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Eco-friendly fungicide based on chitosan and pecan nut oil: development and evaluation in anthracnose control

Gabriela Xavier Giacomini[1], Glaucia de Figueiredo Nachtigal[2], Carlos Roberto Martins[2], Adriane Roedel Hirdes[1], Ricardo Alexandre Valgas[2] and Aline Joana Rolina Wohlmuth Alves dos Santos[1*]

[1]Centro de Ciências Químicas, Farmacêuticas e Alimentares, Universidade Federal de Pelotas, Campus Universitário, 96160-000, Capão do Leão, Rio Grande do Sul, Brazil. [2]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil. *Author for correspondence. E-mail: alinejoana@gmail.com

ABSTRACT. Industrial processing of pecan nuts results in edible oil – with fractions of fatty acids, polyphenols and phytosterols - that may be used for managing phytopathogens. Besides, chitosan may interfere with pathogen development directly and/or activate mechanisms of defense in plant tissues. This study aimed at developing a novel, natural and eco-friendly fungicide based on pecan nut oil immobilized in chitosan and at evaluating its activity against *Colletotrichum gloeosporioides*, an agent of anthracnose in guava trees and other fruit trees. Changes in the immobilized material exhibited at bands were identified by infrared spectroscopy. Additionally, micrographs by scanning electron microscopy (SEM) showed efficient changes in the immobilized material, by comparison with the polymer chitosan. Decreases in *Colletotrichum gloeosporioides* sporulation in vitro were 37% in the case of pecan nut oil immobilized in chitosan (IO) and 39% in the case of non-immobilized pecan nut oil (NIO). These results are positive because this fungus produces a large number of spores which may disseminate and survive on surfaces of plant tissues infected by anthracnose. Immobilization of pecan oil in chitosan showed benefits in the synthesis of a novel, stable and eco-friendly material which may be applied to guava trees to control anthracnose.

Keywords: *Colletotrichum gloeosporioides*; guava tree; *in vitro*; antimicrobial; biological activity.

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Introduction

The pecan nut tree (*Carya illinoinensis* (Wangenh.) K. Koch) (Jungladaceae), which is native to the United States and Mexico, has been predominantly grown in temperate regions in the northern hemisphere (Walker et al., 2016). Its popularization triggered its cultivation in several countries in different continents, not only in China, South Africa and Australia, but also in South American countries, such as Uruguay, Argentina, Chile, Peru, and Brazil (Wells, 2017). In Brazil, pecan nut crops stretch over temperate regions in southern Brazil.

Pecan nuts have beneficial effects on health due to their unsaturated fats, proteins, fibers, vitamins (folic acid, niacin and vitamin E), minerals (magnesium, potassium and calcium), phytochemicals (flavonoids, carotenoids and phytosterols) and compounds with cardioprotective, anticarcinogenic, anti-inflammatory and antioxidant properties (Kornsteiner, Wagner, & Elmadfa, 2006).

Pecan nut production aims mostly at consumption *in natura* and in processed forms, but pecan nuts have also been added to yogurt, dairy beverage, ice cream and other products from the dairy industry. In industries, pecan nut pressing results in oil, while its by-product – husks – is sold to the tea market (Oro, Ogliari, Amboni, Barrera-Arellano, & Block, 2008). The edible oil has compounds with high added value that represent between 69 and 79% of total fruit weight, depending on the variety and cultivation conditions (Bouali et al., 2013). It has fractions of phytosterols, mainly b-sitosterol (about 64%), followed by stigmasterol (16.8 - 21.74%) and campesterol (8.4 - 8.7%) [11], besides being rich in fatty acids (around 50%) and phenolic compounds with antioxidant activity (Atanasov et al., 2018).

Organic compounds, such as fatty acids, compose the class of molecules involved in energy storage, membrane structuring and several basic processes, such as activity on cell membrane phospholipids (Avis, 2007; Liu et al., 2008). They interfere with the stability of fungal and bacterial microorganisms and are able to control diseases (Pohl, Kock, & Thibane, 2011; Hulankova & Borilova, 2020).

