Article

Biological effect of nanoparticles loaded with microbial indoleacetic acid on tomato morphometric parameters

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Abstract

The tomato is one of the vegetables that have the highest production worldwide, so for its fertilization and pest control products of synthetic origin are used, which affect the ecosystem where they are applied, for this reason biologically sustainable alternatives are sought. One of these alternatives is the use of microbial metabolism, an example of this is *Botryodiplodia theobromae*, a phytopathogenic fungus capable of producing phytohormones from its metabolism, such as indolacetic acid (IAA). However, exogenous application of this phytohormone exhibits accelerated degradation when in contact with environmental factors. An alternative to minimize this effect is encapsulation with the use of biopolymeric materials that have the ability to coat the phytohormone and at the same time allow to increase the effectiveness of the product. In this work, the encapsulation efficiency (EE) of IAA from microbial broth was evaluated by liquid fermentation of *B. theobromae* in alginate/chitosan nanoparticles (Np) (ALG/QS); as well as its biological effectiveness represented in morphological development patterns in tomato plants. The results showed that IAA was produced from the microbial metabolism of *Botryodiplodia theobromae*, which once encapsulated reached an EE of 90%, as well as various particle sizes. Regarding the biological effectiveness in tomato plants, it was observed that the Np loaded with microbial broth and synthetic grade IAA showed a significant difference in most of the morphological parameters evaluated compared to the absolute control. Therefore, nanosystems loaded with biostimulants are shown as a future alternative for the application of biostimulants in vegetables such as tomatoes.

Keywords: *Botryodiplodia theobromae*, indolacetic acid, nanoencapsulation.

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Introduction

The tomato (*Solanum lycopersicum*) is one of the vegetables that is most cultivated worldwide as a national level for its fresh and industrial consumption, in Mexico the production is up to 2 875 164 tons (SIAP, 2018). However, the crop faces a series of difficulties such as inadequate management of fertilization, varieties with short production cycles, attack by pests and diseases.

Currently the solution to these problems is the excessive use of synthetic products that increase the yield of tomato production, one of the main biological assets are stimulants and within this group plant growth regulating hormones stand out for their versatile application (Terry *et al*., 2010). Growth regulators are defined as natural or synthetic compounds that interfere in plant development processes, these when applied exogenously in commercial crops increase and improve the production and quality of the crops and products obtained, which is why they are widely used in agriculture (Rademacher, 2015).

These substances play an important role within plants, which use them in low concentrations, acting at the cellular level during different stages of development, within this group, the most relevant so far are gibberellins, auxins, cytokinins, jasmonic acid and ethylene (Santo-Pereira *et al*., 2017).

Indoleacetic acid (IAA) is the most important natural auxin present in most plants. It is a plant hormone that regulates various processes such as: growth, cell division and root formation, among others. Currently the most used auxins are those obtained by chemical synthesis; however, due to the environmental pollution problems that exist, its use begins to be limited, so that obtaining this hormone through systems that are more committed to the environment becomes relevant.

An alternative to this problem may be metabolic synthesis from mycoorganisms (Castillo *et al*., 2005). Studies have shown that there are microorganisms capable of producing phytohormones as part of their metabolism, a representative of this type of microorganisms is the *Botryodiplodia theobromae* fungus, which has the ability to produce phytohormones and one of the most prominent is IAA, which when applied exogenously, it can be efficient to increase plant growth and development (Rohwer and Erwin, 2010; Andolfi *et al*., 2014).

A great disadvantage in the use of phytohormones is that they can lose their biological efficiency due to environmental factors such as oxidative degradation, humidity, UV light, temperature and the leaching of compounds due to rains (Armendariz-Barragan *et al*., 2016; Bakry *et al*., 2016). Due to this problem, it is convenient to explore and investigate new technologies in order to develop alternatives regarding their application, one of these is the use of nanotechnology (Lugo *et al*., 2010; Agrawal and Rathore, 2014; Campos *et al*., 2014).

