In vivo study: effect of essential oils and lipopeptides from *Bacillus tequilensis* EA-CB0015 on the growth of the phytopathogenic fungus *Colletotrichum Acutatum* EAHP-012.

Joana Paola Orozco Vergel

Trabajo de grado presentado como requisito para optar al título de BIÓLOGO

Asesor: Luis Alejandro Gómez Ramírez, Ph.D.

[a]Pregrado en Biología
Departamento de Ciencias Biológicas
Escuela de Ciencia
Universidad EAFIT

Medellín, 18 de mayo de 2021

ABSTRACT

In the search of enhancing antifungal effects of *Bacillus tequilensis* EA-CB0015 and finding new fungicidal biological agents, the capacity of different concentrations of *Bt* EA-CB0015 lipopeptides was evaluated *in vitro* and *in vivo* to tests if its effect on the growth of one of the most destructive phytopathogens worldwide recognized to produce Anthracnose (*Colletotrichum acutatum*; EAHP-012, 008 strains) were consistent in both conditions, on the other hand, an initial *in vivo* assay was made to test the effect of three commercial essential oils: tea tree oil (*Melaleuca alternifolia,* mint oil (*Mentha piperita*) and cinnamon oil (*Cinnamomum zeylanicum*), on the growth of *C. acutatum* EAHP-012 in tamarillo fruits. Lipopeptides concentrations under 16ppm exhibited partial growth inhibition in both models, these results are in line with previous reports of metabolic studies made to *Ca* EAHP-08 strain. The inhibitory effect of the three essential oils showed significant lesion reduction (more than 6mm) caused by anthracnose in tamarillo fruit. This study is a first step forward to a series of evaluations using essential oils complementing the powerful antifungal activity of *Bt* EA-CB0015 lipopeptides.

Keywords

*Colletotrichum acutatum, Bacillus tequilensis***,** essential oils, lipopeptides, biological control

1. Intoduction

Colletotrichum sp. is known for being a large genus of Ascomycete fungi, characterized by its wide host range worldwide and containing species that are amongst the most successful plant pathogenic fungi (Wharton and Dieguez-Uribeondo, 2004). This genus has been ranked within the top ten most devastating fungal pathogen, for causing significant economic damage to crops in tropical, subtropical, and temperate regions (Dean et al., 2012). The species comprised in the Colletotrichum sp. complexes are associated with quiescent infections, pre-and post-harvest diseases (Damm et al., 2012; Wharton and Dieguez-Uribeondo, 2004), which means that crops can be jeopardized even during storage conditions, generating crop losses as high as 50% or more and significant economic losses equivalent to hundreds of billions of dollars annually (Afanador-Kafuri et al., 2014; Aranzazu and Rondon, 1999; MinAgricultura and DANE, 2014).

Distinctively, *Colletotrichum acutatum* is one of the most pathogenic species causing Anthracnose; a disease that causes dark, sunken lesions or blights of aerial plant parts and fruit rotting post-harvest (Gregory et al., 2008). The damage caused by *C. acutatum* affects staple food crops and a variety of fruits, including strawberry, avocado, citrus, mango, tamarillo, and blackberry. Colombia is among the main exotic fruit exporters along with a large local market, whereby the control of

phytopathogenic fungi, such as *C. acutatum,* have always been a leading challenge. Anthracnose disease can decrease the productive period of different fruits, in the specific case of tamarillo has decreased from 5 years to 2 or 3 years with subsequent abandonment of the crop (Aranzazu and Rondon, 1999; MinAgricultura and DANE, 2014). Additionally, Anthracnose control still relies heavily on chemical fungicides, which represents from 15% to 45% of the total operational costs (Oliveira et al., 2019; MinAgricultura and DANE, 2014), furthermore, the application of chemical fungicides brings potential risks to human health, the environment, and contributes to the selection of resistant plant pathogen strains (Ishii et al., 2016; Matrose et al., 2021). In search of mitigating the negative impacts, there is a strong need of implementing alternative, more eco-friendly strategies for Anthracnose control (Oliveira et al., 2019).

Those eco-friendly alternatives are recognized as biological control strategies. Several natural compounds such as active metabolites produced by microorganisms and plant extracts have shown potential as antimicrobial agents against a wide range of microorganisms including phytopathogens alike *C. acutatum* (Juarez et al., 2015). Within the *Bacillus* genus, the strain *Bacillus tequilensis* EA-CB0015 was isolated in Urabá, Colombia for its remarkable ability to inhibit the growth of *Mycosphaerella fijensis* in banana plants related to the production of lipopeptides (Ceballos et al., 2012). Lipopeptides isolated from *B.tequilensis* EA-CB0015 were found to have astounding activity against different *C. acutatum* strains (Arroyave-Toro et al., 2017) and were recently tested *in vitro* to evaluate if its inhibitory effect is related to an alteration of mitochondrial function, showing that even though decreasing its growth rates and biomass production, it does not result in a detriment of the pathogen respiratory capacity (Tobón-Ospina and Gómez-Ramírez, 2019).

Essential oils (EOs) have been gaining more attention as alternative biological control agents, due to their extended antimicrobial capacity. Moreover, their complex composition and a variety of mechanisms of action lower the risk for the development of pathogen resistance (Oliveira et al., 2019; Jahani et al., 2020). Some studies have revealed the antifungal activity of EOs against some phytopathogens and common fungi including *C. acutatum*, *F. oxysporum, A. niger, and A. oryzae* (Sharma et al., 2017; Hu et al., 2019). Furthermore, some EOs establish a membrane potential across the cell wall, causing cell wall damage through the disruption of ATP assembly and there has been evidence of interference in the Electron Transport System (ETS) pathway by disintegrating mitochondrial membrane (Tariq et al., 2019). Considering its potential for disrupting such important pathways, EOs could be tested along with *Bt* EA-CB0015 lipopeptides to evaluate if their combination can lead to a synergy against fungal activity.