Even though antioxidant and antimicrobial activities of extracts from pecan nut tree husks and leaves have already been reported to manage different bacterial strains (Prado et al., 2014) associated with high levels of fatty acids, polyphenols and phytosterols, the use of pecan nut oil in phytopathogen management has not been deeply studied (Kornsteiner et al., 2006). Thus, it provides opportunities for innovation.

Another compound that has shown promising activity against fungal diseases is chitosan (Kritchenkov et al., 2021; Mehta, Mahajan, & Hivrale, 2021). This natural polymer may carry out two functions, i. e., it interferes directly with pathogen development and/or activates mechanisms of defense in plant tissues (Liu et al., 2017; Liu et al., 2008). Chitosan is capable of either bonding or interacting with metal ions and organic molecules. Besides, when it is applied to plants, it may exhibit eliciting capacity, thus, interfering with and helping plants to yield toxins and degrade enzymes that act against microorganisms which cause diseases (Zahid et al., 2014). Concerning the use of chitosan as a support to immobilize compounds, it should be highlighted that it is capable of releasing them fractionally, slowly and durably on plant surfaces (Verma, Kumar, Das, Randhawa, & Chamundeeswari, 2020).

The use of natural compounds, such as pecan nut oil and chitosan, to formulate products of interest is a little aggressive strategy for human health and the environment. The search for products that employ antimicrobial bioactivity and/or the ability to elicit plant defense, for example, against *Colletotrichum gloeosporioides*, the etiological agent of anthracnose in guava trees, may be interesting since it meets environment-friendly requirements (Moraes, Tanaka, & Júnior, 2013).

Anthracnose has been considered one of the most severe diseases in guava trees since the fungus that infects plants and fruit in different developmental stages, mainly in hot and humid environment, causes significant damage throughout blooming, maturation and post-harvest stages. The disease is also responsible for significant productivity losses in other crops, such as mango (Chala, Getahun, Alemayehu, & Tadesse 2014; Feng et al., 2019; Li et al., 2019; Tovar-Pedraza et al., 2020), passion fruit, apple (Khodadadi et al., 2020), olives (Rodrigues et al., 2019), pepper (Khalimi, Darmadi, & Suprapta, 2019) and pomegranate (Cara et al., 2020). They have been reported in several countries, such as Ethiopia (Chala et al., 2014), Mexico (Tovar-Pedraza et al., 2020), China (Li et al., 2019), Indonesia (Khalimi et al., 2019), and United States of America (Khodadadi et al., 2020). Regarding post-harvest diseases, prevention of infections in fields is an interesting strategy because they may also occur in young fruit and keep quiescent up to maturation (Nachtigal, Martins, Nachtigal, & Giacomini, 2017).

This study aimed at developing a novel, natural and eco-friendly fungicide based on pecan nut oil immobilized in chitosan and at evaluating its activity against *Colletotrichum gloeosporioides*, a causative agent of anthracnose in guava trees and other fruit trees (Figure 1).

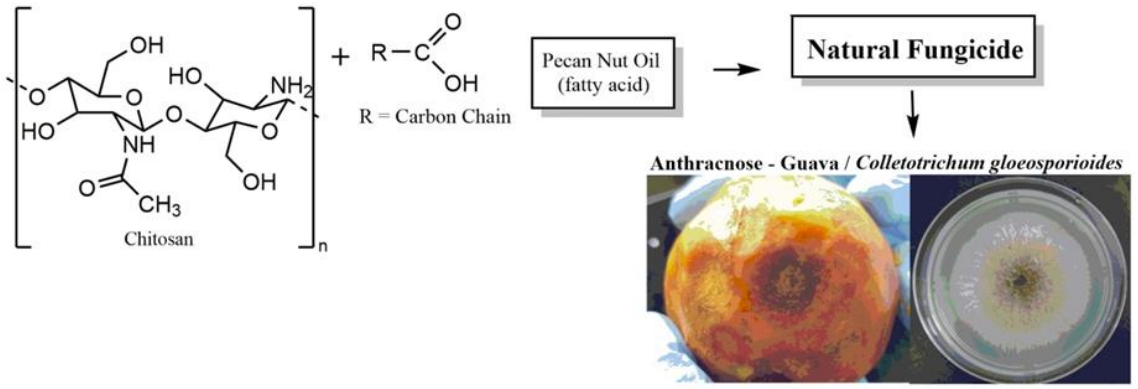

Figure 1. Development of a natural fungicide against *Colletotrichum gloeosporioides* in fruit trees. Degree of deacetylation of chitosan is above 50%.