That thanks to its virtues makes the application of doses efficient, managing to decrease the amount of active ingredient, as well as guarantee a lower impact on the environment (Nuruzzaman *et al*., 2016). Within nanotechnology, the production of nanoparticles (NP) for encapsulation of active

ingredients using natural polymers such as chitosan and alginate that allow controlled release and protection of the active ingredient emerges as an option for the application of phytohormones (Bakry *et al*., 2016).

There are various methods for the production of Np, among which nano-precipitation, drying spray, ionic pre-gelation, coacervation and extrusion stand out, all of which are effective for the encapsulation of compounds, but differing in costs and times in terms of production process.

The ionic regulation method is extremely attractive due to the use of biopolymers that can be acquired naturally (Nedovic *et al*., 2011; El Asbahani *et al*., 2015; Armendariz- Barragan *et al*., 2016; Jia *et al*., 2016). The objective of this work was to produce Np of chitosan and alginate loaded with IAA of microbial origin, as well as the evaluation of its biological efficiency in tomato model plants.

Materials and methods

B. theobromae fermentation process for the production of phytohormones

Obtaining the *B. theobromae* strain

The *B. theobromae* strain was obtained from the microbiological collection of the Laboratory of Mycology and Biotechnology of the Department of Parasitology of the Autonomous Agrarian University Antonio Narro. The strain was isolated from cocoa fruits from the southeast of Mexico. The conservation increase and development of the microorganism was carried out using the potato dextrose agar (PDA) culture medium.

Production by liquid fermentation of IAA

The fermentation process to obtain the IAA was carried out using the methodology proposed by Michelena (2001). 500 mL Erlenmeyer flasks were used with 250 mL of Miersh medium (Sucrose 50 g L^{-1}, KNO_3 3 g L^{-1}; $MgSO_4$ $7H_2O$ 0.2 g L^{-1}; KCl 0.1 g L^{-1}; $FeSO_4$. $7H_2O$ 0.01 g L^{-1}; $ZnSO_4$. $7H_2O$ 0.01 g L^{-1}; $MnSO_4$ 0.001 g L^{-1}; Na_2MoO_4 $2H_2O$ 0.001 g L^{-1}; $CuSO_4$ $5H_2O$ 0.001 g L^{-1} and yeast extract 0.1 g L^{-1} modified).

The flasks with the culture medium were autoclaved at 15 lb pressure for 15 min and inoculated with three portions of mycelium with culture medium 5 mm in diameter of the *B. theobromae* strain and incubated in complete darkness by 15 days at a constant temperature of 28 °C without agitation or aeration (Eng *et al*., 2008).

IAA quantification

For the calibration curve, IAA reactive grade from the commercial company Sigma Aldrich was used. The determination and quantification of IAA from the fermentation of *B. theobromae* was initially performed with a chemical concentration of the microbial broth, using the broth supernatant to which an equal volume of ethyl acetate was added as solvent.

Subsequently, the concentration of the liquid was carried out to absolute drying using a lyophilizer, once the dry material was obtained, it was suspended in methanol and the quantification was performed using the microplate technique proposed by Anguiano *et al*. (2017). For the calibration curve, 100 µL of solvent (methanol) were placed in the row of a microplate, then 100 µL of stock solution at 100 ppm were added to the first line.

To obtain a first point of 50 ppm, 100 µL was mixed and transferred to the second line (25 ppm). This procedure continues until line 11 is the concentration 0. Add 100 µL of the developer Salkowski to each well, incubated for 30 minutes and read at 520 nm. Each of the determinations was made in triplicate (Anguiano, 2017).

Np production

For the production of Np, the ionotropic pregelation method proposed by Sarmento (2006) was used. For this, 3.75 mL of $CaCl_2$ solution was added to 59 ml of sodium alginate solution (0.037% pH 4.9) with a peristaltic pump (Ismatec, EW-78000-03) under constant and vigorous agitation, later with the same peristaltic pump 12.5 mL of chitosan solution (0.07%, pH 4.6) were added to the $CaCl_2$ and sodium alginate solution and maintained at again in constant vigorous stirring for 90 min. This procedure was performed with the presence of the hormone (AG/QS/IAA), with the microbial broth (AG/QS/broth) and without the presence of the hormone (AG/QS).