To determine if there is a comparable *in vivo* response to that demonstrated on the *in vitro* metabolic functional study; where different concentrations of *Bt* EA-CB0015 lipopeptides (32ppm, 16ppm, 10ppm, and 8ppm) were tested on *C. acutatum* liquid culture. The lipopeptide concentrations were evaluated on tamarillo (*Cyphomandra betacea*) as an *in vivo* pathosystem. Both evaluations are consistent that under 32ppm demonstrated significant growth inhibition by a reduction of biomass production, yet without affecting its metabolic function. Lipopeptide production is somehow limited, that is why exploring alternatives compounds that can be used to enhance antifungal activity is the focus of this work. Taking that into account, the *in vivo* activity of three commercial essential oils: tea tree oil (*Melaleuca alternifolia),* mint oil (*Mentha piperita*), and cinnamon oil (*Cinnamomum zeylanicum*) against *C. acutatum* EAHP-012 was evaluated to determine the effect on the growth of the phytopathogen, showing promising results by reducing Anthracnose symptoms on tamarillo fruit.

2. Material and methods

2.1 Microorganisms and culture conditions

Colletotrichum acutatum strains EAHP- 008 and EAHP-012 are kept stored in folded filter paper at 4°C and need to be activated before any experimental use by placing it on Potato Dextrose Agar (PDA by Oxoid) plate culture for 7-9 days at room temperature. *Bacillus tequilensis* EA-CB0015 (GenBank accession number KC006063) was stores at -80°C in Tryptic Soy Broth (TSB, Oxoid) with 20% glycerol and was activated on half-strength Tryptic Soy Agar (TSA, Oxoid) plate culture at 30°C for 48 h.

2.2 B. tequilensis EA-CB0015 lipopeptides

Production, extraction, and purification of the *B. tequilensis* EA-CB0015 lipopeptides were performed following previously described protocol by Villegas-Escobar et al. (2013), which consist of 3 major phases: fermentation, a solid phase extraction, and purification through RP-HPLC. The lipopeptide mixture was kept at 4°C until its use.

2.3 Essential oils

The three pure essential oils: tea tree oil (*Melaleuca alternifolia) (FUNAT),* mint oil (*Mentha piperita*) (FUNAT), and cinnamon oil (*Cinnamomum zeylanicum*) (FUNAT) were store-bought.

2.4 B. tequilensis EA-CB0015 lipopeptides activity on C. acutatum kinetics in vitro

A liquid culture titration was performed in microtitration plates as described by Wiegand, Hilpert, and Hancock (2008), which consist of measuring the turbidity of the medium, understanding that the greater the turbidity, the greater the growth of the microorganism. For the present case, fungal spores of *C.acutatum* EAHP-008 at a concentration of 1 x 10^5 (spores/ml) were added and cultured in a 96-well microtiter plate, with each well containing 100µl of Sabouraud broth mixed with different concentrations of purified lipopeptides with a final concentration of 8ppm, 16ppm, 32ppm, and 64ppm. Microtiter plates were incubated for 156 hrs at 26°C, and an optical density was measured using an iMark™ Reader (Biorad) at 595nm every 12h. Three independent experiments were performed, with six replicates for each lipopeptide concentration used. Amongst the concentrations tested, 32ppm and 64ppm completely inhibited *C. acutatum* growth as previously reported; 32ppm was recognized as the MIC (Arroyave-Toro et al., 2017). Four more independent experiments were performed as described, only modifying the final lipopeptide concentrations ranging from 8ppm to 14ppm to evaluate the effect of lower lipopeptide concentrations on the fungal growth. The optical density measurements were used to build up the kinetic growth of *C. acutatum* with and without lipopeptide treatments. Furthermore, the last concentrations used were selected as treatments over *C. acutatum* for respirometry assays and *in vivo* trials to evaluate whether lipopeptides effects are consistent in both cultured conditions.

2.5 Fruit trials

To evaluate if the *in vitro* effects of lipopeptides on *C. acutatum* growth is consistent or can be reproduced *in vivo* tamarillo fruits were artificially inoculated as indicated by Arroyave-Toro et al.

(2017) using only the lipopeptide mixture and matching the spore suspensión to that used for the *in vitro* and bioenergetic assays (1 x 10^5 spores/ml). Briefly, tamarillo fruits were sterilized by immersion in 0.1% bleach for 1 min and 70% ethanol for 2 mins, rinsed with water wounded with a width and depth of 1 x 2 mm respectively. Afterward, 15µl of fungal spore suspension was used to inoculate tamarillo fruits through the wounds. After 24h, 15µl of water or lipopeptides were added to the wounds. The treated fruits were incubated for 6 days in humid chambers, with ~ 85 - 90% humidity and 25°C. Lesion diameter was measured every 24h. Five independent experiments were performed with three replicates for each lipopeptide concentration used.

2.6 Fruit trials: effect of essential oils (EOs)

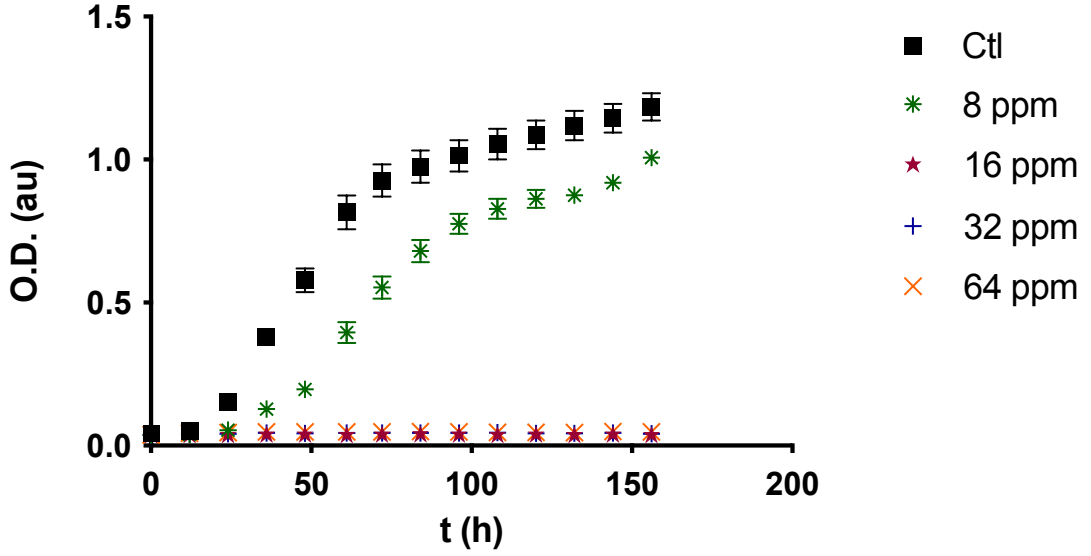
To determine the effect of EOs on *C. acutatum* infection in tamarillo fruit, the same process as 2.5 was followed. In this specific case, the lesion diameters were only measured after 6 incubation days and just 2 treatments corresponding to 1:16 and 1:20 dilutions were tested. Six independent experiments were performed with three replicates for each treatment. The two dilutions were chosen for being among the highest concentrations that did not produce any damage to the fruit skin.

