientos con el QuitoMax® presentaron las plantas más vigorosas y las de mayor altura fueron las de menor concentración del producto. El número y longitud de las raíces fueron similares en los tratamientos con AIA (2 mg L^{-1}) y QuitoMax (1 mg L^{-1}).

Palabras clave: *Capsicum annuum*, cultivo de tejidos, quitosano, supervivencia.

*Author for correspondence: dayabo124244@gmail.com

Received: 19/10/2020

Accepted: 20/06/2021

INTRODUCTION

The pepper belongs to the genus *Capsicum* of the family Solanaceae, it includes an average of 29 species. It has its center of origin in the tropical and subtropical regions of America. Its main limitation is altitude, since it is rare to find it above 1,000 meters above sea level (m a.s.l.) (1).

It is the second most consumed horticultural product in the world after tomatoes (2). It is successfully cultivated in many countries (2,3), where the continent with the largest area of land dedicated to this crop is Asia, with China, Turkey and Indonesia being the main producers (4).

It is also the second most important vegetable for Cuba, due to its great demand for both fresh consumption and industrial use. In addition, it can be used as a medicinal plant and as a condiment. The area harvested and in production in 2019 was 190 954 ha with an agricultural production of 2 673 245 t of food for an agricultural yield of 13.15 t ha^{-1}, of which 6 438 ha correspond to pepper cultivation with an agricultural production of 71 528 t, for an agricultural yield of 12.09 t ha^{-1} (4).

'YAMIL' is an open-pollinated pepper cultivar, with a 130-day productivity cycle; it is recommended for the winter season (September 15 to February 15) and reaches a 45-65 cm height. It flowers at 29 days after transplanting (dat), achieves its massive flowering at 34 dat, reaches physiological maturity and becomes deep red in its maturity for consumption; the mass ranges between 200-230 g and the yield is 30 t ha^{-1}. It has an acidity (0.16 %), °Brix (4.5-4.6), pH of 5.5-5.6 and 170-175 mg 100 g^{-1} of vitamin C content (5).

As a result of the *in vitro* environment, plants present a different anatomy and physiology than those grown under field or grow house conditions (6-8). The disorders observed affect all plant organs, although not all have the same weight on *ex vitro* behavior. Among these disorders are the poor development of the photosynthetic apparatus, of the leaf cuticle, the emission of non-functional roots without connection with the conductive bundles and others that can affect the survival of plants in the acclimatization phase (9).

The term acclimatization is defined as the environmental adaptation of plants obtained by tissue culture or in vitro propagation that have been moved to a new environment, greenhouses or field. During acclimatization, the environment of the plants is changed gradually over time, starting with the near *in vitro* environment and ending with the near greenhouse or field environment. Acclimatization performed in the greenhouse or field under shaded conditions is called "*ex vitro* acclimatization" (10).

However, all studies previously conducted for *in vitro* propagation of pepper reveal that the main drawbacks for propagating this species are rooting and acclimatization of plants in the greenhouse (11). Survival can reach values of 70 % at 16 weeks in chili pepper plants previously propagated by cotyledon and hypocotyl culture.

It is essential that the plants form a good root system, since their nutrition will depend to a large extent on the functionality of their roots. This is why new biotechnological strategies must be established to increase the efficiency of in vitro propagation protocols for pepper plantlets.

The introduction of active substances of national production in the methodology of *in vitro* regeneration of pepper plants could constitute an alternative to improve rooting and *ex vitro* survival. QuitoMax® (12) is one of these domestically produced active substances.

QuitoMax® is a biostimulator of growth obtained from chitin of marine origin (12). The application of chitosans in culture media constitutes a favorable alternative for the *in vitro* propagation of plants, since they significantly improve cauline and root development in plantlets in general and in the *Catleya* spp. orchid in the short term (13). They also develop antifungal activity in different crops (14,15).

Then, the aim of this work was to determine the QuitoMax® effect on the acclimatization of pepper plantlets (*Capsicum annuum* L.) cultivar 'YAMIL'.

MATERIALS AND METHODS

Plant material

Plantlets of pepper (*Capsicum annuum* L.), cultivar 'YAMIL' from the *in vitro* multiplication phase (third subculture), obtained in DM + 6-BAP (2 mg L^{-1}) culture medium and without roots, all from the same multiplication lot, were used.