Np characterization

Np with and without the presence of the hormone was characterized in terms of size, distribution and zeta potential. For measurements, samples were diluted to a concentration suitable for analysis, dilutions were made with distilled water. The distribution and sizes of the nanoparticles were determined using the dynamic light scattering technique (DLS), (NanoSight NS 300, Malvern).

The samples were analyzed in triplicate, at 25 °C, with the scattered light detected at a 90° angle. For the determination of the zeta potential (mV) the samples were evaluated in triplicate, at 25 °C, using the equipment (ZETA-check, Colloid metrix). Regarding the pH concentration, a previously calibrated potentiometer (ion meter 450, Corning) was used. Finally, to determine the dry weight, the samples were centrifuged and then lyophilized to remove excess liquid, once dry they were weighed on an analytical balance.

Encapsulation efficiency

To determine the encapsulation efficiency (EE), the solution was centrifuged with Np at 14 000 rpm, at 10 °C for 15 min, then a sample of the supernatant was taken and lyophilized. To determine the concentration of the hormone in the supernatant. The reading was made in a UV-vis spectrophotometer at a wavelength of 520 nm. This was done in triplicate. Encapsulation efficiency was evaluated using the formulas proposed by Wohlfart *et al*. (2011). $EE\% = \frac{\text{AIA weight loaded in Np}}{\text{AIA weight initially used}} \text{ x100}$ $LC\% = \frac{\text{AIA weight in the formulation.}}{\text{AIA weight - loaded NP}} \text{ x100}$

Biological effectiveness of Np in tomato plants

The experiment was established in a bioclimatic chamber, at a temperature of 26 °C, with photo periods of 12 h and a relative humidity of approximately 70%. Under these conditions and in germination trays with a sterile substrate, tomato seeds (*Solanum lycopersicum*), variety Río Grande, were sown. When they reached a height of 10-15 cm, they were transplanted into plastic containers.

To determine the biological effectiveness, 6 treatments were evaluated: T1= Np without loading; T2= Np loaded with IAA reactive grade 140 mg L^{-1}; T3= Np loaded with 140 mg L^{-1} microbial broth; T4= IAA reactive grade 125 mg L^{-1}; T5= 125 mg L^{-1} microbial broth and T6= absolute control, a plant was taken as the experimental unit and each of the treatments had eight repetitions. The treatments were applied at the time of the transplant, as well as at 15, 30 and 45 days after the first application.

These were carried out with the help of atomizers by spraying directly on the foliage of the plants and the amount used was about to spray, the concentration of the treatments was estimated in relation to the dry weight obtained from the production of nanoparticles and the content of hormone encapsulated, so that the treatments were prepared from the dissolution of the product obtained from drying by lyophilization and subsequently resuspended in an estimated volume of water from the expense generated in a preliminary test to completely cover the foliage of a tomato plant with the previously established conditions.

Data were collected regarding the morphometric parameters presented by the plants which were: stem diameter, plant height, number of leaves and chlorophyll content expressed by SPAD 502 units (Minolta). The results obtained were analyzed using the Tukey mean comparison test at 0.05% significance, using the SAS statistical program.

Results and discussion

IAA production by liquid fermentation

The production of IAA from *B. theobromae* was achieved by liquid fermentation in a medium rich in carbohydrates. According to the IAA quantification obtained by the strain used, it was determined that it produces, on average, a final concentration of 125 ppm.

Research has shown that the *B. theobromae* strain has the ability to produce this hormone, Castillo (2014) who carried out a similar work to this work with two strains of *B. theobromae* obtained different production values of 176 and 130 ppm of IAA. This indicates that the production of IAA is influenced by the capacity that different strains of *B. theobromae* may have to produce this hormone.

Np characterization

The size of the Np ALG/QS presented values of 123 ±23.5 nm, for the Np of ALG/QS/IAA of 247 ±26.6 nm and finally for the Np of ALG/QS/broth the values obtained were 220 ±10.1 nm, suggesting that possibly the addition of the synthetic hormone, as well as the addition of the fermentation broth filtrate did not affect the structure of the Np, however, it did increase in size compared to the uncharged Np.

