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ARTICLE

Compositional analysis of cashew (*Anacardium occidentale* L.) peduncle bagasse ash and its *in vitro* antifungal activity against *Fusarium* species

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ABSTRACT: (Compositional analysis of cashew (*Anacardium occidentale* L.) peduncle bagasse ash and its *in vitro* antifungal activity against *Fusarium* species). Cashew (*Anacardium occidentale* L.) is a plant with a highly social and economic importance in the Northeast Region of Brazil. Cashew peduncle bagasse is one of the greatest sources of residues (90–94%) produced by the cashew agronomic industry. In this study, we prepared cashew peduncle bagasse ash and submitted it to compositional analysis and *in vitro* tests for antifungal activity against *Fusarium* species. This analysis indicated a crystallinity of around 73%, corresponding to the following soluble phases: potassium bicarbonate - $KHCO_3$ (39.54%), potassium sulfate - K_2SO_4 (24.87%), and struvite-K - $MgKPO_4{\cdot}6H_2O$ (8.59%). The amorphous phases (around 27%) were identified as the insoluble fraction of the ash. The solution showed high antifungal activity against *F. oxysporum*, *F. moniliforme* and *F. lateritium*. The activity of this product was greater than that of Cercobin® (thiophanate-methyl), indicating that this material could possibly be used as a non-toxic antifungal agent.
Key words: antifungal activity, compositional analysis, *Fusarium*, cashew peduncle, residues.

RESUMO: (Análise da composição das cinzas do bagaço do pedúnculo do cajú (*Anacardium occidentale L.*) e sua atividade antifúngica *in vitro* contra espécies de *Fusarium.*). O Cajueiro *(Anacardium occidentale* L.*)* é uma planta com uma grande importância social e econômica no Nordeste do Brasil. O bagaço do pedúnculo do caju é uma das maiores fontes de resíduos (90-94%) produzidos pela indústria cajueira. Neste estudo, foram preparadas cinzas do bagaço e submetidas à análise da composição e a testes de atividade antifúngica *in vitro* contra espécies de *Fusarium.* Esta análise indicou uma cristalinidade em torno de 73%, correspondendo às seguintes fases solúveis: bicarbonato de potássio - $KHCO_3$ (39,54%), sulfato de potássio - K_2SO_4 (24,87%), e estruvita-K - $MgKPO_4 \bullet 6H_2O$ (8,59%). As fases amorfas (cerca de 27%) foram identificadas como a fração insolúvel de cinzas. A solução apresentou alta atividade antifúngica contra *F. oxysporum, F. moniliforme e F. lateritium.* Sua ação foi maior do que o Cercobin® (tiofanato metílico), indicando uma possível utilização como um agente antifúngico não tóxico.
Palavras-chave: atividade antifúngica, caracterização, pedúnculo do caju, *Fusarium*, resíduos.

INTRODUCTION

The cashew tree (*Anacardium occidentale* L.) occupies an important position among tropical fruit trees because of the growing commercialization of its main products: the nut and the cashew "apple" (i.e., the peduncle) (Fig. 1A). Cashew is one of the major crops in the Northeast Region of Brazil, where it is grown mainly in the states of Ceará, Rio Grande do Norte and Piauí. The cashew peduncle bagasse (Fig. 1B), the by-product of the juice extraction process, represents approximately 20% of the total weight of the peduncle (AGRIANUAL 2000). It is one of the greatest sources of residues (90–94%) produced by the cashew agronomic industry (Santos *et al.* 2007), and its exploitation is restricted to its use as a nutritional supplement in animal food. However, this utilization is limited because of its fast degradation, making it impossible to store. An alternative to recycling this agro-industrial residue involves its conversion to inorganic compounds through an incineration process (ash production) (Ogada & Werther 1996). Santos *et al.* (2007) synthesized potassium bicarbonate ($KHCO_3$), potassium sulfate (K_2SO_4), struvite-K – ($MgKPO_4{\cdot}6H_2O$), and small amounts of $Mg_3(Si_2O_5)(OH)_4$ and $NaAl(HPO_4)_2$ by burning cashew peduncle bagasse. Potassium bicarbonate is classified by the FDA (Food and Drug Administration) as a food ingredient "generally recognized as safe." It is used in various ways, for example, in the production of crystals, special glass, ceramics, enamels, potassium silicates, fertilizers and fire extinguishers, in the reduction of cation loss in acid and neutral soils, in drug synthesis (vitamins and penicillin), and in the food industry. It is also used as an antacid and fungicide ($KHCO_3$ Handbook

