Evaluation of bio-detoxification of *Jatropha curcas* seed cake and cottonseed cake by basidiomycetes: nutritional and antioxidant effects

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Abstract

Poultry and swine are the major proportion of the livestock industry in terms of output value. To meet the growing need for protein sources in these sectors, the use of biomasses coming from agro-industrial residues can be an interesting option in the coming years. This study aimed to evaluate the capacity of seven basidiomycetes to grow, detoxicate, increase protein content, and also its antioxidant activity when grew in pure *Jatropha* seed cake (JSC) and cottonseed cake (CSC) biomasses and mixtures containing 50% of lignocellulosic biomasses from coconut husks and *Acrocomia aculeata* (macauba cake). Results showed that five basidiomycetes were able to grow in these substrates. *F. hepatica*, *P. lecomtei*, and *P. pulmonarius* presented the highest bio-detoxification capacity. All treatments showed a reduction in total phenolic compounds (TPC) and antioxidant activity, but treatments with coconut husks showed lower reductions. Results also indicated that there are molecules produced by basidiomycetes responsible for antioxidant activity other than phenolic compounds. These results indicated that basidiomycetes could detoxify JCS and CSC biomasses, suggesting their possible use in animal feed and that the addition of coconut husks in JSC and macauba cake in cottonseed cake can promote greater colonization by fungi.

Statement Of Novelty

Globally, there is an increasing demand for sustainable protein sources for animal feed. The use of agro-industrial wastes appears as a viable, economic and ecological alternative. In this context biomasses from *Jatropha curcas* seed cake (JSC), cottonseed cake (CSC). However, JSC and CSC contain toxic compounds that can impair animals' digestibility. Bio-detoxification with macro-fungi is a cheap and efficient process to reduce the concentration of these substances and improve protein content, besides generating edible mushrooms and lignocellulosic enzymes. Our work evaluated the colonization of seven different basidiomycetes in pure and mixed biomasses with lignocellulosic agro-industrial wastes aiming to select a suitable one for animal feed, focusing on reducing producer's cost and avoiding environmental disposal.

1. Introduction

As the human population grows, the demand for food increases proportionately. To help address the global food challenge, researchers have developed alternatives sources of animal feed for poultry and swine industries, the most consumed meats in the world. Among these new options for animal consumption, oil press cakes have been widely used as sources of proteins and carbohydrates.

Amongst the biomasses with potential for use in animal nutrition, *Jatropha curcas* seed cake and cottonseed cake, residues of oil extraction, both oilseeds suitable for biodiesel production, are good candidates especially due to their high protein content. However, toxic compounds such as phorbol esters and free gossypol, present respectively in *Jatropha* cake and cottonseed cake, make these materials unsafe for animal feed. Phorbol esters can act either acutely inducing an intense inflammatory response, or chronically, inducing tumor development [1]. Free gossypol (FG) can cause infertility and toxicity in

young ruminants and monogastric animals [2]. As an alternative to enable the use of these biomasses, colonization with macrofungi (basidiomycetes) offers the advantages of being a low-cost process to bio-detoxicate these substrates and also the production of value-added products, such as edible mushrooms and lignocellulosic enzymes [3]. Besides, the cultivation of mushrooms in agro-industrial wastes can improve their protein content, produce bioactive molecules such as phenolic compounds, polysaccharides, sterols, and reduce lignin and cellulose content, improving their digestibility for animal feed [4]. A good balance of carbon-nitrogen is important to promote the better growth of fungi [5]. So, the combination of *Jatropha* or cottonseed cakes as protein sources, and coconut husks or *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. (palm) cakes as carbohydrates source, is interesting for basidiomycetes cultivation as well since the last ones make the carbon-nitrogen balance increases. Finally, carotenoids of *Acrocomia aculeata* and the high phenolic content of coconut husks might help the antioxidant activity of final biomasses products [6].

Basidiomycetes are a class of fungi with species that produce visible fruiting bodies commonly known as mushrooms [7]. They have high commercial value due to their nutritional composition and for being an alternative source of quality proteins and essential amino acids [8, 9] as well as because some species present medicinal properties. Among basidiomycetes used for bio-detoxification purposes, some species stand out. *Agaricus subrufescens* (syn. *A. blazei*, *A. brasiliensis*), also known in Brazil as “Sun mushroom” is widely consumed for its medicinal activities [10] being known for preventing a range of diseases such as cancer, diabetes, arteriosclerosis, and others [11]. *Lentinula edodes* is popularly known as Shiitake and it is the second most-consumed mushroom in the world. Shiitake has high nutritional value and medicinal properties, being pointed as a good choice to reduce cholesterol, modulation of the immune system, and by its anti-inflammatory properties [12]. *Schizophyllum commune* is one of the most common mushrooms in nature. Although usually classified as a non-edible species due to its fibrous texture, it is consumed traditionally in Mexico [13]. *S. commune* gained pharmaceutical visibility after the discovery of its derived polysaccharide, schizophyllan, which has anticancer activity [14].

Another species of basidiomycetes applied in bio-detoxication is *Pleurotus spp.* popularly known as oyster mushroom. *P. ostreatus* is widely consumed, while *P. eryngii* and *P. pulmonarius* are less consumed. These two species present, however, anticancer, anti-inflammatory, and antimicrobial properties already recognized mainly for their polysaccharides, peptides, proteins, triterpenes, and nucleotides [15]. *Fistulina hepatica* is another basidiomycete known for its medicinal properties. Its antimicrobial and antioxidant activities have already been studied and polysaccharides and phenolic compounds have been identified as bioactive molecules [16, 17]. *Panus lecomtei* is a mushroom traditionally consumed in Brazilian indigenous communities and Japan. Little is known about this species, however, some authors [18, 19] have already reported that *P. lecomtei* has anticancer and antimicrobial activities. Finally, *Ganoderma lucidum* also called Reishi mushroom, the “immortality mushroom”, has traditionally been used in Chinese medicine to prevent hepatitis, nephritis, hypertension, and asthma, and also to stimulate the immune system [20]. The bioactive compounds identified in *G. lucidum* include triterpenoids, polysaccharides, nucleotides, sterols, and proteins; being these molecules responsible for anticancer, antimicrobial, anti-inflammatory, antioxidant, antifungal, and anti-viral

properties of the species [21]. Nowadays, global concern about antibiotic resistance and residues in animal products has resulted in the search for alternatives to growth promoters and veterinary drugs (such as prebiotics, probiotics, and other feed additives), focus on reducing the use of antimicrobials in animal production [22].

Therefore, this work aimed to evaluate growing, detoxicating efficiency, protein gain, and improvement of antioxidant activity of four basidiomycetes with medicinal properties (*Agaricus subrufescens*, *Schizophyllum commune*, *Fistulina hepatica*, and *Ganoderma lucidum*), and three edible mushrooms (*Lentinula edodes*, *Pleurotus* spp, and *Panus lecomtei*) cultivated in pure biomasses of *Jatropha curcas* seed cake and cotton seed cake and combined with coconut husks and macauba cake biomasses. Results obtained here can be used in poultry and swine industries as a guide to select biomasses suitable for animal feed enriched with bioactive compounds, reducing meat-producing costs, and avoiding the disposal of these agro-residues in the environment.

2. Materials And Methods

2.1 Plant Material

Four different biomasses for basidiomycetes growth were used in this experiment: pure *Jatropha* seed cake (JSC) and cottonseed cake (CSC) and in mixtures containing 50% of lignocellulosic biomasses from coconut husks (CH) and *Acronomia aculeata* (macauba cake-MC). Seeds from *Jatropha curcas* were provided by Embrapa Cerrados (Planaltina – DF, Brazil). After mechanically extract the oil, the cake was dried for 30 min, at 100°C, in rotatory dryer. Cottonseed cake was provided by Farmotec Industry (Buritis – MG, Brazil).

Coconuts husks were obtained from local sellers (Brasília – DF, Brazil), crushed, and subsequently oven-dried for 1 week, at 60°C. Macauba (*Acronomia aculeata*) fruits were provided by Agrotech/Soleá Company (João Pinheiro – MG, Brazil). Macauba mesocarp had its oil extracted mechanically, and residual solid biomass was dried for 30 min, at 100°C.

