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Research Article

A Versatile Bio-packing Based on Natural Extract from *Calotropis procera* a Sustainable Alternative for Agriculture and Biofouling Applications

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Abstract: Background and objective: Despite the situation in the north of Minas Gerais being semiarid, this region presents a plant with low water demand, *Calotropis procera*. This plant has a great potential as an antifungicide supply, although other important characteristics. In this context, this research proposes an extraction of natural material from *C. procera* to produce a bio-packing for agriculture and anticorrosive applications as a sustainable alternative. This bio-packing is eco-friendly, low-cost, widely available, and quickly produced. The antifungicide and anticorrosive activity were evaluated as the control of fruit ripening based on *in vivo* tests. **Materials and methods:** This research is based on the production of bio-packing based on natural extracts from *C. procera* seeds for agricultural and anticorrosive applications. The material was characterized by Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR) and surface contact angle. In addition, *in vitro* and *in vivo* biological experiments were performed to analyze the antifungal activity and control of fruit ripening. Moreover, the anticorrosive property was also analysed. **Results:** The results indicated the presence of proteins in the composition of the product based on natural extract, which may contribute to the antifungal and anticorrosive characteristics. Furthermore, the natural extract demonstrated anticorrosive protection in simulated seawater. The *in vivo* analysis of bananas showed that the extract concentration of 50 $\mu L\ mL^{-1}$ presented the best results, controlling the fruit ripening and protecting against diseases. **Conclusion:** Thus, for the first time, these products extracted from *C. procera* seeds presented a great future for applications as a versatile action for agriculture and anticorrosive applications.

***Keywords*:** biomolecules, antifungicide, anti-biofouling, sustainable agriculture

1. Introduction

Although water is the most important element of nature and is crucial for all animal life, at the same time, disposable water is the biggest factor limiting agricultural productivity [1-3]. In this context, the state of Minas Gerais in

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Brazil has been facing a great water crisis for the past few years, which has deteriorated very quickly and has left over 180 cities in an emergency state, the semiarid region [4]. Aggravating this scenario, the semiarid region has sparse rain during the year and undergoes desertification, which improves their sense of the water crisis. Some cities were at risk of having their main economic activities disrupted due to water shortages [4]. For this reason, new approaches to agro-industry are developed and measures are taken to expedite the implementation of projects for sustainable agriculture processes.

Banana is one of the main fruit linked to the international agribusiness due to its worldwide consumption. Its production generates approximately US$ 5 billion/year and Brazil is the second-largest producer [5]. This culture is very lucrative for Brazilian agriculture as it is developed throughout the national territory, having great socioeconomic and cultural importance [5]. Just the north of Minas Gerais state produces over 346 tons in an area of 16,000 hectares [6]. However, despite the high production, several factors make production difficult, such as the water crisis and many types of fungi that hinder fruit storage [6].

Many fungi provoke terrible loss to the plantation, this loss is close to 50% of the plantation [7, 8]. One of these fungi that cause many problems to the plantation is *Colletotrichum musae* (known as the anthracnose disease). Just the north of Minas Gerais, there is a loss of 30 to 40% of marketable fruit [8]. Moreover, during the fruit exportation and transportation, a major loss to the fruit was due to the fungal infection or by fast maturation of the fruit even before reaching its destination. Hence, it is essential to control the fruit ripening during transportation, mainly considering the parameters established by some regulatory bodies, as injuries and the degenerative process cannot over 10% of the fruit [9, 10].

Despite the situation of the north of Minas Gerais being semiarid, this region presents a plant with low water demand, *Calotropis procera* (Asclepiadaceae). This plant has great potential as an antifungicide among other important characteristics. *C. procera* is a plant much common in semiarid regions, majorly from Asia (Afghanistan, Arabia, India, Iran and Pakistan) but growing around the world in semiarid and arid regions. This species grows with enormous facility and produces fruits around the year. In addition, it can be successfully grown in arid, degraded, and nutrient-poor soils [11]. In some areas, the dry biomass of *C. procera* is used as animal feed, in addition, some studies reported that *C. procera* accumulates several chemical elements [12-14]. Furthermore, its antifungal property has already been studied in several types of fungi, such as *Alternaria alternata* [14], *Candida albicans* [15], *Epidermophyton floccosum* [16], *Tricophyton gypseum* [16], *Fusarium oxysporum* [17] and *Colletotrichum dematium* (URM 3315) [18]. Moreover, fungal attacks are very difficult to control and the development of materials that promotes excellent protection against these fungal diseases as well as making it eco-friendly is extremely crucial. In addition, although the literature has already indicated the antifungal activity of *C. procera*, as reported by Amini et al. [13], there is a lack of comprehensive antimicrobial characteristics of *C. procera*.