The plantlets from the *in vitro* culture were planted in polyethurane trays of 70 alveoli, which had a capacity of 115 cm^3 and contained a substrate composed of organic matter (cachaça) and compacted eutrophic Ferrallitic Red soil (16), in a proportion in volume of 75 and 25 %, respectively. This soil was not sterilized. Plantlets were irrigated by misting in the first 10 days, to achieve a high relative humidity (90-95 %): they were irrigated four times a day (8:30 am, 10:30 am, 1:30 pm and 3:30 pm), between 2-3 minutes each time. A black mesh (70 % sunlight reduction) was used for shading. The chemical characteristics of the substrate are shown in Table 1.

Roots of the plantlets were immersed for 15 minutes (I) and 15 days after planting, they were foliar sprayed (A) with QuitoMax® at a rate of 2 mL per plantlet.

The treatments were as follows:

1.- Control (without I or A).

2.- I+A with IAA (2 mg L^{-1})

3.- I+A with QuitoMax® (1 mg L^{-1})

4.- I+A with QuitoMax® (5 mg L^{-1}) 5.- I+A with QuitoMax® (10 mg L^{-1})

where:

I: immersion of plantlet roots for 15 minutes in QuitoMax®.

A: foliar spraying of plantlets with QuitoMax® 15 days after planting.

Evaluations were carried out at 7, 14, 21 and 28 days. The following variables were evaluated:

- Survival percentage: total number of live plants with respect to the total number of plantlets transferred to the acclimatization phase for each treatment.
- Plant height (cm): measured with a graduated ruler from the base of the stem to the last extended leaf.
- Number of roots per plant: the number of roots was counted and then the mean was calculated for each treatment.
- Root length per plant (cm): measured with a graduated ruler from the base of the stem where the roots are inserted to the longest root.
- Plant vigor: was determined according to the proposed scale (17), where: 1.- Not very vigorous, 2.- Vigorous and 3.- Very vigorous.

A completely randomized design was used with 20 plantlets per treatment and the experiment was repeated three times over time. The data were processed by means of a Single Classification Analysis of Variance (ANOVA) with the SPSS 11.5 program for Windows (SPSS, Inc., Chicago, IL) and the comparison between means was carried out according to Tukey's test ($p \leq 0.05$). In all cases, normal distribution (KolmogorovSmirnov) and homogeneity of variance (Bartlett) were previously checked (18).

Se empleó un diseño completamente aleatorizado con 20 plántulas por tratamiento y el experimento se repitió tres veces en el tiempo. Los datos se procesaron mediante un Análisis de Varianza de Clasificación Simple (ANOVA) con el programa SPSS 11,5 para Windows (SPSS, Inc., Chicago, IL) y la comparación entre las medias se realizó de acuerdo a la prueba de Tukey ($p \leq 0,05$). En todos los casos, previamente se chequeó la distribución normal (Kolmogorov-Smirnov) y la homogeneidad de varianza (Bartlett) (18).

RESULTS AND DISCUSSION

The results related to the percentage survival of pepper plants (*Capsicum annuum* L.) cultivar 'YAMIL' are shown in Table 2. As can be seen in general, there were significant differences between treatments at 7; 14; 21 and 28 days. Plant survival was high in treatment 3 (I+A with QuitoMax® [1 mg L^{-1}]), with 100 % survival and, in the same period of time, plants obtained with treatment 1 (Control) were those with the worst result.

A similar survival between 85-95 % was obtained in four genotypes of *Capsicum* (19). Similar results were reported by other authors in different species of the genus *Capsicum* (20,21).

Other authors obtained 70 % survival at 16 weeks in chili pepper (*Capsicum annuum* L.) plants previously propagated by cotyledon and hypocotyl culture (22).

When evaluating the influence of an oligogalacturonide on the acclimatization of banana (*Musa* spp.) clone 'FHIA-18' (AAAB) vitroplants, several authors observed 90 % plant survival with concentrations of 1 and 5 mg L-1 (23).

As can be seen, there were significant differences between treatments for the height and vigor of pepper plants (*Capsicum annuum* L.) cultivar 'YAMIL'. The best treatment was treatment 3 (I+A with QuitoMax® [1 mg L^{-1}]), with a height of 3.99 cm, and the plants were very vigorous (reaching a value of 3); the lowest results were obtained by the plants of treatment one (Control [without I+A]), as shown in Figure 1.

Similar results in *Capsicum annum* L. were observed by several authors, who reported that plants with vigorous appearance during the acclimatization phase when they were kept in a growth chamber and 16 weeks later transferred to soil, reached 6 cm in length (22).