2.2 Media preparation and basidiomycetes cultivation

Fungi tested were *Agaricus subrufescens* (strain CC414), *Lentinula edodes*, *Schizophyllum commune* (strain FPB117), *Pleurotus pulmonarius* (strain EF88), *Fistulina hepatica* (strain CC102), *Panus lecomtei* (strain CC40), and *Ganoderma lucidum* (strain CC351). Samples were obtained from the Microorganisms and Microalgae Collection (Coleção de Microrganismos e Microalgas Aplicados Bioferrefinaria – CMMABio) from Embrapa Agroenergy (Brasília – DF, Brazil). Strains were preserved in potato dextrose agar plates (PDA) at 4°C. The inoculum was prepared by transferring disks of 5 mm of diameter from the preserved cultures to another PDA plate, and incubating it in a BOD incubator, for 7 days, at 28°C.

Growth medium assayed were pure *Jatropha* seed cake (JSC) and cottonseed cake (CSC), *Jatropha* seed cake with coconut husks (JSC+CH), cottonseed cake with coconut husks (CSC+CH), *Jatropha* seed cake

with macauba cake (JSC+MC), and cottonseed cake with macauba cake (CSC+MC). In each 10 cm jar (225 ml of volume), 15 g of each biomass was weighted, totaling 30g for mixtures. For JSC and CSC puree, 30 g were added to each jar. Biomasses humidity was adjusted to approximately 65% by adding water and sterilized in an autoclave for 45 min, at 121°C. After cooling, 4 disks (5 mm of diameter) of each basidiomycete's inoculum were added to each jar. Also, jars without inoculum were incubated at the same conditions as uncolonized control samples. Cultures were prepared in triplicate. Jars were incubated for 15 days at 28°C, in a BOD incubator. After incubation time, cultures were dried with air circulation for 48 h, at 40°C, milled, and stored at -20°C until further analysis.

2.3 Growth rate

During the 15 days of incubation time, every 3 days, mycelium growth was measured. At the end of the cultivation period, the growth rate for each fungus at each different biomass was estimated in cm/day. Basidiomycetes incapable of growing in the biomasses assayed were excluded from further analysis.

2.4 Phorbol ester and free gossypol quantification

Phorbol ester quantification in JSC was performed in triplicate. The applied method was adapted from the original protocol described in the literature [23]. Thus, 15 mL of methanol was added to falcon tubes containing 3 g of cultures grown with jatropha seed cake and then crushed with Teflon sticks for 1 min. After that, they were ultrasonicated (USC480A, Unique, SP, Brazil) at an ice bath, for 3 min. Tubes were centrifuged at 9000 rpm, for 8 min, and the supernatant was transferred to a round bottom flask and was evaporated in a rotary evaporator under vacuum (Rotavapor R210/215, Büchi Labortechnik, Flawil, Swiss), at 40°C. Phorbol esters were resuspended with 5 mL of methanol and centrifuged at 14000 rpm, for 8 min, at 8°C. Chromatographic analysis was performed at a UPLC (Ultra-Performance Liquid Chromatography) System (Acquity UPLC H-Class System, Waters, Massachusetts, USA) with a PDA detector, using a reverse-phase column (Waters, Acquity UPLC HSS-T3), pre-column (VanGuard HSS-T3) at 45°C. An elution gradient was used, and the detector was adjusted at 280 nm. A calibration curve was performed with a standard solution of phorbol myristate acetate (PMA) (Sigma-Aldrich) and results of the samples plotted on this curve to obtain phorbol esters content.

FG quantification was performed according to a method described in the literature [24]. In a falcon tube, 1 g of milled and dried samples was added. The extraction was performed with two sequential steps in a cold ultrasonic bath: the first one with 3 mL of ultrapure water and the second one with 7 mL of acetone. The tubes were centrifuged at 9.000 rpm, for 5 min, at 8°C, and finally, 1 mL of supernatant was transferred to 2 mL sample vials. The chromatography analysis was conducted on a Water Acquity UPLC H-Class system (Waters, Milford, USA) with a PDA detector using a reverse-phase column C18 (100 x 2.1 mm, 2.6 µm, KINETEX®). A calibration curve was performed with a standard solution of gossypol (≥ 95% purity HPLC, Sigma-Aldrich) and results of the samples plotted on this curve to obtain free gossypol content.

The degradation rate of both phorbol ester and free gossypol was obtained considering 100% the amount of these toxic compounds in the uncolonized biomasses.

2.5 Ergosterol content

Ergosterol, a primary sterol present in cell membranes of filamentous fungi is widely used to estimate fungal biomass [25]. Ergosterol quantification was performed in triplicate, applying a method adapted from a published protocol [26]. Briefly, 10 mL of methanol and 5 mL of ethanol were added to 400 mg of dried milled culture with 2 g of KOH in assay tubes. Saponification was carried out in a water bath at 70°C, for 30 min. After cooling of samples, 5 mL of water and 10 mL of hexane were added. Tubes were ultrasonicated (USC480A, Unique, SP, Brazil) for 1 min and supernatants were collected. Extraction was performed twice using 5 mL of hexane each time. Extracts were pooled and rota-evaporated (Multivapor Büchi, Flawil, Swiss) with bath temperature of 40°C. Extracts were resuspended in methanol and centrifuged at 13500 rpm, for 5 min. Quantification was performed by injection of extracts in a UPLC system (Acquity UPLC H-Class System, Waters, Massachusetts, USA) with a PDA detector and a 2.6 µm C18 column (Kinetex, Torrance, CA, USA). A calibration curve was made with an ergosterol standard (Sigma-Aldrich) and results of the samples plotted on this curve to obtain ergosterol content.

2.6 Crude protein content

Two mg of milled and dried colonized biomasses were weighted in a Perkin Elmer AD-6 Auto Balance (Massachusetts, EUA) and nitrogen content was quantified with a CHN (Carbon, Hydrogen and, Nitrogen) analyser (2400 CHNS/O Series II, PerkinElmer, Massachusetts, EUA). Helium was used as carrier gas. Acetanilide (C = 71.09 %, N = 10.36 %, H = 6.71 %) was used to calibrate the instrument. For crude protein content, nitrogen content was multiplied by the standard default conversion factor 6.25. This analysis was performed in triplicate [27].

2.7 Preparation of extracts

To carry out analyses of antioxidant properties, it was necessary to produce extracts of samples. Extracts of milled and dried colonized biomasses were obtained in triplicate, as reported below to each sample, 80% ethanol was added (1:20 w/v), and the solutions were placed in an ultrasonic bath (room temperature) (USC480A, Unique, SP, Brazil) for 20 min. After that, samples were shaken at 180 rpm, for 30 min, and finally centrifuged at 4000 rpm, for 10 min. Then, the supernatant was collected, filtered with Whatman no. 41 filter paper, and evaporated (Rotavapor R210/215, Büchi Labortechnik, Flawil, Swiss) with bath temperature of 40°C. Residual extracts were freeze-dried (K120, Liotop, São Carlos, SP, Brazil) and stored at -20°C in the dark, until analyses.

2.8 Antioxidant activity

Antioxidant activity was evaluated in extracts resuspended with methanol (5 mg/mL) by two different methods of free radical scavenging activity, one with radical DPPH● (2,2-difenil-1-picrilhidrazyl) and the other with ABTS^{•+} (2,2'-azino-bis 3-etilbenzotiazolyn-6-sulfonate) radical.

DPPH radical antioxidant activity was performed as described by previous authors [28], with modifications. To carry out the reaction, 22 µL of extract were added to a 200 µL of DPPH solution (150 µM). The absorbance of the samples was read at 520 nm (Molecular Devices, Spectramax 190, California, USA). Results of the samples were plotted on a calibration curve performed with Trolox (6-hidroxi-2,5,7,8-tetrametilchroman-2-carboxilic acid) at 0.5 mg/mL.