Although various pieces of research in the literature are based on bio-packings produced by many methods [19-21], to the authors' best knowledge, the antifungal property of *C. procera* seed against *C. musae* as well as the evaluation of *in vivo* tests have not yet been published, demonstrating that its potential of not only for disease control but also to control the fruit ripening, a significant parameter for fruit storage and transport, in addition to anticorrosive properties, is being studied for the first time.

In this context, this research proposes an extraction of natural material from *C. procera* seed by aqueous route to produce a bio-packing for agriculture and anticorrosive applications as a sustainable alternative. This bio-packing is eco-friendly, low-cost, widely available, and quickly produced. The antifungicide and anticorrosive activities were also evaluated to control fruit ripening via *in vivo* tests. In addition, an extensive characterization of bio-packing was performed using different spectroscopy analyses, Bradford, hydro-distillation extraction, and its toxicity was evaluated. The results showed that this bio-packing has excellent potential for agriculture and anti-biofouling in seawater applications.

2. Materials and methods

Study area: The natural extract was obtained from the seeds of *C. procera*. The seeds of *C. procera* were collected at 15° 48' 10" S 43° 18' 32" W in the Janaúba municipality in the north of Minas Gerais, Brazil, in different seasons of a year (summer, spring, autumn and winter, in the months of December, October, June and April, respectively).

Seeds processing: The seeds were packed, crushed using a knife mill (MA350, Marconi, Brazil) and macerated with 1 L of deionized water using an industrial blender. The natural materials were dialyzed against deionized water for 7 days at room temperature (RT) to remove unreacted species. Finally, they were put into a rotary evaporator at 45 ± 2 ˚C to remove all remaining water and stored in a refrigerator at 4 ± 2 ˚C for further use. Fourier transform infrared (FTIR) spectra using an attenuated total reflectance method (Nicolet 6700, Thermo Fisher, United States) and nuclear magnetic resonance (NMR) spectroscopy to the hydrogen nucleus (^{1}H NMR) with spectrometer (Bruker 400 MHz, Bruker, United States; Varian 400 MHz, Varian, United Kingdom) to characterize the natural extract. Moreover, the hydrophilic/hydrophobic characteristics were performed by contact angle measurements as this parameter may indicate whether the bio-packing has a greater affinity with water, and there is a greater proliferation of microorganisms in more humid environments [22, 23]. Furthermore, *in vitro* antifungicide analysis was performed against *C. musae* isolated from bananas. The antifungal analyses were produced using four different concentrations (25, 50, 75 and 100 µL mL^{-1}) diluted in deionized water. Finally, the antifungal activity was evaluated at 1, 2, 3, 4 and 5 days. In the case of *in vivo* analysis, unripe bananas were chosen as the fruit model. The bananas previous prepared were immersed in different concentrations, similarly to *in vitro* tests. The ripening and antimicrobial activities were evaluated at 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days. The anticorrosive properties were evaluated using steel nails that were properly prepared and immersed into the product for 5 days. In sequence, a corrosive medium was prepared using agar diffusion and 1.0 mol L^{-1} of sodium chloride (NaCl), simulating the seawater medium. Finally, the anticorrosive property was evaluated at 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days.