2.7 Statistical analysis

Statistical analyses were done using GraphPad Prism 8. A two-way ANOVA was used to evaluate differences among treatments means for the *B. tequilensis* EA-CB0015 lipopetides effect on the growth of *C. acutatum*. While one-way ANOVA was used for the EOs assays. Tukey's multiple comparison test, with a 95% confidence level, was used in all assays for the pairwise comparisons of the treatment means.

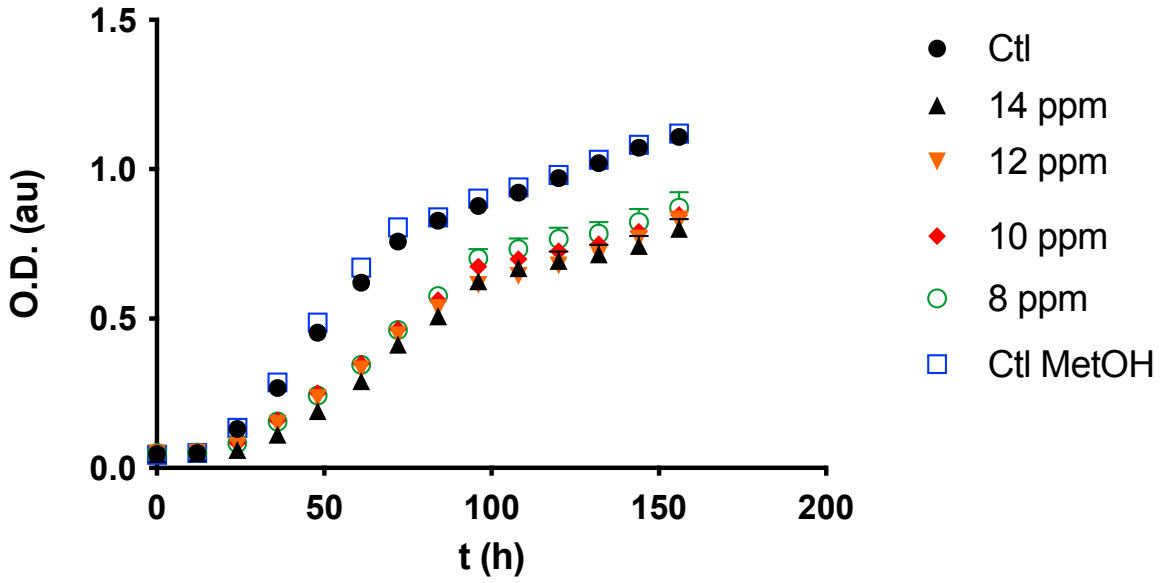
3. Results and discusión

3.1 C.acutatum EAHP-008 growth is partially inhibited by B. tequilensis EA-CB0015 lipopeptide concentrations under 16ppm



Firure 1. Representative figure of n = 3 independent tests, with 6 replica for each time. Ctl denotes *C. acutatum* without treatments, 8, 16, 32, 64 ppm denotes the lipopeptide concentrations used.

After having evaluated a wide *B. tequilensis* EA-CB0015 lipopeptide concentration treatments ranging from 1 – 1025ppm, it was noted that starting from concentrations of 16ppm and above, the growth *C. acutatum* EAHP-008 was almost completely inhibited. Consistent results were obtained after focusing the assays to lesser concentrations of 8, 16, 32, 64ppm (**Figure 1)**, we confirmed that there was a significant growth inhibition starting from 16ppm. On the other hand, 8ppm showed a growth reduction regarding the control, less biomass was produced, but *C. acutatum* EAHP-008 was still functional and kept growing. As a total growth inhibition was not our main goal, we decided to explore different lipopeptide concentrations in between 16ppm and 8ppm (**Figure 2)** to evaluate whether *C. acutatum* EAHC-008 reduction growth could produce enough biomass to assess and study its response to the treatments and if the results from a previous metabolic assay were maintained.



Firure 2. Representative figure of n = 4 independent tests, with 6 replica for each time. Ctl denotes *C. acutatum* without treatments, 8, 10, 12, 14ppm denotes the lipopeptide concentrations used and Ctl MetOH denotes the vehicle control.

In concentrations within 8 – 16ppm growth reduction contrasting the controls was observed. These results indicate that even when its growth is partially inhibited the fungus is still active, capable of growing and it should be ought to generate infection. These results are of interest to us not only to evaluate *C. acutatum* EAHP-008 behavior under lipopeptides treatments but to determine if there is a comparable result with that obtained in previous metabolic studies. It is noticeable that there is an abrupt response from the inhibition growth effect during 14ppm and 16ppm treatments. There seems to be a threshold where the growth it's heavily affected but we do not know what changes or processes are responsible for such an abrupt response, more evaluations using concentrations in between 14ppm

to 16ppm are needed to determine what is causing that threshold and the metabolic responses that might be causing the sudden decrease of biomass production within the two concentrations.