This behavior can be attributed to the interaction that occurred between the biopolymers and the added hormones, when the compound is mixed with an alginate solution at pH 7, it acquires a cationic form that strongly interacts with the negatively charged alginate, it is believed that the ionic interaction generated is contributing to the decrease in size and dispersion (Table 1) (Choe *et al*., 2005).

Table 1. Values obtained of particle size, Z potential, PH and NP weight formed by ALG/QS, ALG/QS/IAA and ALG/QS/broth.

Characteristics	ALG/QS	ALG/QS/IAA	ALG/QS/BROTH
Tamaño (nm)	123 ± 23.5	247 ± 26.6	220 ± 10.1
Potencial zeta (mV)	-31.1 ± 0.57	-30.33 ± 2.3	-33.63 ± 4.09
pH	3.5 ± 0.26	3.13 ± 0.2	3.10 ± 0.26
Peso (g)	0.08 ± 0.01	0.1 ± 1.69	0.37 ± 0.46

±= standard deviation; ALG/QS= alginate/chitosan; ALG/QS/IAA= alginate/chitosan/indolacetic acid; ALG/QS/BROTH= alginate/chitosan/microbial broth.

The literature mentions different sizes of ALP/QS Np, using the same technique; an example is reported by Azevedo *et al*. (2014), who obtained Np's with sizes of 100-120 nm, while Rampino *et al*. (2013), produced nanoparticles of 500nm and 4000nm size which show larger sizes than those obtained in this work. This may have been due to chemical interaction with the negatively charged alginate.

The Np characterization study found that on average the Zeta potential values for the ALP/QS Np's was -31.1 mV (Table 1). The zeta potential indicates colloidal stability, taking as a reference that particles with Zeta potential above +30 mV or below -30 mV are generally considered stable (Silva *et al*., 2011).

The charges are related to the components that were used in the production of Np, there are reports that the Np negative Zeta potential of ALG/QS is due to the carboxyl groups of sodium alginate, which are ionized at pH 4.9 (Costa *et al*., 2015). Regarding the chemical stability of the polymers evaluated, the final pH of the Np's ALG/QS, ALG/QS/IAA and ALG/QS/ BROTH was 3.5, 3.13 and 3.1 respectively, these values being similar between them without any change significant, indicating the absence of hydrolysis of the systems used.

The ability of the ALG/QS Np to encapsulate IAA as well as the fermentation broth was evaluated through the determination of encapsulation efficiency and loading capacity (CC). EE of IAA in Np was 90%, similar behavior was presented in the study carried out by Zhang *et al*. (2010), who obtained an EE between 58 and 80%, while Azevedo *et al*. (2014) obtained 55% of EE.

The high levels of encapsulation efficiency in biopolymers with bioactive substances and the smaller size of the Np obtained in this work can be attributed to the chemical interaction that occurs between the Ca_2+ ions of the sodium alginate and chitosan molecules (De Melo *et al*., 2013).

Biological effectiveness

The results obtained from the morphometric measurements showed that, in relation to the plant height variable, the treatments showed a statistical difference against the absolute control, but not between them (Figure 1).

