

Mexican Journal of Biotechnology

Journal homepage: <u>www.mexjbiotechnol.com</u> ISSN:2448-6590



SHORT COMMUNICATION

Mexican Journal of Biotechnology 2025, 10(2):105-114



Evaluating *Debaryomyces hansenii* in combination with chitosan or salicylic acid for the control of *Botrytis cinerea* in blueberry

Evaluación de *Debaryomyces hansenii* en combinación con quitosano y ácido salicílico para el control de *Botrytis cinerea* en arándano

Surelys Ramos-Bell¹, Juan A. Herrera-González², Luis G. Hernández-Montiel³, Porfirio Gutiérrez-Martínez^{1*}

¹Laboratorio Integral de Investigación en Alimentos, Tecnológico Nacional de México/Instituto Tecnológico de Tepic, Tepic, Nayarit, 63175, Mexico. ²Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Uruapan, Uruapan, Michoacán, 60150, Mexico.

³Centro de Investigaciones Biológicas del Noroeste, S.C., La Paz, Baja California Sur, 23096, Mexico.

*Corresponding author E-mail address: <u>pgutierrez@ittepic.edu.mx</u> (P. Gutiérrez-Martínez)

Article history:

Received: 17 February 2025 / Received in revised form: 12 April 2025 / Accepted: 18 April 2025 / Published online: 30 April 2025. https://doi.org/10.29267/mxjb.2025.10.2.105-114

ABSTRACT

Global consumption of blueberries (*Vaccinium corymbosum* L.) has increased during the last twenty years due to increased recognition of their health benefits. However, postharvest losses of blueberry due to pathogenic fungi are significant. The traditional control methods involving continuous application of synthetic fungicides pose diverse risks, both to health and to the environment. Therefore, environmentally friendly control methods are sought. Therefore, the use of a combined treatment of *Debaryomyces hansenii* with chitosan (CH) and with salicylic acid (SA) was proposed to inhibit the growth of *Botrytis cinerea* in blueberries during postharvest storage. The *in vitro* results showed great potential as the percentage of mycelial inhibition was significantly different to that of the control, with values of 75 and 87% for *D. hansenii*-chitosan and *D. hansenii*-salicylic acid, respectively. The incidence of the disease caused by *B. cinerea* on fruits decreased significantly when

applying *D. hansenii*-chitosan and *D. hansenii*-salicylic acid with a mean reduction of 46.67 and 37.77%, respectively. The microscopy analysis showed damaged *B. cinerea* hyphae due to the effects of the treatments. The combination of *D. hansenii* with chitosan and salicylic acid can mitigate the development of gray mold in postharvest blueberries.

Keywords: antagonistic microorganism, gray mold, postharvest

RESUMEN

El consumo mundial de arándano (*Vaccinium corymbosum* L.) ha aumentado debido al mayor reconocimiento de sus beneficios para la salud. Sin embargo, por ser un fruto pequeño las pérdidas por hongos fitopatógenos son considerables. Las tácticas de control tradicionales implican la aplicación continua de fungicidas de síntesis química y se busca implementar estrategias de control amigables con el ambiente. Por ello, se propuso el uso de un tratamiento combinado de *Debaryomyces hansenii* con quitosano y ácido salicílico para inhibir el crecimiento de *Botrytis cinerea* en arándanos en postcosecha. El porcentaje de inhibición micelial *in vitro* resultó significativamente diferente con respecto al control con valores de 75 y 87 % para *D. hansenii*-quitosano (CH) y *D. hansenii*-ácido salicílico (SA), respectivamente. La incidencia de la enfermedad disminuyó significativamente al aplicar *D. hansenii*-quitosano (CH) y *D. hansenii*-ácido salicílico (SA) en un 46.67 y 37.77 %, en su orden. El análisis microscópico mostró hifas de *B. cinerea* dañadas debido a los efectos de los tratamientos. La combinación de *D. hansenii* con quitosano y ácido salicílico puede mitigar el desarrollo del moho gris en arándanos postcosecha.