3. Results and discussion

Figure 1 shows all the chemical groups involved in the bio-packing developed from *C. procera* seeds by infrared spectroscopy (A) and NMR (B). The band at 1717 cm^{-1} corresponds to carbonyl (C=O) groups which may be related to xylan components of hemicellulose and amino acids in protein backbone chains, e.g. glutamine was visualized in the spectra in the infrared analysis. In addition, bands observed between 2950 - 2830 cm^{-1} were associated with methylene group (CH_2) vibrations which may be related to groups in threonine (Thr) presented into serine/threonine-protein kinase, as bands related to in-plane scissoring of the methyl group (CH_3) in the Thr at 1374 - 1396 cm^{-1}. O-H stretching vibrations of cellulose and cellulose nanocrystal (CNC) was perceived at 3339 cm^{-1}. Adding, at 1463 cm^{-1} and 1429 cm^{-1}, bands connected to C-C stretch relating to fatty acids, Iβ crystalline cellulose of CNCs and an anomeric carbon linked to double-linked cardiac glycoside at cardenolides derivatives, such as calactin and calotoxin (Figure 1A) [15, 24].

Additionally, in the ^{1}H NMR (Figure 1B), the band connected to Thr in the serine/threonine-protein kinase was visualized at 1.258 ppm. Moreover, aliphatic chains at 1.3 - 1.0 ppm (greater δ related to α -CH_2- and β -CH_2-, and smaller δ for -$(CH_2)_{d\text{-}i\text{-}}$) linked to acyl chain protons were confirmed. Similar to FTIR spectra, bands related to CH groups in the xylan components of hemicellulose were observed at 3.513 ppm, close to 2.6 ppm, and 2.050 ppm. Furthermore, near 2.6 ppm, a signal associated with the two-chain monoclinic structure, Iβ was observed. Finally, at 4.30 ppm an anomeric carbon is perceived in relation to double-linked cardiac glycoside linked to cardenolides derivatives (calactin and calotoxin) [15, 25].

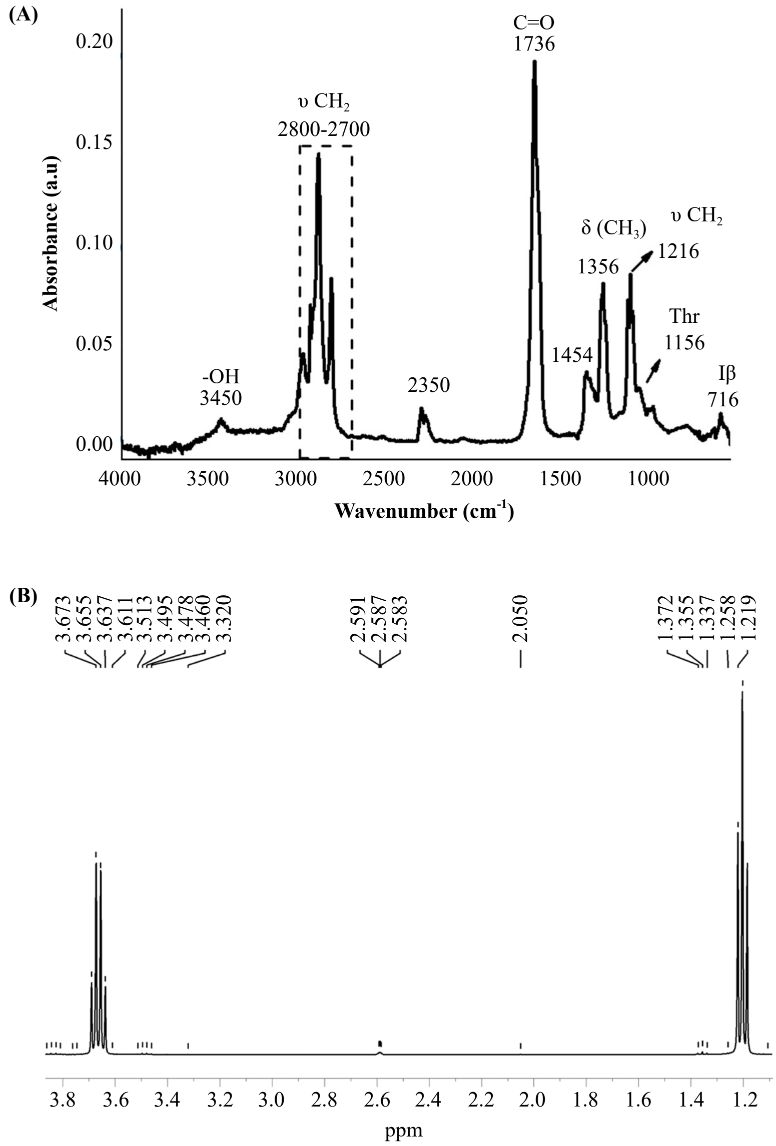
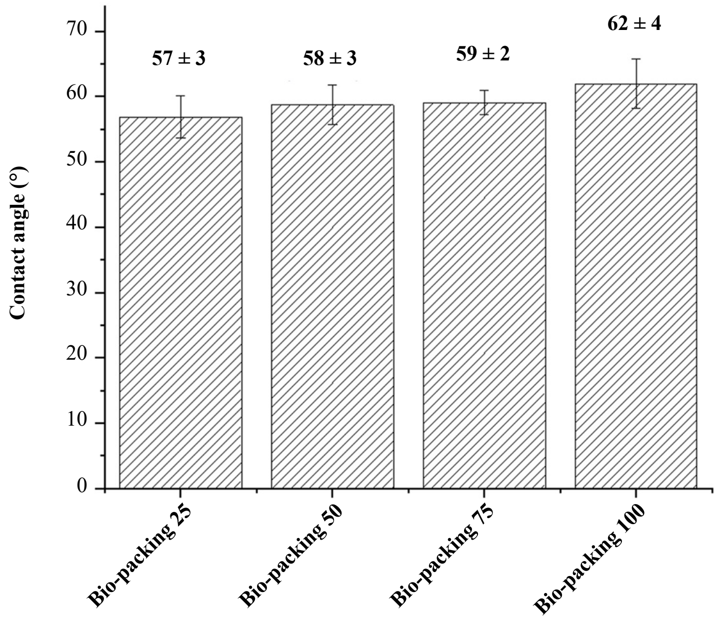