3.2 B. tequilensis EA-CB0015 lipopeptide concentrations under 16ppm results in Anthracnose lesion reduction in tamarillo fruits

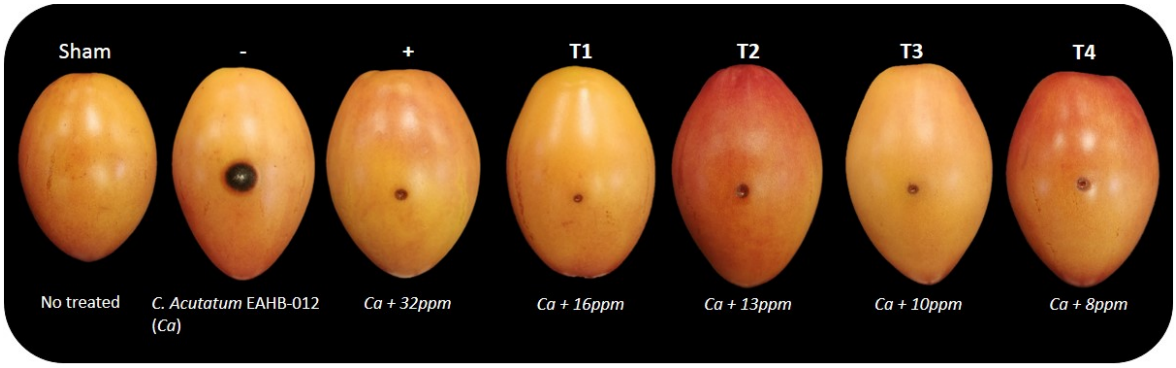
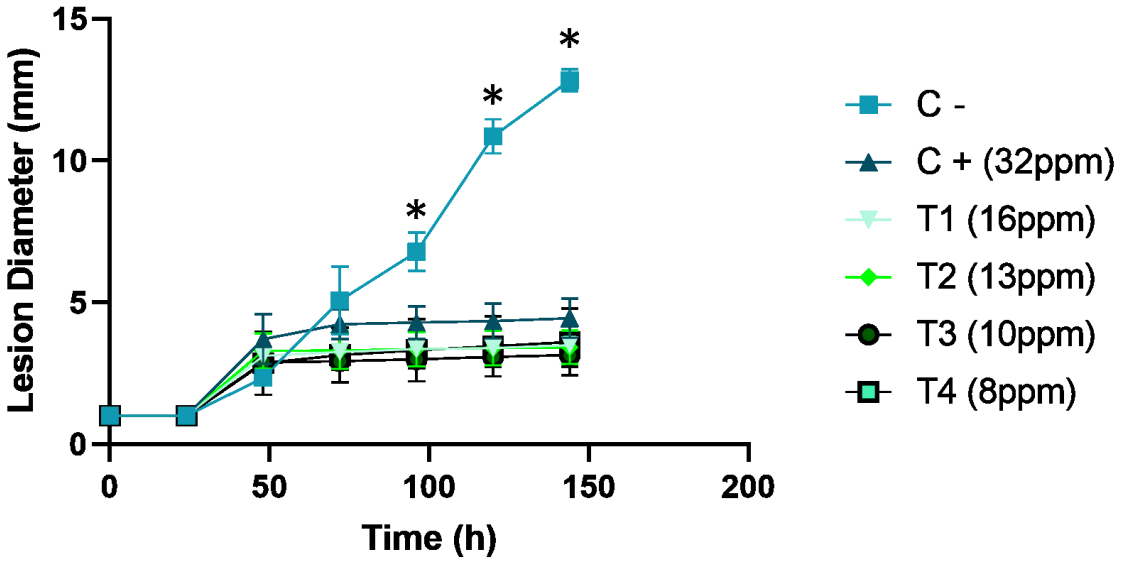


Figure3. Representative figure of n = 5 independent tests, with 5 replicas for each treatment. *Ca* denotes *C. acutatum* EAHP-012. *In vivo* anthracnose lesion reduction on treated tamarillo fruits with *B. tequilensis* EA/CB0015 lipopeptides.

To determine if the inhibitory activity observed in 3.1 was maintained *in vivo,* chosen lipopeptide concentrations between 8 up to 16ppm were evaluated in tamarillo fruit to observe the treatments effect on the development of Anthracnose lesions. All tamarillo fruits treated with lipopeptides exhibited a significant reduction of lesion development (figure 3). Lesion diameters were approximately 9.6mm smaller than the control (-) (figure 3, 4). Suggesting that *in vitro* results are consistent with the *in vivo* disease development reduction. However, *in vivo* 32, and 16ppm treatments did not show a complete inhibition as reported by Arroyave-Toro et al. (2017) and Tobon-Ospina and Gomez-Ramirez. (2019).

In contrast to the microtiter results, *in vivo* assays showed that at 16ppm and 32ppm lipopeptide treatments *C. acutatum* was able to grow (figure 4), which could mean that some other interactions or fruit conditions also play an important role in fungal pathogenesis. Nonetheless, consistent results were found with respect to its activity in previous studies by Tobon-Ospina and Gomez-Ramirez. (2019), such as lipopeptide concentrations in between 8 – 16ppm reduces *C. acutatum* growth, at 96h all treatments effects start to show statistical significance respect to the non-treated control, kinetics shows similar growth behavior to that presented *in vitro* (Figure 2) and in the metabolic evaluations. Besides, the time where statistical significance starts to appear (96h), is around the time (~100h) where in previous kinetics assays the stationary stage starts (Arboleda-Restrepo, 2018). Prior to 96h, there is none to low significance between the treatments respect to the control, this is interesting because it means that during the first stages of the exponential growth phase, lipopeptide concentration treatments are still somehow ineffective, or that lipopeptides have a conditioning period to start its fungicidal effects, giving some time to the phytopathogen to grow before affecting its biomass production. In particular, a microbial biological control agent (MBCA) product based on *Bt* EA-CB0015 cells and lipopeptides was developed and proven effective against Sigatoka disease on banana plants by direct interaction via antibiosis (Villegas-Escobar et al., 2016; Cuellar-Gaviria et

al., 2021). If there were a requirement to use lesser lipopeptides concentration to make the MBCA product more accessible without reducing its antifungal activity, it is necessary to evaluate and integrate new biological agents into the formula that can help to fulfill that purpose.