To analyze ABTS^{•+} antioxidant activity, a method described by previous authors [29] and adapted by others [30] was applied. ABTS^{•+} solution was prepared by adding 88 µL of potassium persulfate solution (140mM) to 5 mL of ABTS solution (7 mM), kept overnight in the dark. This solution was diluted in methanol (approximately 1:40 v/v) till the absorbance at 734 nm reached 0.70. 10 µL of the extract was added to 290 µL of ABTS^{•+} solution and incubated at 30°C, for 6 min, in the dark. The absorbance of the samples was read at 734 nm (Molecular Devices, Spectramax 190, California, USA). Results of the samples were plotted on a calibration curve, performed with Trolox (6-hidroxi-2,5,7,8-tetrametilchroman-2-carboxilic acid) at 0.5 mg/mL, to obtain antioxidant activity.

2.9 Total phenolic compounds (TPC)

To carry out total phenolic compounds quantification, the method was adapted from a published protocol [31]. Extracts were resuspended with ethanol 80% (3 mg/mL). Thus, 500 µL of Folin-Ciocalteu (FC) reagent were added to 100 µL of extract diluted in 7 mL of distilled water. Samples were vortexed and incubated for 8 min, at room temperature. Then, 1.5 mL of Na₂CO₃ 20% (w/v), 900 µL of water were added to the tubes, and incubated for 2 h, at room temperature, in the dark. Sample's absorbance was read at 765 nm in 1 mm cuvettes (Agilent, Cary 60, California, USA). To obtain TPC content, the results of the samples were plotted on a calibration curve performed with Gallic Acid (0.5 mg/mL).

2.10 Statistics

All essays were performed in triplicate. The data were submitted to analysis of variance (ANOVA). In the case of $p < 0.05$, the data were submitted to the Tukey test. Statistics analyses were performed using RStudio software [32] and graphs were made using GraphPrism 8 (GraphPad Software, La Jolla, California, EUA). Pearson's correlation test was performed grouping in one block all experiments with JCS and another with all experiments with CSC ($p < 0.01$). Analytic Hierarchy Process (AHP) was used to rank the most promising treatments.

3. Results

3.1 Growth rate in Jatropha seed cake (JSC), and cottonseed cake (CSC)

The results of the growth rate (GR) showed that of the 7 selected basidiomycetes, *A. subrufescens* and, *L. edodes* were not able to grow in the jars with biomasses containing Jatropha seed cake (JSC), Jatropha seed cake with coconut husks (JSC+CH), and Jatropha seed cake with macauba cake (JSC+MC) (Figure 1). For substrates containing cottonseed cake (CSC), cottonseed cake with coconut husks (CSC+CH), and

cottonseed cake with macauba cake (CSC+MC), *A. subrufescens* was also unable to grow, while *L. edodes* showed a very low growth rate (Figure 2). Considering the inability of both species (*A. subrufescens* and *L. edodes*) to grow in the tested substrates, they were excluded from further analysis.

All the other basidiomycetes species (*S. commune*, *P. pulmonarius*, *F. hepatica*, *P. lecomtei*, and *G. lucidum*) were capable to grow completely in jars for 15 days. *P. lecomtei* and *S. commune* presented the highest growth rate in Jatropha seed cake (JSC) (Figure 1a) and cottonseed cake (CSC) (Figure 2a) respectively, showing GR of 0.66 and 0.69 cm/day. These two species also showed higher GR in JSC+MC (Figure 1c) and CSC+MC (Figure 2c), differing statically from the other 5 basidiomycetes species.

As for substrates containing coconut husks (CH), in JSC+CH, *S. commune*, *F. hepatica*, and *P. lecomtei* presented the highest growth rate (0.56, 0.51, and 0.46 cm/day, respectively), statically differing from the other tested species (Figure 1b). In CSC+MC, diverse growth rates were obtained (Figure 2b) with *P. lecomtei* and *S. commune* presenting higher GR (0.59 and 0.58 cm/day, respectively), followed by *F. hepatica* (0.34 cm/day).

3.2 Phorbol ester, gossypol, ergosterol, and crude protein contents

3.2.1 Jatropha seed cake (JSC)

Results of phorbol ester content in JSC showed the that control sample (uncolonized) presented 1.16 mg/g of phorbol ester, while mixtures of JSC+CH and JSC+MC presented 0.42 mg/g and 0.47 mg/g, respectively. Colonized samples presented phorbol ester contents in JSC varying from the lowest value of 0.02 mg/g, to the highest amount of 0.19 mg/g, identified in *P. lecomtei* and *S. commune* colonization, respectively. In JSC+CH colonized samples, phorbol ester values varied from 0.02 mg/g of *P. lecomtei* and *P. pulmonarius* to 0.24 mg/g for *G. lucidum* colonization. In JSC+MC, values varied from 0.01 mg/g for *P. lecomtei* and *P. pulmonarius*, and 0.06 mg/g for *S. commune* and *F. hepatica* colonization (Table 1). Finally, in colonized JSC substrates degradation percentage ranged from 53.8% for *G. lucidum* (JSC+CH) to 98.2% for *P. lecomtei* (JSC) (Table 1).

Ergosterol content analysis in uncolonized biomasses of JSC, JSC+CH, and JSC+MC showed contents of 52.5, 40.9, and 35.5 ppm, respectively. Meanwhile, in colonized materials, ergosterol was quantified in contents up to 1268.7 ppm (JSC+MC) and 1261.4 ppm (JSC+CH) for *S. commune*, followed by colonization of *P. pulmonarius* in JSC+CH (763.1 ppm) and JSC+MC (694.1 ppm) biomasses. Overall, for all other species, JSC biomass containing CH presented higher ergosterol content when compared to JSC, and JSC+MC (Table 1).

Since CH and MC are biomasses lower in protein content, higher crude protein content was identified in uncolonized pure JSC biomass than in the mixtures. Uncolonized biomasses mixtures presented different protein contents: 27.9 g/100g in JSC, 11.4 g/100g in JSC+CH, and 16.9 g/100g in JSC+MC. Usually, the protein content of cultivation medium increases because of syntheses of fungal protein and also due to consumption of lignocelulitic material during growth, leading to an effect of protein concentration due to

depletion of other components. The crude protein content of colonized JSC biomass ranged from 20.8 g/100g for *P. pulmonarius* to 30.1 g/100g for *G. lucidum*. For JSC mixed coconut husks (CH), crude protein content varied from 17.3 g/100g for *S. commune* to 19.7 g/100g for *F. hepatica*. For JSC+MC, CP data ranged from 18.7 g/100g for *P. pulmonarius* to 22.5g/100g for *F. hepatica* (Table 1).

Although presenting average ergosterol content, which is an indicator of fungi growth, *P. pulmonarius* and *S. commune* species were not capable to concentrate protein by bioconversion in pure JSC, showing decreases in protein content of colonized biomass, -16.9 and -25.7%, respectively (Table 1). The coefficient r of Pearson between initial protein content and % of increase of protein was inversely proportional ($r=-0,88$; $P<0.01$). For all other species, lower conversions were identified in JSC, being 1.2, 7.6 and, 7.8%, respectively for *P. lecomtei*, *F. hepatica*, and *G. lucidum*. Higher protein bioconversion was identified in JSC+CH biomass. Although there was no statistical difference between fungi treatments, an increase of 72.0% was identified for *F. hepatica* colonization, followed by an increase of 66.5% for *G. lucidum*, 56.6% for *P. pulmonarius*, 51.5% for *S. commune*, and 47.5% for *P. lecomtei* (Table 1). In JSC+MC biomass, the most notable increase was 33.7% in *F. hepatica* colonized material, while *P. pulmonarius* showed the smallest increase (10.8%).

3.2.2 Cottonseed cake (CSC)

Results of FG content in uncolonized biomasses varied between 2170.8 ppm (CSC), and 607.2 ppm (CSC+MC). In colonized biomasses, FG content varied from 59.4 ppm for *P. pulmonarius* to 423.0 ppm for *S. commune* colonization in CSC. In CSC+CH, FG content ranged from 90.56 ppm with *F. hepatica* colonization to 658.4 ppm with *S. commune* colonization. In CSC+MC samples, FG content varied from 3.5 ppm for *P. lecomtei* colonization, to 454.6 ppm with *S. commune* (Table 2).