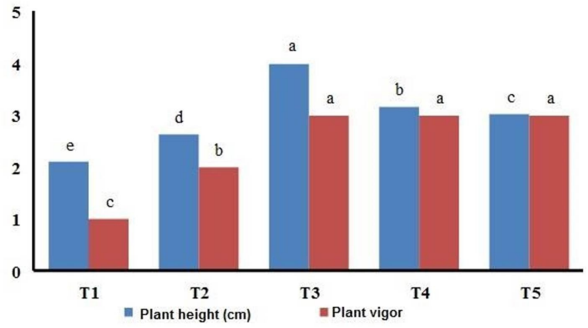
Table 1. Chemical characteristics of the substrate used for acclimatization of *in vitro* plantlets of pepper.(*Capsicum annuum* L.) cultivar 'YAMIL'.

Substrate	pH H_2O	O.M (%)	P (ppm)	K	Ca	Mg	Na
				(cmol kg^{-1})			
Cachaça: soil (75 %:25 %)	7,1	13,01	2700	4,92	28,9	8,39	0,46

Table 2. Percentage survival of pepper plants (*Capsicum annuum* L.) cultivar 'YAMIL', at 7; 14; 21 and 28 days during the acclimatization phase (n =60).

No.	Treatments	Plantlet survival after 7 days (%)	Plantlet survival after 14 days (%)	Plantlet survival after 21 days (%)	Plantlet survival after 28 days (%)
1	Control (without I + A)	60,0 c	60,0 c	65,0 c	70,0 c
2	I + A with IAA (2 mg L^{-1})	85,0 b	90,0 ab	90,0 ab	90,0 ab
3	I + A with QuitoMax® (1 mg L^{-1})	100 a	100 a	100 a	100 a
4	I + A with QuitoMax® (5 mg L^{-1})	90,0 ab	90,0 ab	90,0 ab	90,0 ab
5	I + A with QuitoMax® (10 mg L^{-1})	80,0 b	80,0 b	85,0 b	85,0 b
S.Ex (±)		2,23***	2,24***	2,24**	3,16*
S.D		14,18	12,69	10,59	9,18

I: immersion of roots for 15 minutes; A: foliar spraying of the plantlets 15 days after planting; n: total number of plantlets in the experiment in the three replicates
Means with different letters differ statistically according to Tukey's test ($p \leq 0.05$)

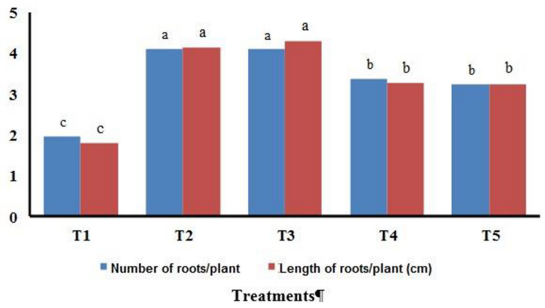


T1.- Control (without I+A) T2.- I+A with IAA (2 mg L^{-1}) T3.- I+A with QuitoMax® (1 mg L^{-1}) T4.- I+A with QuitoMax® (5 mg L^{-1}) T5.- I+A with QuitoMax® (10 mg L^{-1})

I: immersion of roots for 15 minutes; A: foliar spray of plantlets 15 days after planting; n: total number of plants in the experiment in the three replicates

Means with different letters differ statistically according to Tukey's test (p≤0.05) (*** significant for p<0.001).

Figura 1. Height (S.Ex=0.03***; D.S=0.64) and plant vigor (S.Ex=0.00***; S .D =0.80) of pepper (*Capsicum annuum* L.) cultivar 'YAMIL' during the acclimatization phase, at 28 days (n=60)



T1.- Control (without I+A), T2.- I+A with IAA (2 mg L^{-1}) T3.- I+A with QuitoMax® (1 mg L^{-1}), T4.- I+A with QuitoMax® (5 mg L^{-1}), T5.- I+A with QuitoMax® (10 mg L^{-1})

I: immersion of roots for 15 minutes; A: foliar spray of plantlets 15 days after planting; n: total number of plants in the experiment in the three replicates

Means with different letters differ statistically according to Tukey's test (p≤0.05) (*** significant for p<0.001)

Figure 2. Number (S.Ex= 0.05***; S.D= 0.81) and length of roots (S.Ex= 0.06***; S.D= 0.92) per plant of pepper (*Capsicum annuum* L.) cultivar 'YAMIL' during the acclimatization phase at 28 days (n=60).