Palabras clave: microorganismos antagónicos, moho gris, postcosecha

1. INTRODUCTION

The blueberry (*Vaccinium corymbosum* L.) fruit has properties that makes it an ideal highnutrient dense food in a healthy diet. It is a low-caloric fruit, rich in vitamins, amino acids, and high antioxidant capacity due to its anthocyanin content (Kalt *et al.*, 2020). Blueberries are commonly referred to as one of the healthiest foods due to its physicochemical characteristics (Wu *et al.*, 2022). Global consumption of blueberries has steadily increased during the last twenty years in part due to increased recognition of their health benefits (Macha-Huamán *et al.*, 2023). The crop is highly vulnerable to decay caused by various factors, including size, improper handling during harvesting, mechanical damage (Islam *et al.*, 2019), and primarily due to the attack of phytopathogenic fungus, particularly *Botrytis cinerea* (Ramos-Bell *et al.*, 2021). The traditional way to implement postharvest management control of the fungus is based on the application of agrochemicals that have residues with negatives impacts on human health, ecosystems and living organisms (Milinčić *et al.*, 2020).

In this regard, Sautua *et al.* (2019) provided evidence for the resistance of *B. cinerea* to synthetic fungicides applied to blueberry fruits. To counteract this significant issue, eco-friendly alternative treatments are starting to be explored. As antagonistic microorganism, yeast *Debaryomyces hansenii* have been studied and stand out for their effectiveness at low

concentrations, their tolerance to adverse conditions and resistance to synthetic fungicides, and they are perfectly compatible with other fungal control technologies and systems (Hernandez-Montiel *et al.*, 2021). Chitosan, a natural biocompatible polymer with proven antimicrobial properties due to its varied attributes (Herrera-González *et al.*, 2021), emerges as an example of what can be studied as alternative. Chitosan can induce defense mechanisms in plants against phytopathogens, it can chelate metals that are essential in the pathogenicity of the microorganism. Likewise, the chitosan molecule is positively charged, which causes an innate ability to permeabilize the fungal membrane (Riseh *et al.*, 2022). Another example is salicylic acid, which is a naturally occurring phenolic compound that regulates plant growth and has antifungal properties as it can induce the defense mechanism system in fruits by activating salicylic acid signaling pathways (Hamss *et al.*, 2023). To prevent potential resistance, multiple control agents can be used to synergistically inhibit the growth of pathogenic microorganisms, thus avoiding resistance that may arise from using a single molecule.

2. MATERIALS AND METHODS

2.1. Plant material and fungal strain

The blueberry fruits were collected from Nayarit, Mexico at physiological maturity without apparent damage and then transferred to the Integral Food Laboratory and stored at 4 °C for analysis. (Shi *et al.*, 2023). The *Botrytis cinerea* fungus was provided by the Integral Food Research Laboratory of the Technological Institute of Tepic, Mexico.

2.2. Preparation of treatments

The L1 strain of *Debaryomyces hansenii* donated by the CIBNOR Phytopathology Laboratory, commercial grade chitosan (Golden-Shell Co., China) with a molecular weight of 47.5 kDa and 90% deacetylation and reagent grade salicylic acid (Sigma Aldrich, USA) were used in this investigation. The strain was prepared in liquid culture medium PDB (Potato Dextrose Broth, DifcoTM) in constant agitation at 180 rpm for 24 h to obtain a concentration of 10^6 cel mL⁻¹ of *D. hansenii* (Hernandez-Montiel *et al.*, 2018).

Chitosan was prepared at 0.1% by dissolving it in sterile distilled water and 1% acetic acid. Salicylic acid was used at 5 mM from the dissolution in distilled water and 5% glycerin of an initial concentration of 50 mM, according to the methodology described by Ramos-Bell *et al.* (2024).

2.3. Percentage of mycelial inhibition *in vitro*

Botrytis cinerea was seeded in the center of the 0.8 cm diameter disc shaped plate, the plates were incubated for 8 days at 25 °C. The combination of *D. hansenii*–chitosan and *D. hansenii*–salicylic acid was evaluated on the *in vitro* growth inhibition of *B. cinerea*. For this, potato dextrose agar (PDA) medium containing the different treatments was added to 9 cm diameter Petri dishes. Disc of 0.8 cm diameter of *B. cinerea* was placed in the center of a petri dish. Then, the plates were incubated for 8 days at 25 °C (Jiang *et al.*, 2016). The percentage of mycelial growth inhibition (MGI) was determined taking into account the

control and treatment growth according to equation 1. The phytopathogen without treatment was considered as a control. Five replicates per treatment were carried in a completely randomized design and the results were analyzed through an analysis of variance (ANOVA). The means differences were determined by LSD-Fisher (p≤0.05). The Statistica v12.0 program was used (StatSoft Inc., 2013).