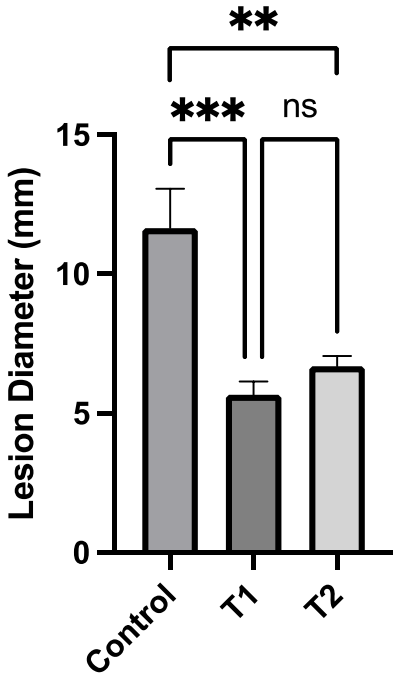
Firure 4. Representative figure of n = 5 independent tests, with 5 replica for each treatment. C - denotes *C. acutatum* without lipopeptides treatments, C + denotes the MIC, T1 to T4 denotes lipopeptide concentration treatments from8 - 14ppm, (*) denotes statistically significant differences respect to the non-treated control at 95% confidence level. P-value = 0.0001.

3.3 Tea tree oil (*Melaleuca alternifolia),* mint oil (*Mentha piperita*) and cinnamon oil (*Cinnamomum zeylanicum*) reduces Anthracnose development in tamarillo fruits

The three essential oils showed a significant growth reduction of *Ca* EAHP-012, which can be observed on the reduced Anthracnose lesion diameter produced by the treatments (Figure 6, 8,10). Notable statistical significance among treatments means respect to the control are presented in figures 5, 7, and 9. The most significative lesion diameters reduction means obtained were: 5.993mm (p-value = 0.0005), 6.602mm (p-value = <0.0001), and 6.585mm (p–value = 0.0035) for Cinnamon oil, Mint oil, and Tea tree oil respectively. These results suggest that these EOs are promising biological agents that could be used to enhance the antifungal or antimicrobial effects of *Bt* EA-CB0015 lipopeptides.

Bt EA-CB0015 lipopeptides have shown growth inhibitory activity against black Sigatoka disease in banana plants (Urabá, Colombia) under *in vitro*, green-house, and field conditions, reducing up to 42.9% the disease level (Gutierrez-Monsalve et al., 2015). Three lipopeptide families are produced by *Bt* EA-CB0015; iturin A, fengycin C, and surfactin (Gonzalez-Jaramillo et al., 2017), each of them

contributes to the overall inhibition effects observed in this work. Iturine A and fengycin inhibits mycelial growth of *B. cinerea*001 and *Ca* EAHP-008 (Arroyave-Toro et al., 2017) and ascospores germination in *P. fijiensis*, while surfactin by itself lacks antifungal activity (Mosquera et al., 2014). The antifungal activity of lipopeptides have been attributed either to a mitochondrial membrane perturbing action or permeabilization, also, some studies proposed the alteration or impairment of mitochondrial function as another underlying factor for the antifungal activity (Avenot and Michailides, 2010; Robles-Martinez et al., 2014; Nichola et al., 2019). The latest mentioned, was evaluated on a previous bioenergetic study, where the possibility of disruption in the lipid organization of mitochondrial membranes was excluded as coupled respiration was preserved in treated cultures (Tobon-Ospina and Gomez-Ramirez, 2019). Antifungal action through disruption in the cell membrane and mitochondrial damage have been reported as EOs treatment effect on food-borne molds (Prakash et al., 2012), and other fungal pathogens. These effects have been reported to be produced by the three EOs used in these work (tea tree oil, mind oil, and cinnamon oil).



Ca EAHP-012	x	x	x
EO 1:16		x	
EO 1:20			x

Figure 5. Cinnamon. Representative figure of n = 6 independent tests, with 6 replicas for each EO treatment. (*) denotes statistically significant differences respect to the non-treated control at 95% confidence level. P-value = 0.0005.

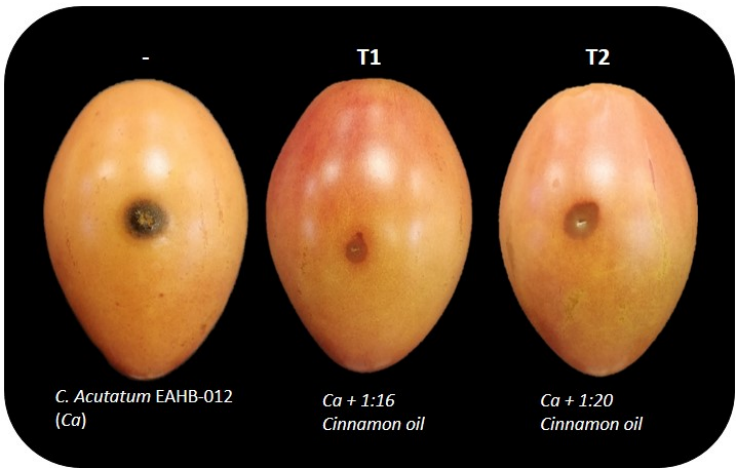
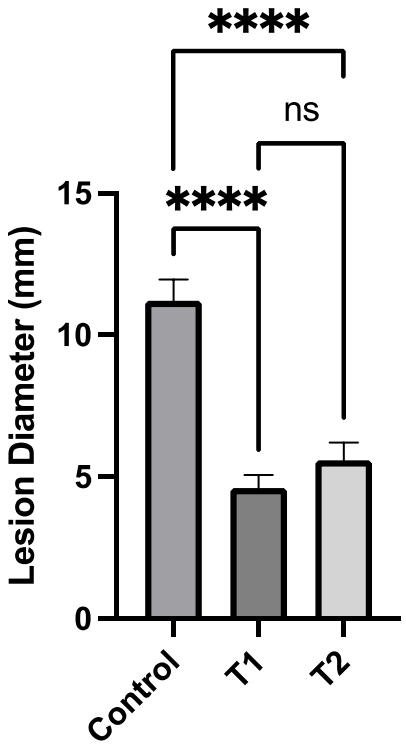


Figura 6. *In vivo* anthracnose lesion reduction on treated tamarillo fruits with cinnamon essential oil.