Figure 1. (A) FTIR and (B) ^{1}H-NMR spectra of bio-packing developed from *C. procera* seeds

Proper and adequate storage of the fruits during transportation, mainly for exportation, is crucial. Parameters such as temperature, humidity, etc. influence the fruit maturation. In addition, these effects conjugated with others provide a conducive environment to the emergence of many diseases, especially those caused by fungus [6]. Then, promoting hydrophobic protection is a viable solution. Figure 2A presents the surface contact angle values, indicating a hydrophobic character of bio-packing, where the *y*-axis is the contact angle of solvent used, in this case, water, indicating the hydrophobic character based on the angle (higher contact angle, $\geq 90°$, is hydrophilic; smaller, is hydrophobic), and the *x*-axis indicates the samples studied (bio-packing produce with 25, 50, 75 and 100 $\mu L\ mL^{-1}$) of natural extract from *C. procera*.

Corrosive environment, e.g. seawater, is a major issue for packaging materials, especially when carbon steel is used. Some parameters are considered crucial in steel corrosion on seawater, such as physical, chemical and biological factors in the medium [15]. Considering that, this research studied the anticorrosive protection of the material in a seawater-simulated medium, as the biological protection of the product (Figure 2B). The results showed satisfactory anticorrosive protection after 20 days, in which no corrosive process started, indicating its potential to apply as anti-biofouling.

(A)



(B)

i ii

Figure 2. (A) The surface contact angle of natural extract at various concentrations (25, 50, 75 and 100 μL mL^{-1}); (B) i) Steel nails after 20 days in corrosive medium without any protection and ii) With bio-packing protection at 25 μL mL^{-1} of natural extract concentration

In the case of *in vitro* antifungal behaviour, the reticence of fungus micellar advance was proportional to the increasing concentration, as it is perceived in Figure 3A and S1, where the *y*-axis indicates the diameter of mycelial growth of each bio-packing studied (bio-packings produce with 25, 50, 75 and 100 μL mL^{-1}) of natural extract from *C. procera* on 5th day, described by the *x*-axis, compared with control (commercial antifungicide). The concentration of 100 μL mL^{-1} demonstrated the best effect, even when compared with commercial antifungals. These results may be associated with the flavonoid increasing with the concentration, as the presence of proteins, e.g. serine/threonine-protein kinase. Differently, the *in vivo* experiments over a period of 20 days for 50 µL mL^{-1} concentration of natural extract indicate the best result. The results demonstrated that the bio-package protected the fruit against diseases and fast maturing.