Material and methods

Pecan nut oil was purchased at Pecanita Alimentos, Cachoeira do Sul, Rio Grande do Sul (RS), Brazil (lot 161PT25K12), while chitosan (molecular mass: 106.104 g.mol^{-1}; degree of deacetylation: 95%) (lot STBG8481) was provided by Sigma-Aldrich. Glacial acetic acid P.A. and Tween 80 (polysorbate 80) were bought from Vetec/Química Fina-Brazil.

Colletotrichum gloeosporioides lineage was got from the Collection of Microorganisms of Interest to the Biological Plague Control that belongs to the Embrapa Clima Temperado (EMBRAPA-Brazil) (access code

CPACT651). It was collected from guavas infected with anthracnose. The colony was kept in PDA (potato, dextrose, and agar) medium at 4ºC.

To prepare the immobilized (IO) material, pecan nut oil (1.53 mL) was mixed with chitosan solution 1.3% (m:v) (Fráguas et al., 2015) and acetic acid 0.25% (v:v) was the solvent. Preparation of non-immobilized (NIO) material was carried out with a mixture of pecan nut oil (1.53 mL) with sterile water (100 mL). Tween 80 (0.046% (v:v)) was added under constant stirring to both to improve emulsification.

Chemical composition of pecan nut oil was determined by GC-MS (70 eV) in an electron-impact Shimadzu, model QP2010, equipped with a chromatographic column RTx-5 (5% phenyl group and 95% dimethylpolysiloxane) which was 30 m long, 0.25 mm in internal diameter and 0.25 µm in film thickness. Heating intervals ranged between 50 and 290ºC at heat ratio of 10°C, keeping maximum temperature for 5 minutes. Interface temperature among the pieces of equipment was 290°C. Then, 1 µL of the solution of pecan nut oil diluted with hexane in the ratio of 1:50 in the splitless mode was injected into the chromatographic column. Helium was the carrier gas. Ionization of compounds found in pecan nut oil occurred by electron impact (EI) (70 eV), while a mass detector was used for generating the chromatogram. Identification of components, mainly the profile of fatty acids, was based on the comparison between relative retention times and fragmentation patterns of mass spectrum and those reported by the literature and data provided by the NIST 08 Software Program.

The analysis of spectroscopy in the infrared region was carried out by a Shimadzu, model IRAffinity-1, to explain structural changes in both IO and NIO material, by comparison with chitosan in dry samples in an oven at 50°C for 24 hours. The analysis was conducted in the region from 400 to 4000 cm^{-1} by a Fourier Transform and infrared attenuated total reflectance.

Analyses of scanning electron microscopy (SEM) were carried out to evaluate material surfaces. Fragments that were about 1 mm in diameter were removed and mounted on stubs with double-sided tape and coated with gold. Images of surfaces were obtained with 1000x (10 µm), 5000x (5 µm), and 8000 x (2 µm) resolution and speed of 10 KeV. The energy-dispersive spectroscopy (EDS) analysis was carried out by a Jeol JSM - 6610 coupled with a Thermo Scientific detector, so as to determine qualitative chemical composition of the material.