Other authors observed different results in the height of chiltepin plantlets in the acclimatization phase with the use of an oligogalacturonide at concentrations of 1, 5 and 10 mg L^{-1}, where the height with the use of this biostimulator was lower than in the control treatments and in the one supplemented with 6-BAP (17).

In addition, the QuitoMax® and chitosan effect at different concentrations on the growth, development and yield of several crops of agricultural interest has been evaluated in field conditions and in cultivation houses, and positive results were obtained, which demonstrates the stimulating characteristic of these products in the growth and establishment of plants (24).

The results related to the number and length of roots per plant of pepper (*Capsicum annuum* L.) cultivar 'YAMIL' are shown in Figure 2. As can be seen, there were significant differences between treatments for the two variables evaluated. Plants achieved the best results with treatments 2 (I+A with IAA [2 mg L^{-1}]) and 3 (I+A with QuitoMax® [1 mg L^{-1}]), with 4.09 and 4.08 roots per plant and 4.12 and 4.28 cm root length, respectively. The lowest results in general were obtained by plants of treatment 1 (Control [without I+A]). Similar results were obtained in tomato (*Solanum lycopersicum* L.) variety 'Mara' under field conditions, where the combined effect of the application by imbibition of seeds and foliar spraying of the bioproduct QuitoMax® at different concentrations (0.1, 0.5 and 1.0 g L^{-1}) showed favorable results in root length in all the concentrations evaluated, so that the different doses of QuitoMax® exerted a positive effect on this growth variable (24).

In addition, when evaluating the Pectimorf® effect on chiltepin plantlets in the acclimatization phase, it was observed that the highest number and length of roots was obtained with the treatment with 10 mg L^{-1} of this oligosaccharin (17).

The response shown by the different growth variables could be explained by the QuitoMax® capacity to stimulate plantlet growth, which also maintains a close relationship with the concentrations used, the molecular size and the product application form to the crop, which includes the contact time with the organ that perceives the application, in this case in the seed, stimulated the germination speed and accelerated growth. It has been shown that chitosans stimulate protein levels in leaves as well as enzyme levels and basal resistance of plants (12,25-30).

Based on the above results, for acclimatization of in vitro plantlets of pepper (*Capsicum annuum* L.) cultivar 'YAMIL', plantlet root immersion for 15 min and foliar spraying of plantlets 15 days after planting with QuitoMax® (5 mg L^{-1}) should be performed.

CONCLUSIONS

The QuitoMax® use enhances the survival, height and vigor of pepper (*Capsicum annuum* L.) plants of the cultivar 'YAMIL' during the acclimatization phase.

BIBLIOGRAPHY

1. Eshbaugh WH. Peppers: History and exploitation of a serendipitous new crop discovery. New crops [Internet]. 1993; Available from: https://hort.purdue.edu/newcrop/proceedings1993/v2-132.html
2. de Morales Dávila Á, de la Paz M. Procesos biológicos implicados en el control de la maduración de los frutos de pimiento (*Capsicum annuum* L.): proteómica funcional y antioxidantes [Internet]. Universidad de Granada; 2015. 130 p. Available from: https://dialnet.unirioja.es/servlet/dctes?codigo=57343