% $MGI = \frac{(Colony \ control \ diameter - Colony \ treatments \ diameter)}{(Colony \ control \ diameter)} x \ 100$ Eq.1

2.4. In vivo combined effect of Debaryomyces hansenii

The analysis was carried out following the methodology described by Ramos-Bell *et al.* (2022). The blueberry fruits were washed and disinfected with a 2% commercial chlorine solution and dried at room temperature to later make a 1x1 cm wound with a sterile needle. The blueberries were immersed for 2 minutes in a solution of *D. hansenii* (1x10⁸ cel/mL) + chitosan (0.1 %), another solution of *D. hansenii* (1x10⁸ cel/mL) + salicylic acid (5 mM) and distilled water as a control. The fruits allowed to dry for 1 h, then 5 µL of spores (1 x 10⁵ mL⁻¹) of *B. cinerea* were inoculated. Once dried, the blueberries with and without treatment were stored at 25 °C and 90 % relative humidity for 8 days and the *in vivo* effect of the treatments was determined according to equation 2, with the percentage of incidence as the response variable. Untreated fruits were considered as controls. The experiment was carried out in duplicate with 30 fruits per treatment and the results were analyzed with a randomized design using an ANOVA. The LSD-Fisher test was used to obtain the means (p≤0.05) using the Statistica v12.0 program (StatSoft Inc., 2013).

% Incidence =
$$\frac{Number infected fruits}{Number total fruits} x 100$$
 Eq. 2

2.5. Scanning electron microscopy

Treated and untreated fruit tissue samples of 8 mm in diameter were placed in a vial in contact with a solution of 3 % glutaraldehyde and 0.1 M Sorensen's phosphate buffer pH 7.2 for 24 h. Then, the tissues were gradually dehydrated in 30-100 % aqueous ethanol solution for 1 h. The samples were dried in a critical point dryer (Samdri – 780A, USA) and coated with gold palladium in an ionizer (Ion Sputter JFC–1100, Jeol). For structural analysis, the tissues were analyzed using a scanning electron microscope (Jeol JSM-6390, USA) at 5 kV.

3. RESULTS

3.1. In vitro percentage of mycelial inhibition

The mycelial growth of *Botrytis cinerea* was significantly affected by all the treatments (Figure 1), with the highest inhibition, 75% and 87%, produced with the combined treatments *D. hansenii*-chitosan (CH) and *D. hansenii*-salicylic acid (SA), respectively.



Fig. 1. Mycelial inhibition of *B. cinerea* due to the effect of chitosan (CH) and the yeast *D. hansenii* combined with chitosan and salicylic acid (SA). Different letters in the bars indicate significant differences between treatments ($p \le 0.05$).

3.2. In vivo combined effect of Debaryomyces hansenii

The percentage incidence of the disease caused by *B. cinerea* in blueberries was significantly lower in the combined treatments of *D. hansenii* with chitosan and salicylic acid, in comparison with that of the control, being these 46.67, 36.66 and 93.33% respectively.

3.3. Scanning electron microscopy

The structures of *B. cinerea* with and without treatment were analyzed in wounded blueberry tissue using a scanning electron microscope (Figure 2). In untreated fruit tissue (Fig. 2 A and B), abundant mycelium and spores of *B. cinerea* are observed intact and without any apparent damage. Meanwhile, in a sample of fruits treated with *D. hansenii* combined with chitosan (Fig. 2C and D), disordered mycelium, broken hyphae and the presence of *D. hansenii* cells adhered to the *B. cinerea* structures were observed. The yeast combined with salicylic acid (Fig. 2E and F) reveals the absence of the germination process of the phytopathogen and the presence of *D. hansenii* is also observed on the infected tissue.



Fig. 2. Scanning electron micrograph of the combined treatment *D. hansenii*-chitosan and *D. hansenii*-salicylic acid on the structures of *B. cinerea* in blueberry fruit. (A and B) Mycelium and spores of untreated *B. cinerea*, (C and D) Structures of *B. cinerea* under the effect of *D. hansenii*-chitosan, (E and F) Structures of *B. cinerea* under the effect of *D. hansenii*-salicylic acid. M: mycelium, S: spores, Y: yeast.

4. DISCUSSION

Similar synergistic results have been obtained when *D. hansenii* and chitosan were applied together to control *Penicillium italicum* (Godana *et al.*, 2022). This was also demonstrated in another study García-Bramasco *et al.* (2022) that evaluated chitosan in combination with *Pichia anomala* to combat the *in vitro* growth of *Penicillium expansum*, obtaining an inhibition growth percentage of 76.74 %. On the other hand, under *in vitro* conditions, the combination of *Rhodotorula mucilaginosa* with salicylic acid resulted in a 50% inhibition of *P. digitatum* (Ahima *et al.*, 2019). Likewise, in this study, the effect of salicylic acid and chitosan is

enhanced by adding the yeast *D. hansenii* with an increase in the fungal control of *B. cinerea*.