In vitro toxicity of IO and NIO against vegetative and reproductive structures of *Colletotrichum gloeosporioides* was determined by bioassays. Firstly, guava fruits with anthracnose symptoms were chosen and immersed into sterilized water. After asepsis, infected portions (0.5 cm in diameter) were cut and placed in Petri dishes containing PDA medium. Dishes were incubated at 25ºC for a 12-hour photoperiod. After 7 days, there was fungal growth and mycelial disks (5 cm in diameter) were cut. Then, IO and NIO aliquots of 100 µL were deposited and spread on the surface of PDA medium in Petri dishes (9 cm in diameter) (Seixas, Castro, Santos, & Cardoso, 2011). Distilled and sterile water was used as the negative control. Mycelial disks were placed in the center of every Petri dish. The dishes were also incubated at 25ºC for a 12-hour photoperiod. Evaluation of fungal growth (vegetative structures) was carried out by daily measurements of colony diameters in two orthogonal axes. Measuring ended on the 5th day when fungal development in the negative control reached 2/3 of total surfaces of dishes. The experiment had a completely randomized design with five replicates. To evaluate reproductive structures, spore production of *C. gloeosporioides* was analyzed on the 18th incubation day. Three mycelial disks were collected on every dish and transferred to tubes with 9 mL sterile water and penicillin. After vigorous vortex agitation, spore concentration in the solution was estimated by a Neubauer chamber (Caligiore-Gei & Valdez, 2015).

In order to analyze fungal activity, linear models were adjusted to represent the expected mycelial growth on every material. Every replicate was observed throughout the evaluation period. The angular coefficient of every straight line, determined by the method of least squares, was evaluated. Afterwards, results were subject to the analysis of variance (ANOVA) and, when the F value was significant, the Tukey's test at 5% probability was applied.

Results and discussion

The GC-MS analysis of pecan nut oil enabled to identify and quantify 24 different chemical constituents (Table 1). Constituents 13 and 14 were precursors of fatty acids, the major ones (31%), while 9,12–octadecadienoic acid (21.06%) and 9–octadecenoic acid (Z) (10.43%) were precursors of linoleic and oleic acids, respectively.

Table 1. Components identified in pecan nut oil.

Signals	Components	Retention time (min.)	Area (%)
1	glycerol acetonide	4.175	15.60
2	Decane	11.756	1.00
3	Hexadecane	12.937	2.80
4	Undecane	13.488	0.72
5	stearyl vinyl ether	13.592	0.52
6	Eicosane	14.059	3.73
7	2,6,10,14-tetramethylheptadecane	14.119	1.54
8	Tetracosane	15.125	3.30
9	Nonadecane	15.221	1.42
10	Octacosane	16.141	3.14
11	14-methylpentadecanoic acid, methyl ester	16.415	7.58
12	Heptacosane	17.109	2.29
13	9,12–octadecadienoic acid, methyl ester	18.019	21.06
14	(Z)-9–octadecenoic acid, methyl ester	18.070	10.43
15	heneicosanoic acid, methyl ester	18.298	4.28
16	oleic acid	18.666	0.84
17	2–hexyl–1–decanol	18.921	1.20
18	1–eicosanol	19.387	0.39
19	2–hexyl–1–octanol	19.776	1.65
20	2–oxo–octadecanoic acid, methyl ester	20.587	0.63
21	(z)–9–octadecanal	20.855	1.23
22	2–methyl–Z,Z–3,13–octadecadiol	21.229	10.28
23	1,2–benzenedicarboxylic acid, ditridecyl ester	21.800	0.72
24	Squalene	23.801	3.61

Ryan, Galvin, O'connor, Maguire, and O'brien (2006) and Salvador, Podestá, Block, and Ferreira (2016) reported that oleic acid was the most abundant fatty acid found in pecan nuts, followed by the linoleic one. Besides, the authors described how derivatives of both linoleic and oleic acids regulate fungal development, even though there is little knowledge about their mechanisms of action and about the activity of oleic acid and its derivatives against the fungus *Magnaporthe grisea* which is found in rice plants.