Higher percentages of FG degradation were identified in CSC+MC biomass for *P. lecomtei*, *F. hepatica*, and *G. lucidum* colonization with percentages of 99.4, 98.9, and 93.9%, respectively. While *F. hepatica* and *P. lecomtei* showed higher degradation in CSC+CH (88.5 and 87.1%, respectively). The lowest percentages of FG degradation were 16.5% for *S. commune* growing in CSC+CH and 25.5% in CSC+MC (Table 2).

Ergosterol content in uncolonized biomasses were 47.3, 37.7, and 51.7 ppm respectively at CSC, CSC+CH, and CSC+MC. In colonized material is expected the ergosterol content increases since it is a primary sterol present in the cell membranes of filamentous fungi and has been used as a biomarker of fungi growth. The highest ergosterol content was 2.321 ppm, identified for *S. commune* at CSC+MC, in agreement with the visual growth rate observed in this specific experiment. In CSC biomass treated with fungi, ergosterol content varied between 621.7 for *F. hepatica* to 1.665.6 ppm for *S. commune*. For CSC+CH fungi treatments, ergosterol content varied from 676.7-1291.0 ppm, for *F. hepatica* and *P. lecomtei*, respectively. For CSC+MC fungi treatments, ergosterol content ranged from 769.6 to 2.321,0 ppm identified respectively, for *P. lecomtei* and *S. commune* (Table 2).

Overall, higher protein contents were identified in pure CSC, followed by CSC+MC and CSC+CH biomasses. Protein contents for uncolonized treatment were 27.6 g/100g, 12.3 g/100g and, 15.1 g/100g

respectively for CSC, CSC+CH, and CSC+MC biomasses mixtures. The protein content of fungi treated CSC varied from 30.4 g/100g for *P. pulmonarius* to 32.4 g/100g for *F. hepatica*. In the CSC+CH biomass mixture, protein content ranged from 14.0 g/100 to 16.8 g/100g, respectively for *G. lucidum* and *S. commune*. Finally, in CSC+MC biomass, protein content varied from 18.8 g/100g to 21.9 g/100g for *P. pulmonarius* and *F. hepatica*, respectively (Table 2).

The highest protein bioconversions were identified for *F. hepatica* and *P. lecomtei* at CSC+MC, 44.7 and 41.6%, respectively; followed by *S. commune* and *P. pulmonarius* at CSC+CH, 36.5 and 36%, respectively. Lower protein conversions were identified in CSC+MC biomass for *G. lucidum* and *P. pulmonarius*, 7.7 and 10.8%, respectively (Table 2).

The highest increments were reached in JSC+CH treated with *F. hepatica* (72%) followed by JSC+CH with *G. lucidum* (66.6%) and JSC+CH treated with *P. pulmonarius* (56.6%). For CSC, best protein increments after fungi colonization were 34 and 36% in JSC+CH treated with *P. pulmonarius* and *F. hepatica* respectively, as well in CSC+MC treated with *Panus lecomtei* and *F. hepatica*, with increments of 41.6 and 44.7%, respectively.

3.3 Antioxidant activity and phenolic compounds

3.3.1 Jatropha seed cake

Results showed that antioxidant activity decreased in all treatments compared to uncolonized (control) biomasses. This could be related to oxidative enzymes produced during fungi growth. Therefore, the best treatment would be those presenting smaller losses in the ability to react with DPPH and ABTS free radicals. In DPPH• assay, TEAC from JCS colonized varied between 2.4 (which was also the lowest data among all biomasses and basidiomycetes tested) and 31.0 µg/mg of extract for *P. lecomtei*, and *S. commune*, respectively. In JCS+CH biomass results varied between 12.8 and 56.0 µg/mg (which was also the highest data among all biomasses and basidiomycetes teste) of extract, for *P. lecomtei*, and *P. pulmonarius*, respectively. In JCS+MC treatment, TEAC ranged from 15.9 µg/mg of extract identified for *P. pulmonarius* to 27.8 µg/mg of extract for *F. hepatica* (Table 3).

In ABTS+• assay, TEAC varied between 18.4 and 62.4 µg/mg of extract, for *P. pulmonarius* and *G. lucidum*, respectively in JCS treatments. In JCS+CH treatment, TEAC ranged from 23.2 to 97.2 µg/mg of extract for *P. lecomtei* and *P. pulmonarius*, respectively. *P. lecomtei* and *G. lucidum* presented respectively, 6.4 and 55.1 µg/mg of extract in JSC+MC, with remaining sample results falling in between these values. From all colonized biomasses in Jatropha seed cake, antioxidant activity from *P. pulmonarius*, in JCS+CH treatment, presented the highest values between all other colonized biomasses, although not differing statistically to other treatments (Table 3).

Total phenolic compounds in uncolonized biomasses was higher in pure JCS when compared to JCS with mixtures (JSC+CH, and JSC+MC) (Table 3). TPC presented a great reduction for colonized treatments. No TPC data was detected for *P. pulmonarius* and *P. lecomtei* at this treatment. The only

treatment that presented TPC similar to untreated materials was JCS+CH biomass with *G. lucidum*, with 31.8 µg of GAE/mg of extract. Phenolic compounds are usually related to antioxidant activity in plant materials, but in fungi treated biomasses this was not observed. The Pearson correlation analysis revealed that the TPC does not present correlations with DPPH• assay or with ABTS+• assay.

3.3.2 Cottonseed cake

In CSC fungi treated samples, TEAC by DPPH• assay varied between 4.2 to 14.1 µg/mg of extract, for *P. lecomtei* and *S. commune*, respectively. In CSC+CH treatment, TEAC ranged between 11.0 and 20.2 µg/mg of extract, respectively, for *P. pulmonarius* and *F. hepatica*. For CSC+MC treatment, higher and lower TEAC were 15.4 and 18.9 µg/mg of extract, identified respectively for *P. lecomtei* and *F. hepatica* colonization. Overall, all treatments showed lower antioxidant activity when compared to uncolonized control (Table 4).

When TEAC was quantified in an ABTS+• assay, in CSC treatment, results varied between 15.3 and 37.2 µg/mg of extract, for *P. lecomtei* and *S. commune*, respectively. In CSC+CH treatment, TEAC varied between 24.6 and 56.6 µg/mg (which was also the highest data among all biomasses and basidiomycetes teste) of extract, identified, respectively, for *P. lecomtei* and *G. lucidum*. Finally, in CSC+MC treatment, TEAC ranged between 13.8 µg/mg of extract for *P. lecomtei* to 48.2 µg/mg of extract corresponding to *S. commune*. The highest TEAC results were obtained with CSC+CH biomass colonized with *G. lucidum*, *S. commune*, and *F. hepatica*, 56.6, 56.2, and 52.6 µg/mg of extract, respectively (Table 4).

Uncolonized biomasses, CSC and CSC+CH presented higher GAE values, 21.3 and 24.3 µg of GAE/mg of extract, respectively, when compared to CSC+MC treatment (16.0 µg of GAE/mg of extract). Among colonized treatments, total phenolic compounds (TPC) were higher in CSC treated with *G. lucidum* (14.0 µg of GAE/mg of extract), in CSC+CH treated with for *F. hepatica* (14.5 µg of GAE/mg of extract), and in CSC+MC treated with *F. hepatica* (17.0 µg of GAE/mg of extract). For *S. commune*, *P. pulmonarius*, *P. lecomtei*, and *G. lucidum*, TPC was not detected (Table 4).

4. Discussion

In the current population growth scenario, demanding an increase in food, agro-industrial residues management appears as an important concern for researchers, who have developed strategies to combine the use of these residues to improve animal feed, reduce meat production costs, and prevent the disposal of this waste in the environment. Among potential residues to be applied for these purposes, *Jatropha curcas* cake (JSC) and cotton seedcake (CSC), both byproducts from the oil extraction industry, show interesting options, considering their high protein content. However, these biomasses present some toxic compounds that can impair animal's digestion. The application of biodegrading microorganisms capable of degrading these toxic metabolites represents a promising strategy for the elimination of these substances [1] enabling the use of these biomasses as nutritious animal feed, in addition to the potential for exploitation in the production of edible mushrooms.