Regarding *in vivo* evaluation, the potential of *D. hansenii* to suppress the growth of other phytopathogens such as *Colletotrichum gloeosporioides*, *P. italicum* and *B. cinerea* has been reported (Hernández-Montiel *et al.*, 2018; Vázquez-Vázquez *et al.*, 2021; Ramos-Bell *et al.*, 2022). Moreover, other authors have explored the potential of combining yeast with other control agents, such as chitosan and salicylic acid (Shao *et al.*, 2019; Godana *et al.*, 2021) as addressed in this study. It has been suggested that the synergistic effects in this type of combination treatments results because chitosan triggers self defense mechanisms in yeast, that allows to self prepare for more challenging environments, and subsequently to combat phytopathogens (Godana *et al.*, 2021). The activity of polyphenol oxidase, catalase, phenylalanine and peroxidase enzymes, appears to be involved in this metabolic reaction, as observed when potentiating *P. anomala* with chitosan in table grapes (Godana *et al.*, 2020). Also, in strawberries when applying *R. mucilaginosa* grown in a medium containing chitosan, the defense system of the fruit was induced through the synthesis of phenolic compounds and provided strength to the cell wall (Gu *et al.*, 2021).

As for salicylic acid, it has been reported that there is a greater effect on the control of green mold when combined with *R. mucilaginosa*, it is mentioned that the yeast obtains an advantage over the pathogen, colonizing the fruit wound quickly through the mechanism of competition for space and nutrients (Gu *et al.*, 2021).

The combination treatments suppressed the germination of *B. cinerea* and significantly damaged its hyphae through possible mechanisms of direct action such as competition, production of hydrolytic enzymes, and metal chelation, among others. On the other hand, the different signals of fruit defense response might be activated by inhibiting fungal activity in the fruit (Ahima *et al.*, 2019).

The treatments evaluated may strengthen the fruit's cell wall and reduce its infection rate and incidence of gray mold. According to Ochoa-Jiménez *et al.* (2023), during the fruit ripening process enzymes act that degrade the cell wall such as pectin methylesterase (PME), polygalacturonase (PG), pectate lyase (PL), among others. Therefore, it is possible that chitosan, salicylic acid, and *D. hansenii* slow the expression of these enzymes.

5. CONCLUSIONS

The results of this initial study assessing combination treatments of *Debaryomyces hansenii* with chitosan and salicylic acid are promising. The synergistic effect of such treatments significantly reduced the mycelial growth of *Botrytis cinerea* and mitigated the gray mold disease on blueberries. Likewise, it was evidenced that the combined treatments damage the structures and inhibit the germination of *B. cinerea* on tissue of blueberry fruit. Further research is warranted as to determine whether this combination treatments affect overall quality traits of blueberry and to demonstrate whether the positive results obtained is due to the activation of the fruit's defense responses through increased activity of enzymes, and higher content of phenolic compounds and other plant secondary metabolites.

ACKNOWLEDGMENTS

The authors thank CONAHCYT (Mexico) for the scholarship granted to Surelys Ramos Bell.

AUTHORS' CONTRIBUTION

Surelys Ramos Bell: Investigation, formal analysis and writing. Juan Antonio Herrera González: Investigation and writing. Luis Guillermo Hernández Montiel and Porfirio Gutiérrez Martínez: Supervision, Validation and Visualization. All authors read and approved the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ahima, J., Zhang X, Yang, Q., Zhao, L., Tibiru, A.M., Zhang, H., 2019. Biocontrol activity of *Rhodotorula mucilaginosa* combined with salicylic acid against *Penicillium digitatum* infection in oranges. Biological Control, 135(May), 23–32. https://doi.org/10.1016/j.biocontrol.2019.04.019

García-Bramasco, C.A., Blancas-Benitez, F.J., Montaño-Leyva, B., Medrano-Castellón, L.M., Gutierrez-Martinez, P., González-Estrada, R.R., 2022. Influence of Marine Yeast *Debaryomyces hansenii* on Antifungal and Physicochemical Properties of Chitosan-Based Films. Journal of Fungi, 8(4). <u>https://doi.org/10.3390/jof8040369</u>