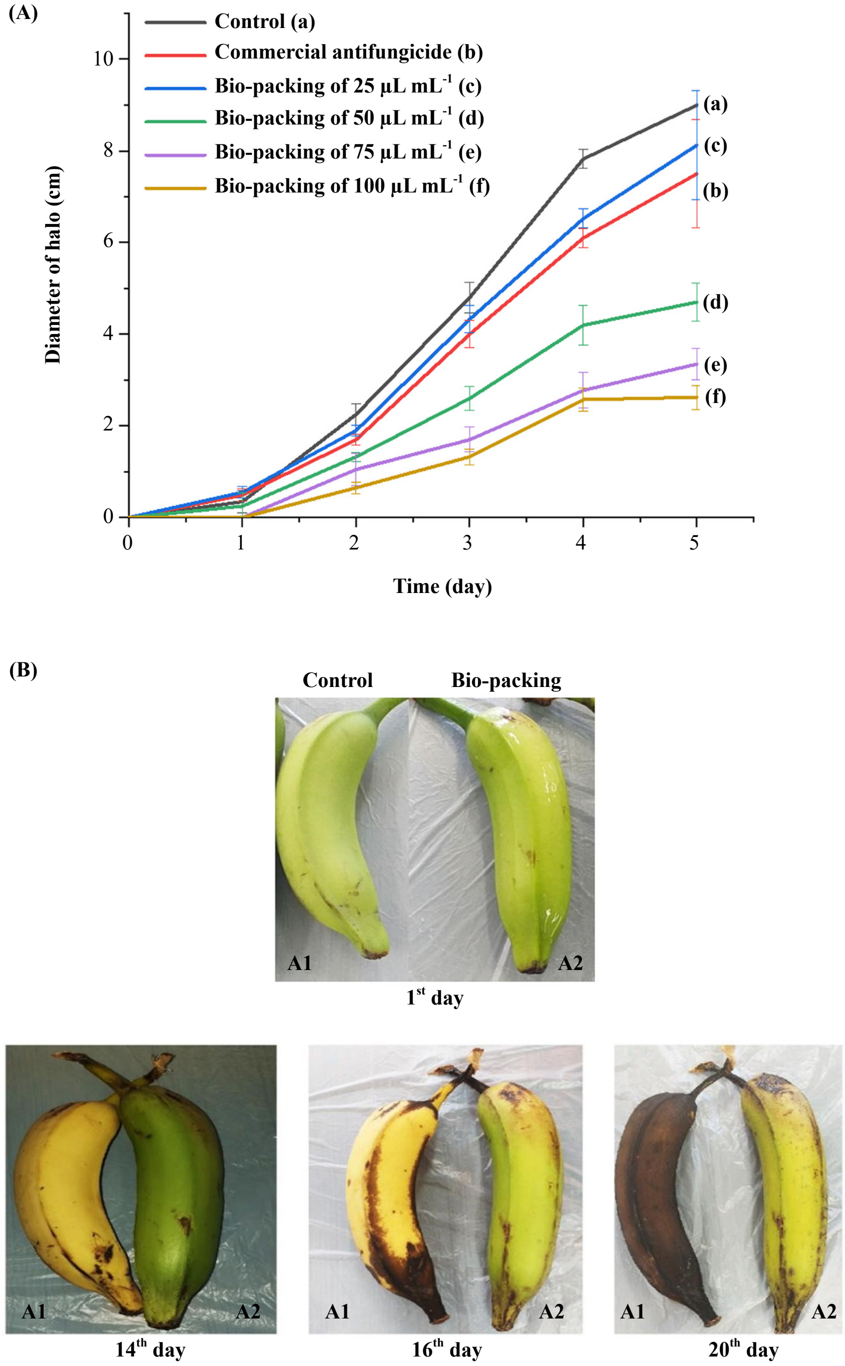


Figure 3. (A) Antifungal activity of (a) Control, (b) Commercial fungicide, (c) Natural extract at 25 µL mL^{-1} concentration, (d) Natural extract at 50 µL mL^{-1} concentration, (e) Natural extract at 75 µL mL^{-1} concentration and (f) Natural extract at 100 µL mL^{-1} concentration; (B) Images of *in vivo* experiment of 50 µL mL^{-1} concentration at 1st day, 14th day, 16th day and 20th day (A1-control samples/without bio-packing; A2-samples with bio-packing)