Here, we evaluated the capacity of 7 basidiomycetes species, also known as white-rot fungi, to detoxicate different protein-rich biomasses and combined with lignocellulosic ones from coconut husks (CH) and *Acrornomia aculeata* (macauba cake - MC). The data obtained here showed that although *A. subrufescens* and *L. edodes* were able to grow in lignocellulosic wastes, as described by some authors [34, 35] who reported growing in composts such as sawdust and wheat straw; these two species were not able to grow in pure JCS and CSC biomasses or combined with CH and MC. The cultivation of *A. subrufescens* has being described as complex, demanding N supplementation during all growth besides requiring strict environmental controls [36] which, may explain why no growth was achieved in the biomasses tested here. For *L. edodes*, as identified here, some authors have previously shown a remarkable growth reduction when cultivated in oak-wood sawdust, a lignocellulose-rich biomass, suggesting that nitrogen content could be a growth-limiting factor for shiitake [37, 38]. All other 5 fungi species grew in the assayed biomasses. Some authors have also reported the growth of white-rot fungi species, in pure biomasses of JSC and CSC and combined with other cellulosic sources for *Pleurotus* species (Oyster mushrooms), including *Pleurotus pulmonarius* [39, 24, 40], *Schizophyllum* [41], *Fistulina hepatica* [42], *Panus lecomtei* [3], and *Ganoderma lucidum* [43, 3].

Besides visual measurements in centimeters, another indicator of fungi colonization is the presence of ergosterol [44, 26]. This molecule, vitamin D₂ precursor, is sterol present in cellular membranes of fungi and commonly used as an indicator of fungal growth [45, 46, 25]. Some authors have also reported higher levels of ergosterol in basidiomycetes species [47, 48, 46], showing a positive correlation between fungi growth and ergosterol content [47, 48], which corroborates the use of this molecule as a fungal biomass growth indicator. It is important to highlight, however, that each fungi species has its pattern relation of growth and ergosterol content [26]. So, ergosterol is a good marker of growth to compare different biomasses or treatment approaches for the same species but showed inconsistencies to comparisons among different fungi. Higher content of ergosterol was identified in colonized JSC biomass mixed with lignocellulosic material (CH and MC), mainly in *S. commune*, and *P. pulmonarius*. As for CSC mixed with lignocellulosic biomasses, all fungi showed higher content of ergosterol, except for *G. lucidum*. These data suggest that biomasses with lignocellulosic material induced a greater growth of the selected basidiomycetes, with coconut husks being a good additive for JSC biomass and, macauba cake for cottonseed cake; proving that a good balance of carbon-nitrogen is important to promote better growth of fungi [5]. Ergosterol is also a precursor of vitamin D₂, and its presence could bring benefits to animal diet. Some authors [49] tested supplementation of 50 µg mushroom vitamin D₂/kg in feed diet of pigs and found increased pork antioxidant status and improved pig growth performance, carcass weight, and color.

To be safely used in animal feed, phorbol ester and FG present, respectively, in JSC and CSC biomasses need to be destroyed. The development of a biological approach using basidiomycetes to decrease the level of toxic compounds meets fundamentals of sustainable processes [4] when compared to other detoxication methods such as chemical, physical and enzymatic treatments. Usually in *Jatropha* seed cake levels of phorbol ester range from 1–3 mg/g, but according to some authors [1], tolerable levels of

phorbol esters must be below 0.09 mg/g to safely be used in an animal diet. Here, levels above 0.09 mg/g were found in *S. commune* in JSC, *P. pulmonarius* in JSC, *F. hepatica* in JSC + CH, and *G. lucidum* in JSC, and JSC + CH, which implies that these treatments didn't reach safe levels. Except for the treatments cited above, all treatments met levels of phorbol esters under the target threshold, reaching a high rate of detoxification, as much as 98,2% using *P. lecomtei* in pure JSC. Many authors have also reported high degradation levels of phorbol ester in biomasses colonized by basidiomycetes. It has been shown [39] a reduction of 99% in phorbol concentration, in JSC incubated for 60 days with *Pleurotus ostreatus*, with the same results being reported after 45-day incubation [50]. Other authors studying 10 different white-rot fungi, including three samples from *Pleurotus* sp. and *Ganoderma lucidum*, showed the degradation of PE in all tested fungi, however, the extent of degradation varied with the fungal culture [43], as observed in this work. A decrease in the concentration of phorbol esters in *Ganoderma* sp was also reported [4]. Authors have pointed that, the efficiency in the degradation of phorbol esters in JSC through white-rot fungi colonization could be attributed to enzymatic complexes produced during the growth process, which have high esterase activities, suggesting that phorbol ester bonds are susceptible to hydrolytic degradation [4].

As for FG content, CSC + CH biomass mixture showed still higher content when colonized with *S. commune*, while CSC + MC and CSC + CH treated with *F. hepatica* and *P. lecomtei*, presented the lowest values, representing up to 99.4% degradation. European Union Directive 2002/32/EC limits FG in cottonseed cake feedstuffs to 20 ppm for laying hens and piglets, 100 ppm for poultry and calves, and 500 ppm for cattle, goats, and sheep. So, these treated biomasses could feed even poultry. All fungi treated biomasses presented high-rate degradation (except for those with *S. commune*) reaching permissible levels for ruminant's feed. Significant reduction in FG content was also reported from other authors [24] working with *Pleurotus* sp., with degradation percentages reaching 100% [51]. In addition, some authors analyzing 06 basidiomycetes including *P. lecomtei*, *F. hepatica*, *Ganoderma* sp., and *Pleurotus* sp. showed that all fungi were able to reduce FG content when colonizing CSC, with degradation percentages ranging from 74 to 94% [52]. These data corroborate the capacity of white-rot fungi to biodegrade this toxic molecule.

As a consequence of fungi fermentation, an increment in protein content occurs [43], which places it as a good strategy for improving the nutritional quality of vegetable biomass to animal feed. Although fungi are not capable to fix nitrogen, increases in crude protein content were observed here. This increase occurs due to the consumption of other nutrients (like lipids and carbohydrates) during metabolism, reduction in the total amount of biomass, and protein concentration. Thus, part of biomass nitrogen is incorporated into fungi protein, and carbohydrates are converted into fungal biomass and CO₂ [53]. Data assayed here showed higher protein increments after fungi colonization in most treatments, comparing to respective untreated material. But some treatments, as in pure JSC with *S. commune* and *P. pulmonarius*, presented a decrease in protein. This can be indicative of protein use in these species' metabolism when they are in excess. According to some authors [33], the ligninolytic enzymes are produced during the secondary metabolism under conditions of limited nitrogen. Since the protein concentration of pure JCS

was the highest of all cultivation medium, probably the consumption of carbon-rich material was impaired. The high protein content available during fermentation could induce fungi to use it for their own metabolism.

The large variation in metabolizing protein among different fungal species identified here was also reported by other authors [53], which showed that after 7 weeks of colonization with *P. eryngii* and *L. edodes*, the total amount of CP increased by 23.3–30.9% in the fungal-treated wheat straw. Authors have also reported increases and decreases in CP in different fungi rather than white-rot species. Thus, treatment of JSC colonized with ascomycetes (*Trichoderma longibrachiatum*, *Trichoderma harzanium*, and *Aspergillus niger*) promoted a decrease of 26% of crude protein content, as identified here for *S. commune* and *P. pulmonarius*. Increments, however, were also reported in treatments of JSC with *Aspergillus niger*, showing an increase of crude protein content of up to 11.3% [54, 55]. A previous study with JSC treated with fungi *Absidia spinosa* and *Mucor rouxxi* presented increases up to 8% in crude protein content [56]. Some authors have also demonstrated that fungi treatment with *Candida capsuligena*, *Candida tropicalis*, *Saccharomyces cerevisiae*, *Aspergillus terricola*, *Aspergillus oryzae*, and *Aspergillus niger* in CSC biomass promoted an apparent crude protein increase between 8.95 and 22.24% [57]. As white-rot fungi, basidiomycetes are very efficient organisms in degrading lignin present into lignocellulosic materials (coconut husks and macauba cake), so the addition of these biomasses in the cultivation of fungi benefits the balance between carbon and nitrogen ideal for fungal growth, besides having more protein diluted, which implies in a preferred degradation, contributing to protein concentration in these biomasses [53, 58]. Finally, as reported in this work, some authors suggested that the increase in protein content may be related to nitrogen fixed in fungi mycelia, being highly related to organic matter losses as well as with other factors such as temperature, pH, nature of fungi, aeration, type of substrate, and heat [59, 60, 61, 62], so variation would be expected among different species. The degradation of free gossypol and lignocellulosic biomasses promoted by basidiomycetes can also be related to the apparent increase of protein content [57], as higher CP concentrations were, overall, identified in colonization and biomasses treatments showing greater phorbol and FG degradation. However, at present work, no significant correlation was found between increase of protein content and growth rate or degradation rate.