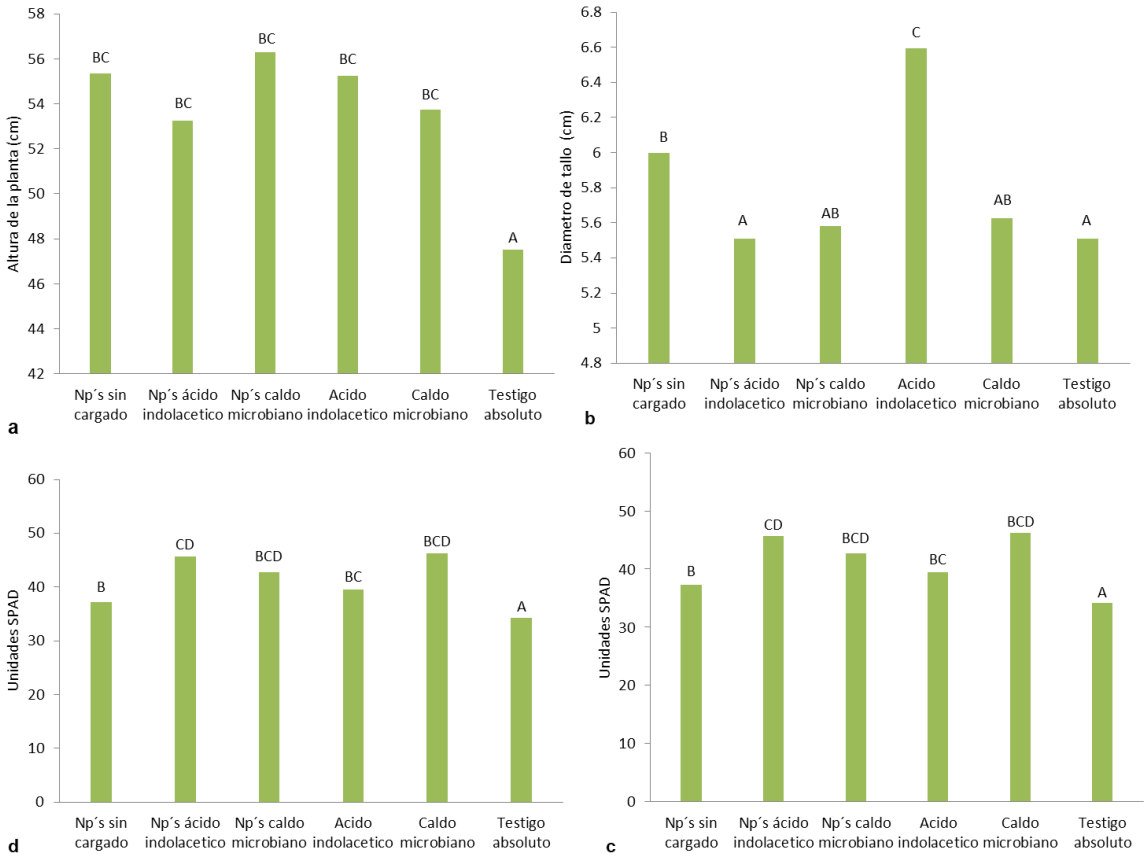

Figure 1. Biological activity of the different treatments on tomato plants 45 days after the first application of the treatments. a) height of the plant; b) stem diameter; c) number of leaves; and d) chlorophyll in leaves. The statistical analysis used was Anova (Tukey, $p \leq 0.05$), where values with the same letter are statistically equal.

All the treatments induced a greater height in relation to the absolute control, being the reactive grade IAA treatment the one that gave the highest numerical height with a value of 56.6 ±3.3 cm followed by the Np loaded with microbial broth and Np unloaded with 55.3 ±3.21 cm.

The higher plant height obtained in the treatments is attributed to the application of auxins, as well as to the compounds with possible unidentified biological activity present in the microbial broth, likewise there are reports that the $CaCl_2$ used in the production of Np reduces stress in plants, increasing their quality and size (Santo-Pereira *et al*., 2017).

Regarding the stem diameter, it was observed that statistical differences between the treatments were presented (Figure 1), with the reactive grade IAA treatment having a larger stem diameter than that obtained by the absolute control, having a diameter of 5.52 ±0.04 cm and the IAA reactive grade 6.74 ±0.34 cm.

The increase observed by the exogenous application of IAA may be due to the fact that it promotes the development and acceleration of cell division in plants (Xu *et al*., 2012), in this sense Hadi *et al*. (2010) observed an increase in plant growth, as well as an increase in biomass due to the application of IAA by spraying. Regarding Np, the increase in diameter can be attributed to the biostimulant action of alginate on various physiological processes in plants, such as: root elongation and cell division (Hussein & Hamideldin, 2014).