4. Conclusion

This work reported the production of a bio-package based on the natural extract from *C. procera* seeds as a sustainable alternative application in agriculture and anticorrosive protection. The results showed that important chemical groups may play an essential role in these functions. The presence of proteins, especially serine/threonine protein kinase, plays a crucial role in these applications. Thus, these bio-packings from *C. procera* seeds presented an outstanding potential for applying in these different areas as a versatile product.

Significance statement

Despite indisputable advances in recent decades in the field of storage, transportation, control of fruit ripening and control of diseases in the agriculture sector, the development of innovative and versatile materials which fulfil all of the requirements is a crucial challenge which needs to be overcome by researchers and professionals. In addition, many types of fruit are widely consumed in the world but around 50% is lost in each harvest, and 30 to 40% of marketable fruit is wasted because of fungal diseases which makes the development of new sustainable materials to protect these fruits crucial. Moreover, a bio-packing with excellent potential for anti-biofouling in seawater applications conjugated with applications in agricultural transport, storage and control of fruit ripening is still unknown. Thus, this research focused on the design of novel bio-packing from *C. procera* seed using a green route for agriculture and anticorrosive applications as a sustainable alternative. The results demonstrated that bio-packing may contribute to the antifungicide and anticorrosive properties. Furthermore, the *in vivo* analysis showed that bio-packing controlled the fruit ripening and also offers protection against fungal diseases. In addition, although the literature has already indicated the antifungicide activity of *C. procera*, there is a lack of comprehensive antimicrobial characteristics of *C. procera*. Additionally, to the authors' knowledge, the antifungal property of *C. procera* seed in the case of *C. musae* has not been explored, as well as the evaluation of *in vivo* tests, demonstrating its potential not only for diseases control but also to control fruit ripening, a significant parameter for fruit storage and transport. In addition, this is the first report which evaluates the anticorrosive property of the bio-package developed. Thus, for the first time, an eco-friendly bio-packing having excellent potential for agriculture and anti-biofouling in seawater applications was successfully developed.

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Author disclosure statement

The authors declare that they have no competing interests.

Author contributions

The manuscript was written through the contributions of all authors. All authors have given their approval to the final version of the manuscript.

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Supplementary material

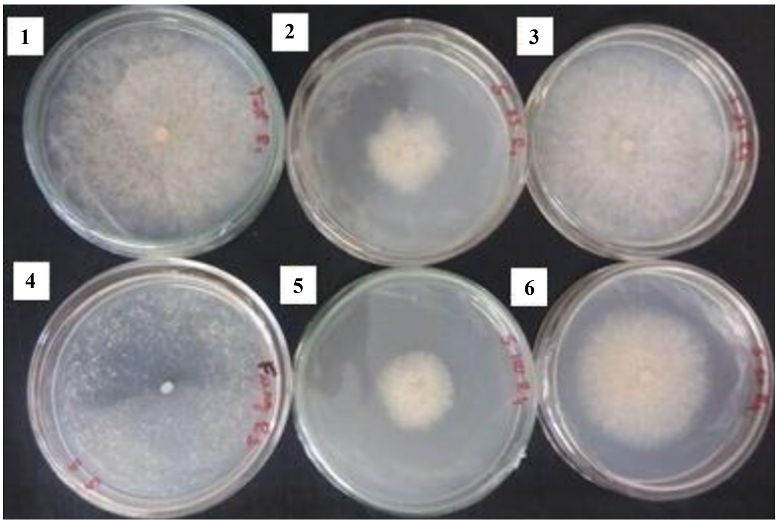

Figure S1. Illustrative images of fungus strain (*C. musae*) for (1) Control, (2) 75 µL mL^{-1}, (3) 25 µL mL^{-1}, (4) 50 µL mL^{-1}, (5) 100 µL mL^{-1} after 20 days and (6) Commercial antifungicide (carbendazim)