3. López-Muñoz NR, Romero-Bastidas M, Arce-Amézquita PM, Hernández-Rubio JS. Antifungal activity of antioxidants derived from four cultivars of *Capsicum* spp. against phytopathogenic fungi. Ecosistemas y recursos agropecuarios [Internet]. 2019;6(18):487-98. Available from: http://www.scielo.org.mx/scielo.php?pid=S2007-90282019000300487&script=sci_abstract&tlng=en
4. Anuario de Producción. FAOSTAT [Internet]. 2019. Available from: https://www.fao.org/faostat/es/#home
5. Rodríguez Y, Depestre T, Rodríguez S, Camejo C. 'YAMIL', nueva variedad de pimiento para campo abierto [Internet]. Cuba. El Productor; 2015. Available from: https://www.grupoagricoladecuba.gag.cu/media/Agrotecnia/pdf/38_2014/2/11.pdf
6. Rashmi P, Ramesh J, Jat BL. *In vitro* propagation of wax oil plant Jojoba (*Simmondsia chinensis* L.). World Journal of Pharmaceutical Research [Internet]. 2016;5(4):1524-58. Available from: https://www.cabdirect.org/cabdirect/abstract/20163186311
7. Nahla AA, Eliraqy M, El-Khasab AA, Ismail RM, Esmail AS, Elsayh SAA, et al. Comparative Studies on Vegetative and *in vitro* Propagation of Elite Selected Jojoba Strains. Asian Journal of Agricultural and Horticultural Research [Internet]. 2018;1-7. Available from: https://www.journalajahr.com/index.php/AJAHR/article/view/220
8. Hernández AF, Plana RR, Bonato RR, Borcioni E. Adaptación de vitroplantas de *Acrocomia aculeata*, con la aplicación de HMA y Biobras-16. Cultivos Tropicales [Internet]. 2018;39(2):34-40. Available from: http://scielo.sld.cu/scielo.php?pid=S0258-59362018000200005&script=sci_arttext&tlng=pt
9. Posada-Pérez L, Padrón-Montesinos Y, González-Olmedo J, Rodríguez-Sánchez R, Barbón-Rodriguez R, Norman-Montenegro O, et al. Efecto del Pectimorf® en el enraizamiento y la aclimatización in vitro de brotes de papaya (*Carica papaya* L.) cultivar Maradol Roja. Cultivos Tropicales [Internet]. 2016;37(3):50-9. Available from: http://scielo.sld.cu/scielo.php?pid=S0258-59362016000300005&script=sci_arttext&tlng=pt
10. Simó-González JE, Ruiz-Martínez LA, Rivera-Espinosa R. Inoculación de hongos micorrizógenos arbusculares (HMA) y relaciones suelo pardo-abonos orgánicos en la aclimatización de vitroplantas de banano. Cultivos Tropicales [Internet]. 2017;38(3):102-11. Available from: http://scielo.sld.cu/pdf/ctr/v38n3/ctr15317.pdf
11. Grozeva S, Todorova V. *In vitro* regeneration in pepper (*Capsicum annuum* L.) and characterization of plantregenerants. Electronic Journal of Biology [Internet]. 2015;11(1):17-22. Available from: https://www.researchgate.net/profile/Stanislava-Grozeva-2/publication/303975592_In_vitro_regeneration_in_pepper_Capsicum_annuum_L_and_characterization_of_plant-regenerants/links/577165b408ae842225ac2518/In-vitro-regeneration-in-pepper-Capsicum-annuumL-and-characterization-of-plant-regenerants.pdf
12. Acosta DL, Menéndez DC, Rodríguez AF. Los oligogalacturónidos en el crecimiento y desarrollo de las plantas. Cultivos Tropicales [Internet]. 2018;39(2):127-34. Available from: http://scielo.sld.cu/scielo.php?pid=S0258-59362018000200020&script=sci_arttext&tlng=pt
13. Vera Alvarado KE. Uso de quitosano en medios de cultivo para el desarrollo en la propagación *in vitro* de la Orquídea *Cattleya* spp [Internet]. Facultad de Ciencias Agrarias Universidad de Guayaquil; 2017. Available from: http://repositorio.ug.edu.ec/handle/redug/17754
14. Rodríguez Pedroso AT, Plascencia Jatomea M, Bautista Baños S, Cortez Rocha MO, Ramírez Arrebato MÁ. Actividad antifúngica *in vitro* de quitosanos sobre *Bipolaris oryzae* patógeno del arroz. Acta Agronómica [Internet]. 2016;65(1):98-103. Available from: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-28122016000100014
15. Rodríguez-Rico D. Determinación preliminar del efecto *in vitro* de nanopartículas de quitosano-Prosopis glandulosa para inhibir *Candida albicans*. Revista de Ciencias Farmaceúticas y Biomedicina (ISSN: 2448-8380) [Internet]. 2018;30-30. Available from: https://rcfb.uanl.mx/index.php/rcfb/article/view/146
16. Hernández-Jiménez A, Pérez-Jiménez JM, Bosch-Infante D, Speck NC. La clasificación de suelos de Cuba: énfasis en la versión de 2015. Cultivos Tropicales [Internet]. 2019;40(1). Available from: http://scielo.sld.cu/scielo.php?pid=S0258-59362019000100015&script=sci_arttext&tlng=pt
17. Izquierdo Oviedo H, Alcaraz Meléndez L, Rodríguez-Álvarez M. Micropropagación de chiltepín (*Capsicum annuum* L. cv.'glabriusculum') mediante el empleo de una oligosacarina de origen péctico. Acta universitaria [Internet]. 2017;27(5):34-43. Available from: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0188-62662017000500034
18. Cochran WG, Cox GM. Diseños experimentales [Internet]. Trillas; 1965. 132-135 p. Available from: https://www.urbe.edu/UDWLibrary/InfoBook.do?id=5068
19. Orlinska M, Nowaczyk P. *In vitro* plant regeneration of 4 *Capsicum* spp. genotypes using different explant types. Turkish Journal of Biology [Internet]. 2015;39(1):60-8. Available from: https://journals.tubitak.gov.tr/biology/abstract.htm?id=15557
20. Sanatombi K, Sharma GJ. Micropropagation of *Capsicum annuum* L. Notulae Botanicae Horti Agrobotanici Cluj-Napoca [Internet]. 2007;35(1):57. Available from: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.962.9872&rep=rep1&type=pdf
21. Aniel Kumar O, Rupavathi T, Subba Tata S. Adventitious shoot bud induction in chili pepper *Capsicum annuum* L. cv. X-235. International Journal of Science and Nature [Internet]. 2012;3(1):192-6. Available from: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.1059.7673&rep=rep1&type=pdf
22. Paz AR, Castañeda GC. Regeneración *in vitro* de plantas de Chile (*Capsicum annuum* L.) mediante cultivo de