The following is intendend as an overview of what is known about the EOs mechanism of antifungal action. Cinnamon essential oil (*Cinnamomum zeylanicum)* has been outlined for its antifungal activity against food borne molds (Prakash et al., 2012; Kiran et al., 2014), plant pathogenic fungi such as *C. gloeosporioides*, *B. cinerea*, *F. graminea* (J. Wang et al., 2020), and its antimicrobial activity against multidrug-resistant strains like *S. aureus*, and *E. faecalis* (Saki et al., 2020). Cinnamon oil antifungal action mechanisms are reported to be caused by an increase of ROS levels and ergosterol content and reducing mitochondrial membrane potential (Prakash et al., 2012; N. Wang et al., 2020). Mint oil (*Mentha piperita)* also shows significant antifungal activity against *Penicillium spp*., *F. oxysporum*, and *A. fumigates* (Rajkumar et al., 2019; Dsam et al., 2019). Tea tree (*Melaleuca alternifolia*) oil have been extensively evaluated against *B. cinerea* and is reported as a highly competent antifungal agent (Li et al. 2017; N. Wang et al., 2020) Its action mechanism has many similarities to those reported for the cinnamon oil: mitochondrial damage, resulting in matrix loss and disruption of the tricarboxylic acid cycle, as well as membrane-related pathways in mitochondrial (Li et al., 2017; Gupta et al.,2018).

EOs studies in general, have demonstrated the antifungal action through alteration of mitochondrial integrity, a mechanism that could be used along the *Bt* EA-CB0015 for an enhanced inhibition effect on phytopathogenic fungi such as *C. acutatum*.



Ca EAHP-012 x x x

EO 1:16	x	
EO 1:20		x

Figura 7. Mint. Representative figure of n = 6 independent tests, with 6 replicas for each EO treatment. (*) denotes statistically significant differences respect to the non-treated control at 95% confidence level. P-value = 0.0001.

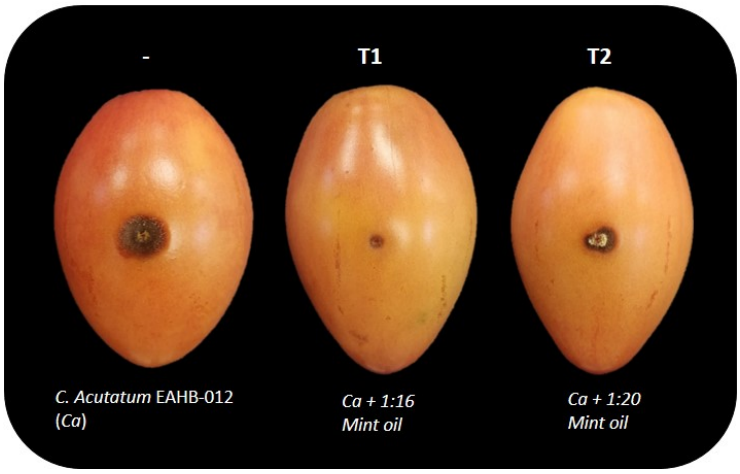
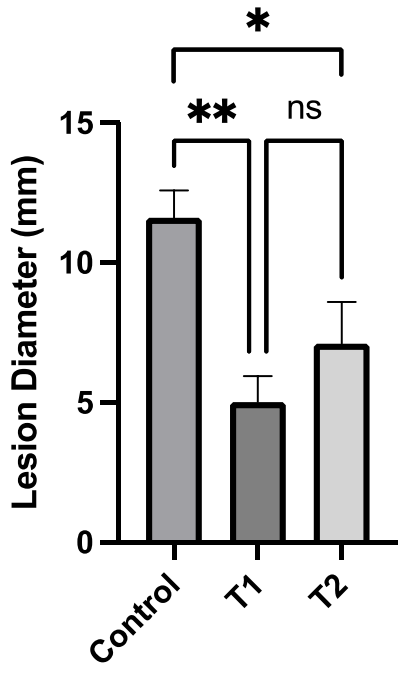


Figura 8. *In vivo* anthracnose lesion reduction on treated tamarillo fruits with mint essential oil.



Ca EAHP-012	x	x	x
EO 1:16		x	

EO 1:20	x

Figura 9. Tea tree. Representative figure of n = 6 independent tests, with 6 replicas for EO treatment. (*) denotes statistically significant differences respect to the non-treated control at 95% confidence level. P-value = 0.0035.

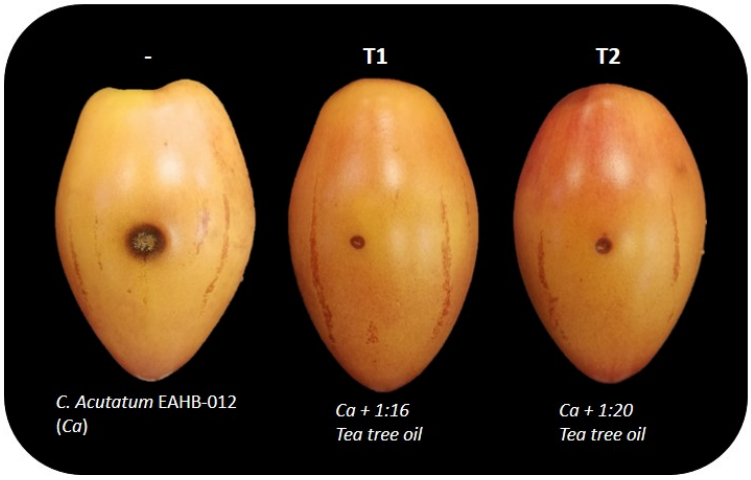


Figura 10. *In vivo* anthracnose lesion reduction on treated tamarillo fruits with tea tree essential oil.