The Fourier-transform infrared spectroscopy (FTIR) analysis (Figure 2, Table 2) showed changes in spectrum bands that give evidence of pecan nut oil immobilization in chitosan by electrostatic interaction among oil constituents and protonated $-NH_2$ groups of chitosan. NIO (Figure 3c) shows characteristic bands of pecan nut oil, i. e., of the mixture of its constituents, such as: 3410 cm^{-1} attributed to [ν(O–H)], from 3006 to 2841 cm^{-1}, which refer to [ν(C–H)] of aromatic and non-aromatic carbons, in this order, 1740 cm^{-1} and 1610 cm^{-1} attributed to carbonyl groups [ν(C=O)], which exist in carboxylic acids and ketones, respectively, 1036 cm^{-1}, which refer to ν[C–O] of alcohols and ethers (Prado et al., 2013; Dalir, Djahaniani, Nabati, & Hekmati, 2020) and 720 cm^{-1} which refer to [δ(C–H)] of aromatic rings of constituents found in pecan nut oil (Chacón-Garza, Losoya-Sifuentes, & Flores-Chávez, 2016).

Chitosan (Figure 1a) has characteristic and specific bands of groups found in chitosan, β-(1-4)-D-glucosamine (Saharan et al., 2015; Chacón-Garza et al., 2016), such as: broad band in the region of 3364 cm^{-1} [ν(O–H)] overlapping [ν(N–H)], 2917 cm^{-1} [ν(C–H)], 2856 cm^{-1} [ν(C–H)] of non-aromatic carbons, 1734 cm^{-1} [ν(C=O)] (amide I), 1640cm^{-1} [δ(N–H)] (amide II), 1450 cm^{-1} [δ(C–H)], 1322 cm^{-1} [ν(C–N)] and wide band at 1066 cm^{-1} [ν(C–O)]. The fact that groups, such as amino ($-NH_2$) and hydroxyl (–OH), are found in the polymeric structure of chitosan enables chemical and/or physical modifications by insertion of cross-linkers, which promotes changes in the structure and in physico-chemical properties of the polymer (Argüelles-Monal, Lizardi-Mendoza, Fernández-Quiroz, Recillas-Mota, & Montiel-Herrera, 2018). Thus, insertion of oil in chitosan shows modifications in bands in the case of IO material. A band in the region of 3006 [ν(C–H)] shows evidence of the incorporation of pecan nut oil, with its aromatic carbons, in chitosan (Figure 1b). In addition, IO material exhibited decrease in intensity on chitosan bands in both regions 1640 cm^{-1} [δ(N–H)] (amide II) and 1450 cm^{-1} [δ_s(C–H)]; it shows electrostatic interaction among oil constituents and protonated $-NH_2$ groups of chitosan (Santos et al., 2016), besides intermolecular interaction among oil constituents and –CH groups of chitosan. The band associated with [ν(C=O)] stretching in IO material is shown in the region of 1742 cm^{-1}, very close to the one that was found for the oil; it was shifted to higher wavenumbers, by comparison with chitosan. The band at 1450 cm^{-1} [δ(C–H)] in the chitosan spectrum is shifted when the oil is incorporated into chitosan, towards higher wavenumber 1464 cm^{-1}. According to Pereira et al. (2019), the fact

that bands appear and shift may be evidence of interaction among functional groups, such as the incorporation of essential oil from clove in the chitosan membrane.

In 1162 cm^{-1}, a band for IO (Figure 2b) and NIO (Figure 2c) material shows that there is immobilization of oil in chitosan. This band has been reported by Chacón-Garza et al. (2016) in different varieties of pecan nut oil in the region of 1160 cm^{-1}, attributed to ν[C–O]. Prado et al. (2013) also showed characteristic bands of ν[C–O], which referred to carbohydrates and phenolic compounds, in pecan nut oil and husks, in the region that ranged between 1000 and 1400 cm^{-1}. In pecan nut extract, Dalir et al. (2020) identified bands in the regions between 1370 and 1055 cm^{1}, which are related to ν[C–O], ν[C–N] and ν[C–C] of phenolic compounds, aliphatic amines and alkanes, respectively. According to Sánchez-Acosta et al. (2019), bands in these regions are characteristic of phenols and aromatic compounds and evidence of tannins. Another band in IO and NIO, which shows that immobilization of oil in chitosan was successful, appears in 720 cm^{-1} [δ(C–H)] and refers to aromatic rings found in constituents of pecan nut oil. Finally, a wide band appears in chitosan, in the region of 1034 cm^{-1}, which is shown in IO shifted to higher wavenumbers. It proves that formation of the material by immobilization of oil in chitosan was successful.