cotiledones e hipocótilos. Revista Fitotecnia Mexicana [Internet]. 2004;27(2):121-6. Available from: https://www.redalyc.org/pdf/610/61027201.pdf

23. Izquierdo H, Núñez M, González MC, Proenza R, Cabrera JC. Influencia de un oligogalacturónido en la aclimatización de vitroplantas de banano (*Musa* spp.) del clon FHIA-18 (AAAB). Cultivos Tropicales [Internet]. 2009;30(1):00-00. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S02585936200900010005
24. Terry Alfonso E, Falcón Rodríguez A, Ruiz Padrón J, Carrillo Sosa Y, Morales Morales H. Respuesta agronómica del cultivo de tomate al bioproducto QuitoMax®. Cultivos Tropicales [Internet]. 2017;38(1):147-54. Available from: http://scielo.sld.cu/scielo.php?pid=S0258-59362017000100019&script=sci_arttext&tlng=pt
25. Malekpoor F, Pirbalouti AG, Salimi A. Effect of foliar application of chitosan on morphological and physiological characteristics of basil under reduced irrigation. Research on Crops [Internet]. 2016;17(2):354-9. Available from: https://www.indianjournals.com/ijor.aspx?target=ijor:rcr&volume=17&issue=2&article=030
26. Morales Guevara D, Dell Amico Rodríguez J, Jerez Mompié E, Hernández YD, Martín Martín R. Efecto del QuitoMax® en el crecimiento y rendimiento del frijol (*Phaseolus vulgaris* L.). Cultivos Tropicales [Internet]. 2016;37(1):142-7. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S02585936201600010020
27. Muñiz LL, Ramírez JG. Efecto de los bioestimulantes Biobras 16 y Quitomax sobre el cultivo del frijol (*Phaseolus vulgaris* L.) Variedad Delicias-364'en la agricultura suburbana de Aguada de Pasajeros. Revista Científica Agroecosistemas [Internet]. 2018;6(2):151-60. Available from: https://ceema.ucf.edu.cu/index.php/aes/article/view/208
28. Costales D, Nápoles MC, Falcón AB, González Anta G, Ferreira A, Rossi A. Influencia de quitosanas en la nodulación y el crecimiento vegetativo de soya (*Glycine max* L. Merrill). Cultivos Tropicales [Internet]. 2017;38(1):138-46. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S02585936201700010018
29. Costales-Menéndez D, Falcón-Rodríguez AB. Combinación de formas de aplicación de quitosano en el desarrollo de soya biofertilizada. Cultivos Tropicales [Internet]. 2018;39(3):71-9. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0258-59362018000300010
30. Jiménez Arteaga MC, González Gómez LG, Suárez Benítez M, Paz Martínez I, Oliva Lahera A, Falcón Rodríguez A. Respuesta agronómica del pimiento California Wonder a la aplicación de Quitomax®. Centro Agrícola [Internet]. 2018;45(2):40-6. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0253-57852018000200006