4. Conclusion

Bt EA-CB0015 lipopeptides antifungal effect is remarkably effective for reducing *C. acutatum* growth with concentrations under the MIC (32ppm) in both *in vitro* and *in vivo*. These evaluations of the growth dynamic under different treatment concentrations are key to a better understanding of how lipopeptides generate their antifungal actions by allowing the study of the state of *C. acutatum* under this specific stress condition, thus, elucidating if there are different pathways or mechanism of action that could be enhanced and how it could be done. In particular, the evidence of a possible alteration of mitochondrial dynamics in *C. acutatum* EAHP-008 treated with *Bt* AC-CB0015 lipopeptides to prevent apoptosis, suggests the potential of fungal cultures to overcome some treatments if not used an appropriate lipopeptide concentration. That is why more biological agents, recognized by their antifungal effects, such as essential oils, should be assessed to develop new combined antifungal formulations where a variety of agents produce a synergetic action against phytopathogens growth with less product needed. In this study we suggest further evaluation of the three EOs used, starting from profiling them to check if they are pure, following with a wider concentrations range tested.

5. References

Afanador-Kafuri, L., González, A., Gañán, L., Mejía, J.F., Cardona, N., Alvarez, E., 2014. Characterization of the colletotrichum species causing anthracnose in andean blackberry in Colombia. Plant Dis. 98, 1503–1513. https://doi.org/10.1094/PDIS-07-13-0752-RE

Aranzazu, L., Rondón, J., 1999. Manejo productivo del cultivo de tomate de árbol y de la antracnosis (Solanum betacea) (Colletotrichum gloeosporioides (PENZ), PENZ & SACC). Corpoica. https://repository.agrosavia.co/handle/20.500.12324/2097

Arboleda-Restrepo, L.M., 2018. Características respiratorias en el hongo fitopatógeno Colletotrichum acutatum EAHP-008: Reserva bioenergética y actividad de la oxidasa alternativa. EAFIT. https://repository.eafit.edu.co/handle/10784/13049

Arroyave-Toro, J.J., Mosquera, S., Villegas-Escobar, V., 2017. Biocontrol activity of Bacillus su*Bti*lis EA-CB0015 cells and lipopeptides against postharvest fungal pathogens. Biol. Control 114, 195–200. https://doi.org/10.1016/j.biocontrol.2017.08.014

Avenot, H.F., Michailides, T.J., 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. Crop Prot. 29, 643–651. https://doi.org/10.1016/j.cropro.2010.02.019

Ceballos, I., Mosquera, S., Angulo, M., Mira, J.J., Argel, L.E., Uribe-Velez, D., Romero-Tabarez, M., Orduz-Peralta, S., Villegas, V., 2012. Cultivable Bacteria Populations Associated with Leaves of Banana and Plantain Plants and Their Antagonistic Activity Against Mycosphaerella fijiensis. Microb. Ecol. 64, 641–653. https://doi.org/10.1007/s00248-012-0052-8

Cuellar-Gaviria, T.Z., González-Jaramillo, L.M., Villegas-Escobar, V., 2021. Role of Bacillus tequilensis EA-CB0015 cells and lipopeptides in the biological control of black Sigatoka disease. Biol. Control 155. https://doi.org/10.1016/j.biocontrol.2020.104523

Damm, U., Cannon, P.F., Woudenberg, J.H.C., Crous, P.W., 2012. The Colletotrichum acutatum species complex. Stud. Mycol. 73, 37–113. https://doi.org/10.3114/sim0010

Dean, R., Van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J., Foster, G.D., 2012. The Top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13, 414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Desam, N.R., Al-Rajab, A.J., Sharma, M., Mylabathula, M.M., Gowkanapalli, R.R., Albratty, M., 2019. Chemical constituents, *in vitro* antibacterial and antifungal

activity of Mentha × Piperita L. (peppermint) essential oils. J. King Saud Univ. - Sci. 31, 528–533. https://doi.org/10.1016/j.jksus.2017.07.013

González-Jaramillo, L.M., Aranda, F.J., Teruel, J.A., Villegas-Escobar, V., Ortiz, A., 2017. Antimycotic activity of fengycin C biosurfactant and its interaction with phosphatidylcholine model membranes. Colloids Surfaces B Biointerfaces 156, 114–122. https://doi.org/10.1016/j.colsurfb.2017.05.021

Gregori, R., Mari, M., Bertolini, P., Barajas, J.A.S., Tian, J.B., Labavitch, J.M., 2008. Reduction of Colletotrichum acutatum infection by a polygalacturonase inhibitor protein extracted from apple. Postharvest Biol. Technol. 48, 309–313. https://doi.org/10.1016/j.postharvbio.2007.10.006

Gupta, P., Gupta, S., Sharma, M., Kumar, N., Pruthi, V., Poluri, K.M., 2018. Effectiveness of Phytoactive Molecules on Transcriptional Expression, Biofilm Matrix, and Cell Wall Components of Candida glabrata and Its Clinical Isolates. ACS Omega 3, 12201–12214. https://doi.org/10.1021/acsomega.8b01856

Gutierrez-Monsalve, J.A., Mosquera, S., González-Jaramillo, L.M., Mira, J.J., Villegas-Escobar, V., 2015. Effective control of black Sigatoka disease using a microbial fungicide based on Bacillus su*Bti*lis EA-CB0015 culture. Biol. Control 87, 39–46. https://doi.org/10.1016/j.biocontrol.2015.04.012

Hu, F., Tu, X.F., Thakur, K., Hu, F., Li, X.L., Zhang, Y.S., Zhang, J.G., Wei, Z.J., 2019. Comparison of antifungal activity of essential oils from different plants against three fungi. Food Chem. Toxicol. 134, 110821. https://doi.org/10.1016/j.fct.2019.110821

Ishii, H., Zhen, F., Hu, M., Li, X., Schnabel, G., 2016. Efficacy of SDHI fungicides, including benzovindiflupyr, against Colletotrichum species. Pest Manag. Sci. 72, 1844–1853. https://doi.org/10.1002/ps.4216

Jahani, M., Pira, M., Aminifard, M.H., 2020. Antifungal effects of essential oils against Aspergillus niger *in vitro* and *in vivo* on pomegranate (Punica granatum) fruits. Sci. Hortic. (Amsterdam). 264, 109188. https://doi.org/10.1016/j.scienta.2020.109188

Juárez, Z.N., Hernández, L.R., Bach, H., Sánchez-Arreola, E., Bach, H., 2015. Antifungal activity of essential oils extracted from Agastache mexicana ssp. xolocotziana and Porophyllum linaria against post-harvest pathogens. Ind. Crops Prod. 74, 178–182. https://doi.org/10.1016/j.indcrop.2015.04.058