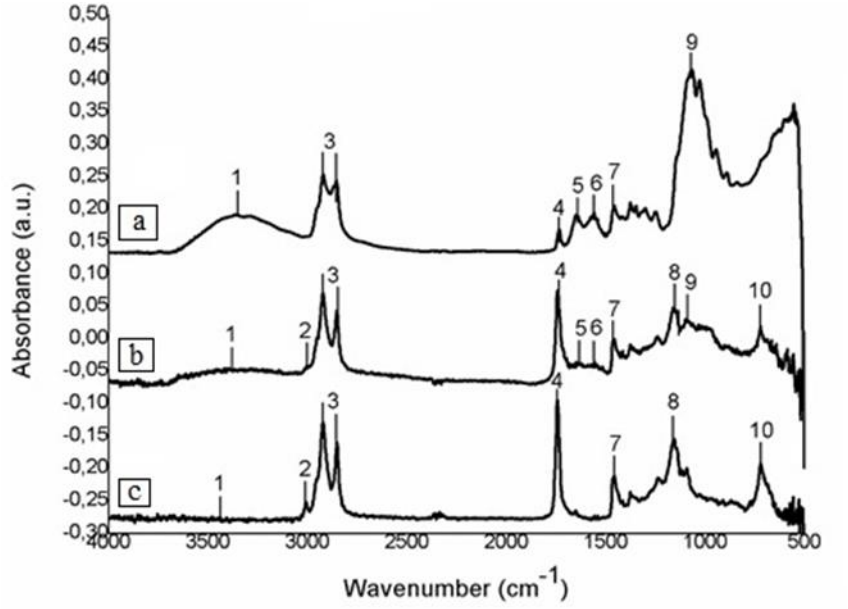

Figure 2. FTIR spectra (ATR). a) Chitosan; b) IO; and c) NIO.

Table 2. Main bands in infrared regions (cm^{-1}).

Signals	Bands	Samples		
		Chitosan	IO	NIO
1	[ν(O–H)]	3364	3378	3410
2	[ν $(C–H)_{arom}$]	-	3006-2921	3006-2930
3	[ν(C–H)]	2856	2848	2841
4	[$ν(C=O)_{Amide\ I}$]	1734	1742	1740
5	[$δ(N–H)_{Amide\ II}$]	1640	-	-
6	[$δ_s$(C–H)]	1450	-	-
7	[ν C–N)]	1322	1378	1372
8	[$ν(C–O)_{Oil}$]	-	1162	1162
9	[$ν(C–O)_{Chitosan}$]	1066	1082	-
10	[$δ(C–H)_{arom}$]	-	720	720

SEM analyses showed considerable change in the micrograph pattern after immobilization of oil to form IO material, by comparison with chitosan (Figure 3). Micrographs of IO showed efficient cross-linking and interaction among constituents in the polymeric matrix since neither cracks nor dents were found in the magnitude under investigation. The result shows the development of coated material with antifungal activity and/or the possibility of becoming a product to manage *C. gloeosporioides* in field conditions. Surfaces with no pores and cracks may ensure more stable, long-lasting and efficient coating of a product in the plant, since little fissures and cracks may affect its protection (Muzzarelli, Stanic, Gobbi, Tosi, & Muzzarelli 2004).

Coating of vegetative and reproductive structures which are susceptible to infection caused by pathogens under normal conditions in fruit farming may ensure decrease in the number of applications needed throughout critical phases of occurrence and expansion. It should be highlighted that anthracnose has been considered one of the most severe diseases in fruit borne by guava trees, since it leads to significant damage in flowering, maturation and post-harvest phases (Nachtigal et al., 2017).