Godana, E.A., Yang, Q., Liu, J., Li, J., Zhang, X., Zhao, L., Wang, K., Zhang, H., 2022. Consumer evaluation of sensory properties of table grapes treated with yeast *Pichia anomala* induced by chitosan. Biological Control, 170(April), 104939. <u>https://doi.org/10.1016/j.biocontrol.2022.104939</u>

Godana, E.A., Yang, Q., Wang, K., Zhang, H., Zhang, X., Zhao, L., Abdelhai, M.H., Guillaume Legrand, N.N., 2020. Bio-control activity of *Pichia anomala* supplemented with chitosan against *Penicillium expansum* in postharvest grapes and its possible inhibition mechanism. Lwt, 124(February), 109188. <u>https://doi.org/10.1016/j.lwt.2020.109188</u>

Godana, E.A., Yang, Q., Zhao, L., Zhang, X., Liu, J., Zhang, H., 2021. *Pichia anomala* induced with chitosan triggers defense response of table grapes against post-harvest blue mold disease. Frontiers in Microbiology, 12(July). <u>https://doi.org/10.3389/fmicb.2021.704519</u>

Gu, N., Zhang, X., Gu, X., Zhao, L., Godana, E.A., Xu, M., Zhang, H., 2021. Transcriptomic and proteomic analysis of the mechanisms involved in enhanced disease resistance of

strawberries induced by *Rhodotorula mucilaginosa* cultured with chitosan. Postharvest Biology and Technology, 172(October 2020), 111355. <u>https://doi.org/10.1016/j.postharvbio.2020.111355</u>

Hamss, H.E.I., Kajad, N., Belabess, Z., Lahlali, R., 2023. Enhancing bioefficacy of *Bacillus amyloliquefaciens* SF14 with salicylic acid for the control of the postharvest citrus green mould. Plant Stress, 7, 100144. <u>https://doi.org/https://doi.org/10.1016/j.stress.2023.100144</u>

Hernandez-Montiel, L.G., Rivas-García, T., Romero-Bastidas, M., Chiquito-Contreras, C.J., Ruiz-Espinoza, F.H., Chiquito-Contreras, R.G., 2018. Potencial antagónico de bacterias y levaduras marinas para el control de hongos fitopatógenos. Revista Mexicana de Ciencias Agrícolas, 20, 4311–4321. <u>https://doi.org/10.29312/remexca.v0i20.1000</u>

Hernandez-Montiel, L.G., Droby, S., Preciado-Rangel, P., Rivas-García, T., González-Estrada, R.R., Gutiérrez-Martínez, P., Ávila-Quezada, G.D., 2021. A sustainable alternative for postharvest disease management and phytopathogens biocontrol in fruit: Antagonistic yeasts. Plants, 10(12), 1–12. <u>https://doi.org/10.3390/plants10122641</u>

Hernandez-Montiel, L.G., Gutierrez-Perez, E.D., Murillo-Amador, B., Vero, S., Chiquito-Contreras, R.G., Rincon-Enriquez, G., 2018. Mechanisms employed by *Debaryomyces hansenii* in biological control of anthracnose disease on papaya fruit. Postharvest Biology and Technology, 139(August 2017), 31–37. https://doi.org/10.1016/j.postharvbio.2018.01.015

Herrera-González, J.A., Bautista-Baños, S., Serrano, M., Romanazzi, G., Gutiérrez Martínez, P. 2021. Non-chemical treatments for the pre-and post-harvest elicitation of defense mechanisms in the fungi–avocado pathosystem. Molecules, 26(22). https://doi.org/10.3390/molecules26226819

Islam, M.Z., Lee, Y.T., Mele, M.A., Choi, I.L., Kang, H.M., 2019. Effect of fruit size on fruit quality, shelf life and microbial activity in cherry tomatoes. AIMS Agriculture and Food, 4(2), 340–348. <u>https://doi.org/10.3934/agrfood.2019.2.340</u>

Jiang, H., Sun, Z., Jia, R., Wang, X., Huang, J., 2016. Effect of Chitosan as an Antifungal and Preservative Agent on Postharvest Blueberry. Journal of Food Quality, 39(5), 516–523. <u>https://doi.org/10.1111/jfq.12211</u>

Macha-Huamán, R., Navarro-Soto, F.C., Ramírez-Ríos, A., Alfaro-Paredes, E.A., 2023. International market concentration of fresh blueberries in the period 2001—2020. Humanities and Social Sciences Communications, 10(1), 1–12. <u>https://doi.org/10.1057/s41599-023-02455-7</u>