Li, Y., Shao, X., Xu, J., Wei, Y., Xu, F., Wang, H., 2017. Tea tree oil exhibits antifungal activity against Botrytis cinerea by affecting mitochondria. Food Chem. 234, 62–67. https://doi.org/10.1016/j.foodchem.2017.04.172

Matrose, N.A., Obikeze, K., Belay, Z.A., Caleb, O.J., 2021. Plant extracts and other natural compounds as alternatives for post-harvest management of fruit fungal pathogens: A review. Food Biosci. 100840. https://doi.org/10.1016/j.fbio.2020.100840

MinAgricultura., DANE., 2014. Antracnosis, importancia y manejo integrado en el cultivo de tomate de árbol (*Cyphomandra betacea*e). Prevención: la mejor alternativa. https://www.dane.gov.co

Nicola, A.M., Albuquerque, P., Paes, H.C., Fernandes, L., Costa, F.F., Kioshima, E.S., Abadio, A.K.R., Bocca, A.L., Felipe, M.S., 2019. Antifungal drugs: New insights in research & development. Pharmacol. Ther. 195, 21–38. https://doi.org/10.1016/j.pharmthera.2018.10.008

Oliveira, J., Gloria, E.M., Parisi, M.C.M., Baggio, J.S., Silva, P.P.M., Ambrosio, C.M.S., Spoto, M.H.F., 2019. Antifungal activity of essential oils associated with carboxymethylcellulose against Colletotrichum acutatum in strawberries. Sci. Hortic. (Amsterdam). 243, 261–267. https://doi.org/10.1016/j.scienta.2018.08.032

Prakash, B., Singh, P., Kedia, A., Dubey, N.K., 2012. Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin, antioxidant activities and *in vivo* efficacy in food system. Food Res. Int. 49, 201–208. https://doi.org/10.1016/j.foodres.2012.08.020

Rajkumar, V., Gunasekaran, C., Christy, I.K., Dharmaraj, J., Chinnaraj, P., Paul, C.A., 2019. Toxicity, antifeedant and biochemical efficacy of Mentha piperita L. essential oil and their major constituents against stored grain pest. Pestic. Biochem. Physiol. 156, 138–144. https://doi.org/10.1016/j.pestbp.2019.02.016

Robles-Martínez, L., Guerra-Sánchez, M.G., Hernández-Lauzardo, A.N., Pardo, J.P., Velázquez-del Valle, M.G., 2014. Effects of chitosan and oligochitosan on development and mitochondrial function of Rhizopus stolonifer. J. Basic Microbiol. 54, 42–49. https://doi.org/10.1002/jobm.201300790

S., K., Kujur, A., Prakash, B., 2016. Assessment of preservative potential of Cinnamomum zeylanicum Blume essential oil against food borne molds, aflatoxin B1 synthesis, its functional properties and mode of action. Innov. Food Sci. Emerg. Technol. 37, 184–191. https://doi.org/10.1016/j.ifset.2016.08.018

Saki, M., Seyed-Mohammadi, S., Montazeri, E.A., Siahpoosh, A., Moosavian, M., Latifi, S.M., 2020. *In vitro* antibacterial properties of Cinnamomum zeylanicum essential oil against clinical extensively drug-resistant bacteria. Eur. J. Integr. Med. 37. https://doi.org/10.1016/j.eujim.2020.101146

Sharma, A., Rajendran, S., Srivastava, A., Sharma, S., Kundu, B., 2017. Antifungal activities of selected essential oils against Fusarium oxysporum f. sp. lycopersici 1322, with emphasis on Syzygium aromaticum essential oil. J. Biosci. Bioeng. 123, 308–313. https://doi.org/10.1016/j.jbiosc.2016.09.011

Tariq, S., Wani, S., Rasool, W., Shafi, K., Bhat, M.A., Prabhakar, A., Shalla, A.H., Rather, M.A., 2019. A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant

microbial pathogens. Microb. Pathog. 134, 103580. https://doi.org/10.1016/j.micpath.2019.103580

Tobón-Ospina, J., Gómez-Ramírez, L.A., 2019. Antifungal activity of lipopeptides isolated from Bacillus su*Bti*lis EA-CB0015 against Colletotrichum acutatum EAHP-008 is not related to an impairment in cellular bioenergetic reserve capacity. EAFIT. https://repository.eafit.edu.co/handle/10784/15357

Villegas-Escobar, V., Ceballos, I., Mira, J.J., Argel, L.E., Orduz Peralta, S., Romero-Tabarez, M., 2013. Fengycin C produced by Bacillus su*Bti*lis EA-CB0015. J. Nat. Prod. 76, 503–509. https://doi.org/10.1021/np300574v

Villegas-Escobar, V., Rey, V., Mosquera, S., Gutierrez, J., Sánchez, D., 2016. Incorporation of a microbial fungicide into a chemical fungicide program for the control of black sigatoka disease in banana plants. Biol. Integr. Control plant Pathog. 117, 74–78.

Wang, J., Su, B., Jiang, H., Cui, N., Yu, Z., Yang, Y., Sun, Y., 2020. Traditional uses, phytochemistry and pharmacological activities of the genus Cinnamomum (Lauraceae): A review. Fitoterapia 146, 104675. https://doi.org/10.1016/j.fitote.2020.104675

Wang, N., Shao, X., Wei, Y., Jiang, S., Xu, F., Wang, H., 2020. Quantitative proteomics reveals that tea tree oil effects Botrytis cinerea mitochondria function. Pestic. Biochem. Physiol. 164, 156–164. https://doi.org/10.1016/j.pestbp.2020.01.005

Wharton, P., Diéguez-Uribeondo, J., 2004. The biology of Colletotrichum acutatum. An. del Jardín Botánico Madrid 61, 3–22. https://doi.org/10.3989/ajbm.2004.v61.i1.61

Wiegand, I., Hilpert, K., Hancock, R.E.W., 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat. Protoc. 3, 163–175. https://doi.org/10.1038/nprot.2007.521