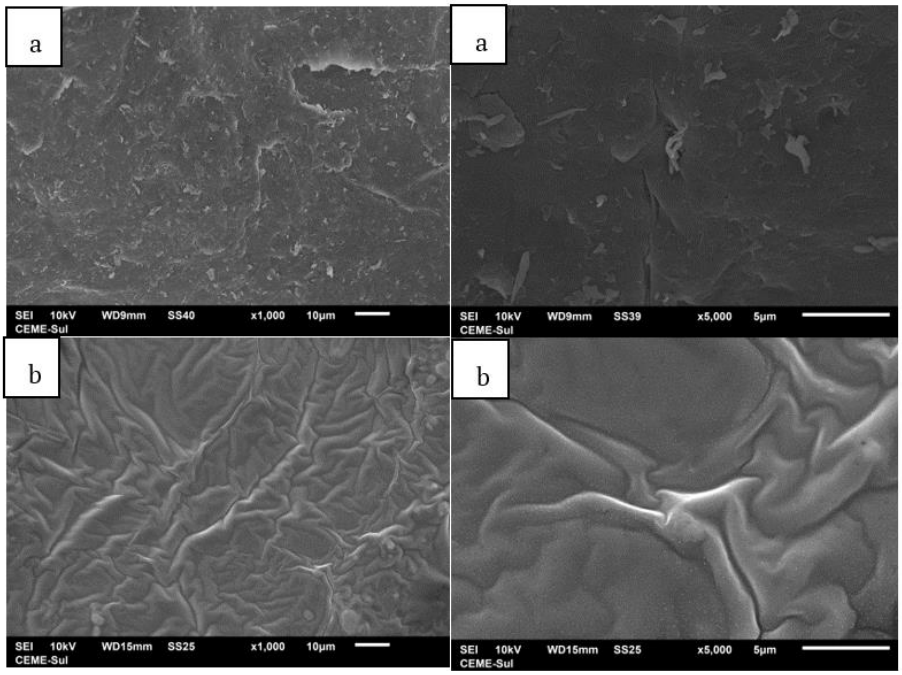

Figure 3. SEM micrographs in 10 µm (left) and 5 µm (right) magnitudes. a) Chitosan; and b) IO.

To complement FTIR and SEM analyses, the EDS spectrum of IO material (Figure 4) showed qualitative demonstration of its chemical composition, with carbon and oxygen which referred to chemical structures of their precursors, chitosan $(C_6H_{11}O_4N)_n$, β-(1-4)-D-glucosamine and pecan nut oil (Saharan et al., 2015).

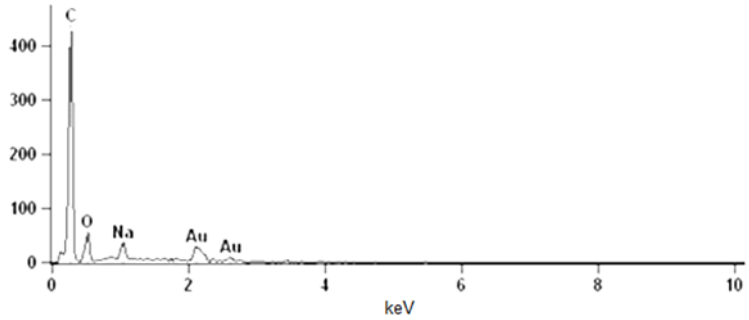

Figure 4. EDS spectrum of IO material.

Evaluation of antifungal potential of both NIO and IO material did not show any antifungal activity in mycelial growth inhibition of *Colletotrichum gloeosporioides*, by comparison with the negative control (Table 3). It partially agrees with reports found in the literature, such as the one published by Liu et al. (2008), who evaluated antifungal activity of different fatty acids, such as oleic and linoleic acids, which are the major constituents of pecan nut oil. No significant inhibitory effect on mycelial growth was found for oleic acid, while, for linoleic acid, decrease of 14-19% was observed in growth of different fungus species: A*lternaria solani, Fusarium oxysporum* f. sp. *Cucumerinum* and *Fusarium oxysporum* f. sp. *lycopersici*.