In the case of number of leaves, the statistical analysis showed a significant difference between the treatments against the absolute control (Figure 1). Of the treatments applied to tomato plants, it was shown that the treatment of Np loaded with microbial broth was the one that induced a greater production of leaves in the plants. In this sense, it has been reported that the increase in aerial biomass can be stimulated for its potentialization with the exogenous application of various concentrations of IAA in a wide variety of plants (García-Corzo, 2015).

In the case of chlorophyll in leaves, a statistical difference was observed between the treatments against the absolute control, while among the treatments, the treatment with Np loaded with reactive IAA was the one that produced the highest level of chlorophyll in the leaves with a value of 48.82 ±6.35 SPAD units. This may be due to the exogenous application of phytohormones such as IAA accelerating biological activity, causing an increase in photosynthetic activity and an increase in leaf chlorophyll (Tucuch-Haas *et al*., 2015).

Conclusions

IAA was produced by liquid fermentation of *B. theobromae*, which was subsequently used optimally as an active ingredient in the formation of Np from ALG/QS. The physical-chemical evaluation of the Np loaded with IAA showed a stability and an encapsulation efficiency level superior to that mentioned by the literature, in the same way the evaluation of biological efficiency under controlled conditions for the development of tomato plants presented superior results with regarding the control treatment used, demonstrating that the application of nanosystems loaded with biostimulants of microbial origin are beneficial in increasing morphological parameters of vegetables of commercial interest.

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Cited literature

Agrawal, S. and Rathore, P. 2014. Nanotechnology pros and cons to agriculture: a review. Int. J. Curr. Microbiol App. Sci. 3(3):43-55. https://pdfs.semanticscholar.org/e24b/0e62cce6e999de300dfae93125ae61417655.pdf.

Andolfi, A.; Maddau, L.; Cimmino, A.; Benedetto, T.; Basso, S.; Deidda, A.; Sierra, S. and Evidente, A. 2014. Lasiojasmonates A-C, three jasmonic acid esters produced by Lasiodiplodia sp., a grapevine pathogen. Fitoquimica. 103:145-153. http://dx.doi.org/10.1016/j.phytochem.2014.03.016.

Anguiano-Cabello, J. C.; Flores-Olivas, A.; Ochoa-Fuentes, Y. M.; Arredondo-Valdés, R. and Olalde-Portugal, V. 2017. Fast detection of auxins by microplate technique. Am. J. Plant Sci. 8(2):171-177. Doi:10.4236/ajps.2017.82013.

Armendáriz-Barragán, B.; Zafar, N.; Badri, W.; Galindo-Rodríguez, S. A.; Dounia-Kabbaj, H. F. and Elaissari A. 2016. Plant extracts: from encapsulation to application. Expert Opinion on Drug Delivery. 13(8):1165-1175. Doi:10.1080/17425247.2016.1182487.

Asbahani, E. l.; Miladi, A.; Badri, K; Sala, W.; Addi, M.; Casabianca, E. A. and Elaissari, A. 2015. Essential oils: from extraction to encapsulation. Inter. J. Pharmaceutics. 483(1-2):220-243. Doi: doi.org/10.1016/j.ijpharm.2014.12.069.

Azevedo, M. A.; Bourbon, A. I.; Vicente, A. A. and Cerqueira, M. A. 2014. Alginate/chitosan nanoparticles for encapsulation and controlled release of vitamin B2. Int. J. Biol. Macromol. Doi:10.1016/j.ijbiomac.2014.05.036.

Bakry, A. M.; Abbas, S.; Ali, B.; Majeed, H.; Abouelwafa, M. Y.; Mousa, A. and Liang, L. 2016. Microencapsulation of oils: a comprehensive review of benefits, techniques, and applications. Comprehensive Reviews Food Sci. Food Safety. 15(1):143-182. Doi.org/10.1111/1541-4337.12179.