In the colonization process of wood, white-rot fungi secrete several free radicals, aiming to activate the peroxidases enzymes system for degradation. As a protection for cells, these fungi also secrete antioxidative enzymes along with low molecular phenolic derivatives and other organic acids in response to ROS (reactive oxygen species) produced and in response to environmental stressors [63]. In the present work, reductions in antioxidant activity and total phenolic content of colonized biomasses were identified when compared to uncolonized biomasses. Overall, extracts of treatments using CH as lignocellulosic biomass presented higher antioxidant activity than treatments of pure JCS, CSC, or with MC added. Concerning the uncolonized biomasses, JCS presented higher TEAC concentrations, since *Jatropha* seed cake is known for its high antioxidant activity [64]. Differences in antioxidant activity results between ABTS and DPPH radical assays were observed. It can occur because some antioxidant agents react rapidly with peroxy radical in ABTS+• assay meanwhile, slowly or even inactive to DPPH•. Furthermore,

DPPH radical discoloration can happen to other reducing agents, contributing to discrepant results [65]. The same results were also observed by other researchers [66], analyzing the colonization of colonized fungi in soybean, with DPPH scavenging free radicals assay varying from 0.6 to 4.4 mg TEAC/mg of extract, and only one colonized sample presented TEAC values higher than uncolonized soybean (3.2 TEAC/mg of extract). Besides, other authors have reported antioxidative activities of other molecules, as polysaccharides isolated from *Ganoderma atrum*, *Agaricus bisporus*, *Lentinus edodes*, *Lentinus polychrous*, and *Phellinus linteus* [67, 68]. This may be the reason why there was no correlation between antioxidant activities and TPC at present work. We hypothesize that the assays applied here are detecting other substances with antioxidant activity besides phenolic compounds, like beta-glucans produced by fungi. Other work had isolated polyphenols with antioxidative properties from the culture broth of *Inonotus xeranticus*, and *Phellinus linteus* [69], corroborating that fungal bioactive compounds have indeed, antioxidant properties, potentially applied in animal food processing.

Frequently, the antioxidant activity is correlated with the phenolic content of biomasses, as the concentration of antioxidant compounds, as a response to the oxidative stress and free radicals production, is expected to increase as fungi grow [70]. However, the majority of colonized biomasses in this work did not show this similarity between antioxidant activity and TPC content. Not all extracts with a higher amount of total phenolic compounds showed high antioxidant activity and vice versa. Therefore, the hypothesis that is suggested, is that oxidative enzyme production by basidiomycetes related to lignin depolymerization could have affected the antioxidant capacity of the biomasses tested. It's known that oxidases and peroxidases are the main enzymes produced being responsible for generating free radicals that can interfere in the measure of antioxidant activity [71]. Besides, the differences between antioxidant activity and total phenolic compounds results are justified by the existence of other compounds produced by basidiomycetes that can promote antioxidant activity, besides phenolic compounds. Basidiomycetes are known to produce compounds other than phenolic compounds, such as carotenoids, C and E vitamins, and β -glucans that also present antioxidant activities [72].

Choose the best treatment for detoxification and enhancement of biomass is a multicriteria decision and needs to be addressed with appropriate tools. Analytic Hierarchy Process (AHP) was applied to establish the weight of each of the dependent variables. The Analytic Hierarchy Process (AHP) is a theory of measurement through pairwise comparisons and relies on the judgments of experts to derive priority scales. It is these scales that measure intangibles in relative terms. The comparisons are made using a scale of absolute judgments that represents, how much more, one element dominates another with respect to a given attribute [73]. The criteria's selected and weights found were degradation rate of toxic compounds (0.48), protein increase (0.19), final CP content (0.13), ergosterol content (0.09) ABTS radical assay (0.06), and DPPH radical assay (0.04). In conclusion, the most promising treatments for JSC were *P. pulmonarius* and *S. commune*; *P. lecomtei* in JCS + CH, and *F. hepatica* in JSC + MC. Treatments with pure JSC did not perform as well as those treatments. In CSC, *F. hepatica* and *P. lecomtei* in CSC + MC were the most promising treatments followed by *P. pulmonarius* in pure CSC. In this sense, those treatments can be further investigated for their use in animal feed.

5. Conclusions

This study showed that most basidiomycetes fungi were able to degrade phorbol esters and free gossypol to acceptable levels for animal feed, in pure JSC and CSC, and mixed with lignocellulosic biomasses. Increases in protein content were also observed, mainly with the addition of other lignocellulosic material to culture media. Ergosterol, a precursor of vitamin D₂ was produced. This bioprocess was proved to be a safe and good choice for the enrichment of non-conventional biomasses for animal feed. *P. pulmonarius*, *F. hepatica*, and *P. lecomtei* presented the best results for both CSC and JSC biomasses.

Antioxidant activity and TPC were reduced after fermentation, possibly due to the production of oxidative enzymes by the fungi. The results of TPC showed that maybe other antioxidant compounds must also have been produced. Therefore, further studies on the quantification of these compounds, possibly carotenoids, vitamins E or C, β -glucans, should be conducted to identify the antioxidant source of basidiomycetes colonization.

Declarations

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Conflicts of interest/Competing interests

The authors declare no conflicts of interest or competing interests.

Availability of data and material

Not applicable.

Code availability

Not applicable.

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Tables

Table 1 Phorbol ester content (mg/g) and degradation (%), ergosterol content (ppm), crude protein content (g/100g d.w.) and crude protein difference (%) of five basidiomycetes colonized in jatropha seed cake, jatropha seed cake with coconut husks and jatropha seed cake with macauba cake.