Milinčić, D.D., Vojinović, U.D., Kostić, A., Pešić, M.B., Špirović-Trifunović, B.D., Brkić, D.V., Stević, M., Kojić M.O., Stanisavljević, N.S., 2020. *In vitro* assessment of pesticide residues bioaccessibility in conventionally grown blueberries as affected by complex food matrix. Chemosphere, 252. <u>https://doi.org/10.1016/j.chemosphere.2020.126568</u>

Ochoa-Jiménez, V.A., Balois-Morales, R., López-Guzmán, G.G., Jiménez-Zurita, J.O., Guzmán, L.F., Berumen-Varela, G., 2023. Changes in quality and gene transcript levels of soursop (*Annona muricata* L.) fruits during ripening. Mexican Journal of Biotechnology 8(2):1-16. <u>https://doi.org/10.29267/mxjb.2023.8.2.1</u>

Ramos-Bell, S., Hernández-Montiel, L.G., González-Estrada, R.R., Gutiérrez-Martínez, P., 2021. Main diseases in postharvest blueberries, conventional and eco-friendly control methods: A review. Lwt, 149(January), 7–12. <u>https://doi.org/10.1016/j.lwt.2021.112046</u>

Ramos-Bell, S., Hernández-Montiel, L.G., Velázquez-Estrada, R.M., Herrera-González, J.A., Gutiérrez-Martínez, P., 2022. Potential of *Debaryomyces hansenii* strains on the inhibition of *Botrytis cinerea* in blueberry fruits (*Vaccinium corymbosum* L.). Horticulturae, 8(12), 1125. <u>https://doi.org/10.3390/horticulturae8121125</u>

Ramos-Bell, S., Diaz-Cayetano., G., Hernández-Montiel, L. G., Velázquez-Estrada, R. M., Montalvo-González, E., Gutiérrez-Martínez, P., 2024. Conservación fisicoquímica de arándanos tratados con quitosano y ácido salicílico en poscosecha. Revista Mexicana de Ciencias Agrícolas. vol.15, n.5, e3391. <u>https://doi.org/10.29312/remexca.v15i5.3391</u>.

Riseh, R.S., Hassanisaadi, M., Vatankhah, M., Babaki, S.A., Barka, E.A., 2022. Chitosan as a potential natural compound to manage plant diseases. International Journal of Biological Macromolecules, 220, 998–1009. https://doi.org/10.1016/j.ijbiomac.2022.08.109

Sautua, F.J., Baron, C., Pérez-Hernández, O., Carmona, M.A., 2019. First report of resistance to carbendazim and procymidone in *Botrytis cinerea* from strawberry, blueberry and tomato in Argentina. Crop Protection, 125(June), 2017–2020. https://doi.org/10.1016/j.cropro.2019.104879

Shao, Y., Zeng, J., Tang, H., Zhou, Y., Li, W., 2019. The chemical treatments combined with antagonistic yeast control anthracnose and maintain the quality of postharvest mango fruit. Journal of Integrative Agriculture, 18(5), 1159–1169. <u>https://doi.org/10.1016/S2095-3119(18)62128-8</u>

Shi, J., Xiao, Y., Jia, C., Zhang, H., Gan, Z., Li, X., Yang, M., Yin, Y., Zhang, G., Hao, J., Wei, Y., Jia, G., Sun, A., Wang, Q., 2023. Physiological and biochemical changes during fruit maturation and ripening in highbush blueberry (*Vaccinium corymbosum* L.). Food Chemistry, 410, 135299. <u>https://doi.org/10.1016/j.foodchem.2022.135299</u>

Vázquez-Vázquez, M.L., Hernández-Montiel, L.G., Sanchez-Viveros, G., Reyes-Pérez, J., Martinez-Hernandez, M., Chiquito-Contreras, R., 2021. Efecto de levaduras de origen marino y ulvan en el control poscosecha de *Penicillium italicum* agente causal del moho azul en limón persa. Biotecnia, 23(3), 78–88. <u>https://doi.org/10.18633/biotecnia.v23i3.1428</u>

Wu, Y., Yang, H., Yang, H., Zhang, C., Lyu, L., Li, W., Wu, W., 2022. A physiological and metabolomic analysis reveals the effect of shading intensity on blueberry fruit quality. Food Chemistry: X, 15, 100367. <u>https://doi.org/https://doi.org/10.1016/j.fochx.2022.100367</u>