Campos, F. S.; Franco, A. C.; Oliveira, M. T.; Firpo, R.; Strelczuk, G.; Fontoura, F. E. and Silva, A. D. 2014. Detection of bovine herpesvirus 2 and bovine herpesvirus 4 DNA in trigeminal ganglia of naturally infected cattle by polymerase chain reaction. Veterinary microbiology. 171(1-2): 182-188. DOI: 10.1016/j.vetmic.2014.03.012.

Castillo, G.; Altuna, B.; Michelena, G.; Sánchez, J. y Acosta, M. 2005. Cuantificación del contenido de ácido indolacético (AIA) en un caldo de fermentación microbiana. Anales de Biología. 27(27):137-142. https://www.um.es/analesdebiologia/numeros/27/PDF/16-CUANTIFICACION.pdf.

Castillo, G.; Torrecillas, A.; Nogueiras, C.; Michelena, G.; Sánchez, J. and Acosta, M. 2014. Simultaneous quantification of phytohormones in fermentation extracts of *Botryodiplodia theobromae* by liquid chromatography-electrospray tandem mass spectrometry. World J. Microbiol Biotechnol. 30(7):1937-1946. Doi:10.1007/s 11274-014-1612-5.

Costa, J. R.; Silva, N. C.; Sarmento, B. and Pintado, M. Potential chitosan-coated alginate nanoparticles for ocular delivery of daptomycin. 2015. Eur. J. Clin. Microbiol. Infect. Dis. 34(6):1255-1262. Doi:10.1007/s10096-015-2344-7.

De Melo, N. F. S.; Campos, E. V. R.; de Paula, E.; Rosa, A. H. and Fraceto, L. F. 2013. Factorial design and characterization studies for articaine hydrochloride loaded alginate/chitosan nanoparticles. J. Colloid Sci. Biotechnol. 2(2):146-152. Doi:10.1166/jcsb.2013.1048.

Eng, F.; Gutiérrez, M. y Favela, E. 2008. Estudio del efecto de la relación carbón: nitrógeno, el tipo tipo de inóculo y la adición de extracto de levadura en la producción de ácido jasmónico con *Botryodiplodia theobromae* Pat. Cepa RC1. Rev. Iberoam. Micol. 25(3):188-192. http://dx.doi.org/10.1016/S1130-1406.

García-Corzo, P. G. 2015. Evaluación de ácidos idolacético e indolbutírico y extracto de algas marinas sobre el desarrollo de follaje de xate jade (*Chamaedorea oblongata*). Universidad Rafael Landívar. Tesis de licenciatura. 53 p. http://recursosbiblio.url.edu.gt/tesiseortiz/2015/06/17/Garcia-Pablo.pdf.

Hadi, F.; Bano, A. and Fuller, M. P. 2010. The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.): the role of plant growth regulators (GA_3 and IAA) and EDTA alone and in combinations. Chemosphere. 80(4):457-462.

Hussein, O. S. and Hamideldin, N. A. H. L. A. 2014. Effects of spraying irradiated alginate on *Solanum tuberosum* L. plants: Growth, yield and physiological changes of stored tubers. J. Agric. Veterinary Sci. 7(1):75-79. Doi:10.9790/2380-07117579.

Jia, Z.; Dumont, M. J. and Orsat, V. 2016. Encapsulation of phenolic compounds present in plants using protein matrices. Food Bioscience. 15:87-104. Doi:10.1016/j.fbio.2016.05.007.

Lugo-Medina, E.; García-Gutiérrez, C. y Ruelas-Ayala, R. D. 2010. Nanotecnología y nanoencapsulación de plaguicidas. El fuerte México. Rev. Soc. Cult. Des. Sust. 6(1):63-67. http://www.redalyc.org/articulo.oa?id=46112896009.

Michelena, G.; Carrera, E.; Bell, A.; Altura, B. y Almeida, G. 2001. Proceso de separacion y decoloracion del ácido jasmónco a partir de *Botryodiplodia theobromae* cepa 715. IMIQ. 16(1):12-19. http://www.redalyc.org/pdf/482/48216103.pdf.

Nedovic, V.; Kalusevic, A.; Manojlovic, V.; Levic, S. and Bugarski, B. 2011. An overview of encapsulation technologies for food applications. Procedia Food Sci. 1:1806-1815. Doi: 10.1016/j.profoo.2011.09.265.