Treatment	Biomass	Phorbol ester (mg/g)	Phorbol ester degradation (%)	Ergosterol (ppm)	CP (g/100g d.w.)	CP difference (%)
Uncolonized	JSC	1.16 ± 0.05 ^{Aa}	-	52.5 ± 7.7 ^{Ba}	27.9 ± 1.2 ^{ABa}	-
	JSC+CH	0.42 ± 0.04 ^{Ab}	-	40.9 ± 1.4 ^{Ca}	11.4 ± 0.4 ^{Bc}	-
	JSC+MC	0.47 ± 0.08 ^{Ab}	-	35.3 ± 0.7 ^{Ca}	16.9 ± 1.6 ^{Cb}	-
<i>S. commune</i>	JSC	0.19 ± 0.02 ^{Ba}	83.4 ± 1.9 ^{Aa}	243.7 ± 138.9 ^{ABb}	23.3 ± 4.5 ^{BCa}	-16.9 ± 16.3 ^{BCc}
	JSC+CH	0.08 ± 0.01 ^{Cb}	80.5 ± 1.8 ^{Aa}	1261.4 ± 136.7 ^{Aa}	17.3 ± 1.2 ^{Ab}	51.5 ± 10.8 ^{Aa}
	JSC+MC	0.06 ± 0.01 ^{Bc}	86.4 ± 3.3 ^{Aa}	1268.7 ± 140.0 ^{Aa}	20.0 ± 2.8 ^{ABCa}	18.5 ± 16.7 ^{ABb}
<i>P. pulmonarius</i>	JSC	0.09 ± 0.03 ^{Ba}	92.1 ± 9.8 ^{Aa}	476.2 ± 96.2 ^{Aa}	20.8 ± 3.4 ^{Ca}	-25.7 ± 12.2 ^{Cc}
	JSC+CH	0.02 ± 0.01 ^{Cb}	94.9 ± 1.2 ^{Aa}	763.1 ± 297.2 ^{ABa}	17.9 ± 2.4 ^{Aa}	56.6 ± 20.8 ^{Aa}
	JSC+MC	0.01 ± 0.00 ^{Bc}	98.0 ± 0.8 ^{Aa}	694.1 ± 137.6 ^{Ba}	18.7 ± 1.6 ^{BCa}	10.8 ± 9.3 ^{Bb}
<i>F. hepatica</i>	JSC	0.06 ± 0.01 ^{Bb}	94.7 ± 3.5 ^{Aa}	289.9 ± 45.4 ^{ABb}	30.0 ± 3.9 ^{Aa}	7.6 ± 13.8 ^{Ac}
	JSC+CH	0.10 ± 0.03 ^{Ca}	75.3 ± 7.1 ^{Bb}	588.2 ± 112.1 ^{Ba}	19.7 ± 1.4 ^{Ac}	72.0 ± 12.2 ^{Aa}
	JSC+MC	0.06 ± 0.01 ^{Bb}	86.9 ± 6.8 ^{Aab}	198.2 ± 46.4 ^{Cb}	22.5 ± 2.1 ^{Ab}	33.7 ± 12.6 ^{Ab}
<i>P. lecomtei</i>	JSC	0.02 ± 0.01 ^{Ba}	98.2 ± 0.5 ^{Aa}	382.0 ± 19.1 ^{Aab}	28.3 ± 1.5 ^{ABa}	1.2 ± 5.3 ^{ABb}
	JSC+CH	0.02 ± 0.00 ^{Ca}	94.2 ± 0.7 ^{Ab}	675.8 ± 184.3 ^{ABa}	16.9 ± 2.3 ^{Ab}	47.5 ± 19.9 ^{Aa}
	JSC+MC	0.01 ± 0.00 ^{Bb}	97.3 ± 0.4 ^{Aa}	309.5 ± 32.4 ^{Cb}	19.6 ± 3.2 ^{ABCb}	16.4 ± 19.2 ^{ABb}
<i>G. lucidum</i>	JSC	0.13 ± 0.03 ^{Bb}	88.7 ± 0.6 ^{Aa}	348.7 ± 19.8 ^{Ab}	30.1 ± 4.2 ^{Aa}	7.8 ± 15.1 ^{Ab}
	JSC+CH	0.24 ± 0.08 ^{Ba}	53.8 ± 2.3 ^{Cb}	845.1 ± 243.4 ^{ABa}	19.1 ± 2.8 ^{Ab}	66.6 ± 24.3 ^{Aa}

JSC+MC	0.05 ± 0.01^{Bc}	89.6 ± 6.5^{Aa}	163.9 ± 30.6^{Cb}	20.3 ± 2.2^{ABb}	$20.6 \pm$ 13.3^{ABb}
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d.w.: dried weight; CP: Crude protein; JSC: Jatropha seed cake; CH: Coconut husks; MC: Macauba cake. Means followed by the same letter are not different by Tukey test ($p < 0,05$). Capital letters represent differences between treatments in the same biomass, and lowercase letters represent differences between biomasses in the same treatment.

Table 2 Gossypol content (ppm) and degradation (%), ergosterol content (ppm), crude protein content (g/100g d.w.) and crude protein difference (%) of five basidiomycetes colonized in cottonseed cake, cottonseed cake with coconut husks and cottonseed cake with macauba cake.

Treatment	Biomass	Gossypol (ppm)	Gossypol degradation (%)	Ergosterol (ppm)	CP (g/100g d.w.)	CP difference (%)
Uncolonized	CSC	2170.8 ± 7.6 ^{Aa}	-	47.3 ± 2.3 ^{Da}	27.6 ± 2.4 ^{Ba}	-
	CSC+CH	788.6 ± 10.0 ^{Ab}	-	37.7 ± 0.0 ^{Da}	12.3 ± 1.1 ^{Cb}	-
	CSC+MC	607.2 ± 10.5 ^{Ac}	-	51.7 ± 10.7 ^{Da}	15.1 ± 2.5 ^{Bb}	-
<i>S. commune</i>	CSC	423.0 ± 94.6 ^{Bb}	80.5 ± 3.4 ^{Ba}	1593.3 ± 259.7 ^{Ab}	30.9 ± 1.4 ^{ABa}	11.7 ± 5.1 ^{Ab}
	CSC+CH	658.4 ± 20.6 ^{Ba}	16.5 ± 2.6 ^{Cb}	1132.0 ± 103.5 ^{Ac}	16.8 ± 1.5 ^{Ac}	36.5 ± 12.2 ^{Aa}
	CSC+MC	454.6 ± 28.9 ^{Bb}	25.5 ± 6.6 ^{Cb}	2321.0 ± 99.0 ^{Aa}	20.3 ± 0.8 ^{Ab}	18.5 ± 16.7 ^{Ab}
<i>P. pulmonarius</i>	CSC	59.4 ± 1.2 ^{CDb}	97.3 ± 0.1 ^{Aa}	808.2 ± 23.7 ^{BCc}	30.4 ± 2.6 ^{ABa}	34.3 ± 5.5 ^{Aa}
	CSC+CH	344.8 ± 37.0 ^{Ca}	56.3 ± 4.7 ^{Bc}	1111.9 ± 203.5 ^{ABb}	16.7 ± 1.9 ^{Ab}	36.0 ± 15.7 ^{Aa}
	CSC+MC	70.5 ± 0.9 ^{Bb}	88.4 ± 0.2 ^{Ba}	1267.5 ± 96.0 ^{Ba}	18.8 ± 2.8 ^{Ab}	10.8 ± 9.3 ^{Ab}
<i>F. hepatica</i>	CSC	57,76 ± 3.4 ^{Dab}	97.3 ± 0.2 ^{Aa}	621.7 ± 25.8 ^{Ca}	32.4 ± 2.3 ^{Aa}	17.0 ± 8.4 ^{Ab}
	CSC+CH	90.56 ± 42.7 ^{Da}	88.5 ± 5.4 ^{Ab}	676.7 ± 33.7 ^{Ca}	16.5 ± 2.1 ^{Ac}	34.0 ± 16.7 ^{Aa}
	CSC+MC	6.5 ± 11.3 ^{Cb}	98.9 ± 1.9 ^{Aa}	844.7 ± 200.6 ^{Ca}	21.9 ± 1.6 ^{Ab}	44.7 ± 10.7 ^{Aa}
	CSC	65.7 ±	95.5 ± 0.8 ^{Aab}	819.7 ± 234.8 ^{BCa}	31.7 ± 1.8 ^{Aa}	14.8 ± 6.5 ^{Ab}

<i>P. lecomtei</i>		17.0 ^{CDa}				
	CSC+CH	102.1 ±	87.1 ± 5.8 ^{Ab}	1291.0 ± 120.1 ^{Aa}	15.3 ± 1.2 ^{ABc}	24.0 ± 9.8 ^{ABab}
		45.6 ^{Da}				
	CSC+MC	3.5 ±	99.4 ± 1.0 ^{Aa}	899.8 ± 208.9 ^{Ca}	21.4 ± 3.5 ^{Ab}	41.6 ± 23.1 ^{Aa}
		7.4 ^{Ca}				
	CSC	206.8 ±	90.5 ± 1.7 ^{Aa}	1090.6 ± 278.9 ^{Ba}	30.9 ± 4.1 ^{ABa}	11.7 ± 14.7 ^{Ab}
<i>G. lucidum</i>		66.1 ^{Cb}				
	CSC+CH	339.4 ±	57.0 ± 4.7 ^{Bb}	871.9 ± 25.5 ^{BCa}	14.0 ± 1.0 ^{BCc}	13.5 ± 8.0 ^{Ab}
		37.0 ^{Ca}				
	CSC+MC	37.2 ±	93.9 ± 3.6 ^{ABa}	869.4 ± 20.5 ^{Ca}	20.8 ± 2.3 ^{Ab}	7.7 ± 3.0 ^{Aa}
		17.3 ^{Bc}				

d.w.: dried weight; CP: crude protein; CSC: cottonseed cake; CH: coconut husks; MC: macauba cake. Means followed by the same letter are not different by Tukey test (p<0,05). Capital letters represent differences between treatments in the same biomass and lowercase letters represent differences between biomasses in the same treatment.