A proposition of mechanism of action for NIO and IO material, more specifically for pecan nut oil, mainly because of fatty acids, refers to their activity on phospholipids in cell membranes of microorganisms, since disturbances caused to their structures tend to be fatal to cell integrity and functioning (Avis, 2007). Fatty acids, such as oleic and linoleic ones, enter lipid bilayers of fungal membranes and cause increase in

membrane permeability; it results in overflow of intracellular content and, consequently, cell death (Avis, 2007; Pohl et al., 2011). Although increase in oxidative stress caused by incorporation of polyunsaturated lipids into membranes may contribute to antifungal activity of fatty acids, this fact does not seem to support data shown by this paper, since it may be the influence of the chemical composition of fatty acids in pecan nut oil and of the pH of the medium used in antifungal evaluation (Altieri, Bevilacqua, Cardillo, & Sinigaglia, 2009). Since knowledge about direct mechanisms of action of fatty acids on vegetative structures of fungi is still very incipient, it cannot be disregarded that they interact with other compounds found in the oil to hinder and minimize its activity. It may also be said about IO material which results from immobilization of pecan nut oil in chitosan. Despite being desirable in the light of application of the product on the field – because it provides mass yield to the resulting product –, it did not favor direct activity of its constituents on vegetative fungal structures.

Regarding the evaluation of antifungal potential under *C. gloeosporioides* sporulation, similar performance was found for IO and NIO, with decrease of 37 and 39% in sporulation, respectively, by comparison with the negative control (Table 3). Lack of effectiveness of IO and NIO material on vegetative structures of fungi and their limited effectiveness on reproductive structures (Table 3) suggest that other mechanisms of action should be explored. Decrease in sporulation is positive in the performance of IO and NIO since a characteristic of this fungus is the production of a large number of spores that may disseminate and survive on surfaces of infected plant tissues (Nachtigal et al., 2017).

Table 3. Effect *in vitro* on sporulation of *Colletotrichum gloeosporioides*.

Material	Sporulation (spores mL^{-1}) ± SEM	Sporulation inhibition (%)
Negative control	159.60 ± 23.409 a[§]	-
IO	100.40 ± 16.167 b	37
NIO	97.400 ± 12.738 b	39

[§]Different letters show statistical difference by the Tukey's test.

Another mechanism that may explain low and limited efficiency of both IO and NIO material on inhibition of mycelial growth and sporulation, respectively, involves the possibility of induction of plant systemic resistance to the fungus against pecan nut oil, a fact that demands further investigation. When induction of systemic resistance may happen, in the case of IO material, chitosan, as an antifungal agent (Xu, Zhang, & Liu, 2020), should be considered, since it has already been evaluated in *Colletotrichum* in papaya and as an inducing agent of plant defense mechanisms against the fungus (Ali et al., 2014; Zahid, Maqbool, Siddiqui, Manickam, & Ali, 2015). The literature states that induction of plant resistance may be detected by increase in activities of enzymes, such as chitinase, β-1,3-glucanase and phenylalanine ammonia lyase (PAL), and in total phenols (Maqbool, Zahid, Ali, & Singh, 2016). Resistance may be able to control anthracnose in pitaya (Zahid et al., 2014), mainly when particles of chitosan, smaller than 600 nm and with low viscosity, potentialized their entry into tissues faster (Zahid, Maqbool, Alib, Siddiqui, & Bhattid, 2019). Material that was synthesized and characterized by this study established a new perspective which is based on this premise, since it did not exhibit the expected antifungal results due to possible induction to resistance. In addition, the eliciting capacity of chitosan (Motelica et al., 2020), which could have potentialized effectiveness of pecan nut oil and directly interfered with *C. gloeosporioides* development, did not occur significantly. This mechanism of inhibition should be studied in order to develop an efficient *in vitro* fungicide that aims at *C. gloeosporioides* management, with positive results in the field to farmers, consumers and the environment.

Conclusion

Pecan nut oil immobilized in chitosan (IO) and non-immobilized (NIO) decreased *Colletotrichum gloeosporioides* sporulation in 37% and 39%, respectively. It is a positive result since a characteristic of this fungus is the production of a large number of spores that may disseminate and survive on surfaces of infected plant tissues. IO showed benefits in the synthesis of a novel, stable and eco-friendly material which may be applied to guava trees to control anthracnose and may show more benefits than NIO, such as, better covering and fixation on guava trees and increase in yield in crop application, due the polymer introduction.

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