Nuruzzaman, M.; Rahman, M. M.; Liu, and.; Naidu, R. 2016. Nanoencapsulation, nano-guard for pesticides: a new window for safe application. J. Agric. Food Chem. 64(7):1447-1483. Doi: 10.1021/acs.jafc.5b05214.

Rademacher, W. 2015. Plant growth regulators: backgrounds and uses in plant production. J. Plant Growth Regul. 34(4):845-872. Doi:10.1007/s00344-015-9541-6.

Rampino, A.; Borgogna, M.; Blasi, P.; Bellich, B. and Cesàro, A. 2013. Chitosan nanoparticles: Preparation, size evolution and stability. Int. J. Pharm. 455(1-2):219-228. Doi: 10.1016/j.ijpharm.2013.07.034.

Rohwer, C. and Erwin, J. 2010. Spider mites (*Tetranychus urticae*) perform poorly on and diperse from plants exposed to methyl jasmonate. Entomol. Exp. Appl. 137(2):1-10. Doi: 10.1111/j.1570-7458.2010. 01043.x.

Sarmento B, Ferreira D, Veiga F, and Ribeiro A. 2006. Characterizacion of insulin-Loaded Alginate Nanoparticles Produceed by Ionotropic Pre-Gelation Through DSC and FTIR studies. Carbohydr. Polym. 66:1.7. DOI: https://doi.org/10.1016/j.carbpol.2006.02.008.

Santo-Pereira, A. E.; Silva, P. M.; Oliveira, J. L.; Oliveira, H. C. and Fraceto, L. F. 2017. Chitosan nanoparticles as carrier systems for the plant growth hormone gibberellic acid. Colloids and Surfaces B: Biointerfaces. 150:141-152. Doi:10.1016/j.colsurfb.2016.11.027.

Silva, M. S.; Cocenza, D. S.; de Melo, N. F. S.; Grillo, R.; Rosa, A. H and Fraceto, L. F. 2011. Alginate nanoparticles as a controlled release system for clomazone herbicide. Química Nova. 33(9):1868-1873. Doi: 10.1590/S0100-40422010000900009.

SIAP. 2018. Servicio de Información Agroalimentaria y Pesquera Anuario Estadístico de la Producción Agrícola del Servicio de Información Agroalimentaria y Pesquera (SIAP). http://infosiap.siap.gob.mx/aagricola_siap_gb/ientidad/index.jsp.

Terry, E.; Ruiz, J. y Díaz M. 2010. Respuesta del cultivo del tomate (*Solanum licopersicon* L.) a la aplicación foliar de un bioestimulante derivado del Vermicompost. Temas de Ciencia y Tecnología.14(41):27-32. http://www.utm.mx/edi_anteriores/temas41/2notas_41_1.pdf.

Tucuch-Haas, C. J.; Alcántar-González, G. y Larqué-Saavedra, A. 2015. Efecto del ácido salicílico en el crecimiento de raíz y biomasa total de plántulas de trigo. Terra Latinoam. 33(1):63-68. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-57792015000100063.

Wohlfart, S.; Khalansky, A. S. and Gelperina, S. 2011. Efficient chemotherapy of rat glioblastoma using doxorubicin-loaded PLGA nanoparticles with different stabilizers. PLoS One. 6(5):1-8. DOI: doi.org/10.1371/journal.pone.0019121.

Xu, Y.; Zhang, Y.; Li, Y.; Li, G.; Liu, D.; Zhao, M. and Cai, N. 2012. Growth promotion of *Yunnan pine* early seedlings in response to foliar application of IAA and IBA. Inter. J. Mol. Sci. 13(5):6507-6520. Doi:10.3390/ijms13056507.

Zhang, N.; Li, J.; Jiang, W.; Ren, C.; Li, J.; Xin, J. and Li, K. 2010. Novel piezoelectric pump with 'E'-shaped valve found from sub-experiments. Int. J. Pharm. 5(2):212-218.