Table 3 Antioxidant activity and total phenolic of pure Jatropha seed cake and Jatropha seed cake with lignocellulosic material (coconut husks and macauba cake) treated with five basidiomycetes.

Treatment	Biomass	DPPH• (ug Trolox/mg extract)	ABTS+• (ug Trolox/mg extract)	TPC (ug GA/mg extract)
Uncolonized	JSC	109.4 ± 1.0 ^{Aa}	147.9 ± 9.3 ^{Ac}	49.3 ± 4.5 ^{Aa}
	JSC+CH	77.3 ± 3.1 ^{Ab}	202.9 ± 0.8 ^{Aa}	36.7 ± 1.7 ^{Ab}
	JSC+MC	80.8 ± 2.5 ^{Ab}	176.4 ± 5.5 ^{Ab}	30.8 ± 3.2 ^{Ab}
<i>S. commune</i>	JSC	31.0 ± 13.7 ^{Ba}	40.4 ± 12.5 ^{Ba}	20.5 ± 0.3 ^{Ba}
	JSC+CH	15.0 ± 4.7 ^{Ca}	64.5 ± 7.9 ^{BCa}	6.8 ± 1.2 ^{Bb}
	JSC+MC	17.8 ± 1.6 ^{BCa}	19.5 ± 9.5 ^{Ca}	7.7 ± 1.5 ^{BCb}
<i>P. pulmonarius</i>	JSC	17.9 ± 4.1 ^{BCa}	18.5 ± 8.8 ^{Bb}	10.5 ± 1.7 ^{Ca}
	JSC+CH	56.0 ± 5.5 ^{Ba}	97.2 ± 23.6 ^{Ba}	8.7 ± 2.5 ^{Bb}
	JSC+MC	15.9 ± 3.6 ^{Ca}	27.0 ± 13.6 ^{Cb}	0.0 ± 0.0 ^{Db}
<i>F. hepatica</i>	JSC	8.0 ± 3.9 ^{BCa}	29.3 ± 8.3 ^{Bb}	4.0 ± 2.3 ^{Cb}
	JSC+CH	14.5 ± 2.6 ^{Ca}	43.7 ± 8.1 ^{BCab}	10.2 ± 1.7 ^{Ba}
	JSC+MC	27.8 ± 5.2 ^{Ba}	55.1 ± 6.3 ^{Ca}	9.2 ± 2.7 ^{Bab}
<i>P. lecomtei</i>	JSC	2.4 ± 0.2 ^{Cb}	42.1 ± 3.7 ^{Ba}	0.0 ± 0.0 ^{Cb}
	JSC+CH	12.3 ± 4.4 ^{Cab}	23.2 ± 3.2 ^{Ca}	14.0 ± 0.7 ^{Ba}
	JSC+MC	23.6 ± 1.1 ^{BCa}	6.4 ± 0.9 ^{Ca}	0.0 ± 0.0 ^{Db}
<i>G. lucidum</i>	JSC	19.1 ± 6.2 ^{BCa}	62.4 ± 13.6 ^{Ba}	17.3 ± 3.0 ^{Bab}
	JSC+CH	54.3 ± 1.7 ^{Ba}	48.7 ± 8.7 ^{BCa}	31.8 ± 6.8 ^{Aa}
	JSC+MC	26.5 ± 5.2 ^{BCa}	35.3 ± 9.5 ^{Ca}	4.0 ± 0.3 ^{CDb}

TPC: Total phenolic content; GA: Gallic acid; JSC: Jatropha seed cake; CH: coconut husks; MC: macauba cake. Means followed by the same letter are not different by Tukey test (p<0,05). Capital letters represent differences between treatments in the same biomass and lowercase letters represent differences between biomasses in the same treatment.

Table 4. Antioxidant activity and total phenolic of pure cottonseed cake and cottonseed cake with lignocellulosic material (coconut husks and macauba cake) treated with five basidiomycetes.

Treatment	Biomass	DPPH• (ug Trolox/mg extract)	ABTS+• (ug Trolox/mg extract)	TPC (ug GA/mg extract)
Uncolonized	CSC	53.5 ± 1.9 ^{Aa}	122.1 ± 7.6 ^{Ab}	21.3 ± 1.2 ^{Aa}
	CSC+CH	34.6 ± 3.7 ^{Ab}	125.9 ± 3.3 ^{Ab}	24.3 ± 1.0 ^{Aa}
	CSC+MC	42.3 ± 3.3 ^{Aa}	147.9 ± 7.0 ^{Aa}	16.0 ± 2.2 ^{Ab}
<i>S. commune</i>	CSC	14.1 ± 9.1 ^{Cb}	37.2 ± 22.6 ^{BCb}	12.0 ± 3.7 ^{Ba}
	CSC+CH	19.5 ± 2.9 ^{BCa}	56.2 ± 7.3 ^{Ca}	9.3 ± 3.3 ^{Ba}
	CSC+MC	16.0 ± 1.2 ^{Ba}	48.2 ± 7.8 ^{Ba}	0.0 ± 0.0 ^{Ba}
<i>P. pulmonarius</i>	CSC	12.3 ± 2.9 ^{BCa}	23.1 ± 7.0 ^{Cb}	5.8 ± 0.7 ^{BCb}
	CSC+CH	11.0 ± 2.4 ^{Ca}	34.9 ± 6.5 ^{Ba}	9.2 ± 0.9 ^{Ba}
	CSC+MC	18.7 ± 10.4 ^{Ba}	15.3 ± 0.9 ^{Cb}	0.0 ± 0.0 ^{Bc}
<i>F. hepatica</i>	CSC	4.8 ± 4.6 ^{Cb}	23.1 ± 7.0 ^{BCb}	12.8 ± 2.0 ^{Aba}
	CSC+CH	20.2 ± 0.6 ^{Ba}	52.6 ± 6.4 ^{CDa}	14.5 ± 3.7 ^{Aba}
	CSC+MC	18.9 ± 0.6 ^{Ba}	30.8 ± 2.4 ^{BCb}	17.0 ± 2.3 ^{Aa}
<i>P. lecomtei</i>	CSC	4.2 ± 3.7 ^{BCa}	15.3 ± 6.2 ^{BCa}	1.7 ± 0.2 ^{Ca}
	CSC+CH	15.4 ± 5.5 ^{BCa}	24.6 ± 3.5 ^{Da}	2.3 ± 1.8 ^{Ba}
	CSC+MC	21.3 ± 9.9 ^{Ba}	13.8 ± 2.6 ^{Ca}	0.0 ± 0.0 ^{Ba}
<i>G. lucidum</i>	CSC	13.1 ± 6.5 ^{Ba}	34.5 ± 6.8 ^B	14.0 ± 5.3 ^{Aba}
	CSC+CH	19.5 ± 3.3 ^{BCa}	56.6 ± 18.2 ^C	7.2 ± 4.5 ^{Bab}
	CSC+MC	19.5 ± 10.8 ^{Ba}	34.1 ± 4.7 ^{BC}	0.0 ± 0.0 ^{Bb}

TPC: Total phenolic content; GA: Gallic acid; CSC: Cottonseed cake; CH: Coconut husks; MC: Macauba cake. Means followed by the same letter are not different by Tukey test (p<0,05). Capital letters represent differences between treatments in the same biomass and lowercase letters represent differences between biomasses in the same treatment.

Supplementary Material

Supplementary material is not available in this version.

Figures

Figure 1

Growth rate (cm/day) of seven basidiomycetes colonized in jatropha seed cake, jatropha seed cake with coconut husks and jatropha seed cake with macauba cake during 15 days in 10 cm jars. JSC: Jatropha seed cake; CH: Coconut husks; MC: Macauba cake. Means followed by the same letter are not different by Tukey test ($p < 0,05$).

Figure 2

Growth rate (cm/day) of seven basidiomycetes colonized in cottonseed cake, cottonseed cake with coconut husks and cottonseed cake with macauba cake during 15 days in 10cm jars. CSC: Cottonseed cake; CH: Coconut husks; MC: Macauba cake. Means followed by the same letter are not different by Tukey test ($p < 0,05$).

Supplementary Files

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