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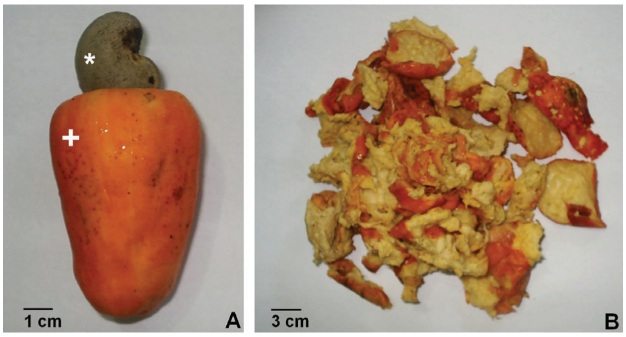

Figure 1. Cashew (A) and peduncle bagasse (B) of *Anacardium occidentale*. The asterisk in (A) indicates the fruit (nut) and the plus sign (+) indicates the cashew "apple" (peduncle).

2004). Potassium sulfate is frequently included as part of chemical mixtures, glues and fertilizers. Struvite-K is considered a premium quality slow-release fertilizer (Bashan & Bashan 2004).

The control of phytopathogenic fungi is of great economic importance since fungal growth inhibits the production of roots, stems, foliage, fruits and seeds, and diminishes the overall quality of a cultivated crop. Because of the vast economic ramifications of fungal propagation in agricultural and horticultural operations, a broad spectrum of fungicidal and fungistatic products has been developed for general and specific applications (McGrath 2009). Selected inorganic salts have been evaluated for their fungicidal properties (Deliopoulos *et al.* 2010). It is known that bicarbonate and carbonate compounds are toxic to several fungi (Arslan *et al*. 2006, Karabulut *et al*. 2006, Nigro *et al.* 2006, Dorn *et al.* 2007).

Fusarium Link ex Fr. is a genus of filamentous phytopathogenic fungi widely distributed in plants and in soil. It is found in the normal mycoflora of commodities, such as rice, bean, soybean, and other crops (Leslie & Summerell 2006). In addition to being a common contaminant and a well-known plant pathogen, *Fusarium* species can cause various infections in humans. *Fusarium* is one of the emerging causes of opportunistic mycoses (Lucca 2007). Some species produce mycotoxins (such as fumonisins and trichothecenes) in cereal crops, which can affect human and animal health if they enter the food chain (Howard 2003, Kokkonen *et al*. 2010). For plants, azoxystrobin, thiophanate methyl, fludioxonil, and triflumizole are the most effective fungicides available against *Fusarium*, but have to be used carefully due to their toxic effects (Elmer & McGovern 2004, Sichel 2007, Hashem *et al*. 2010). In human infections, amphotericin B (AMB) is the drug used for the clinical treatment of fusariosis, despite its nephrotoxicity and its low efficacy (Ortoneda *et al* 2004). However, studies performed by Hang & Woodams (2003) showed that low concentrations of sodium and potassium bicarbonate (0.2 g per 100 mL) inhibit more than 95% of mycelial growth of *F. oxysporum* 950 in Czapek Dox. *Fusarium oxysporum* is one of the most drug-resistant fungi (*in vitro* and *in vivo*) found in disseminated infections (Ortoneda *et al* 2004, Pachón *et al*. 2006).

The aim of this work was to produce and characterize cashew (*Anacardium occidentale* L.) peduncle bagasse ash and to evaluate its efficacy as an antifungal agent for controlling the mycelial growth of *F. oxysporum*, *F. moniliforme*, *F. decemcellulare* and *F. lateritium*.

MATERIALS AND METHODS

Preparation of the ash

The samples of red cashew peduncles used were obtained from plantations in the state of Ceará, Brazil, during the months of September and October, in 2008. After extracting the nut, the peduncles were pressed to remove the juice, producing the cashew bagasse. The bagasse was then dried in an oven at 36ºC for 48 h and ground in an analytical mill. The bagasse powder was burned at 640ºC in a muffle-type oven in an air atmosphere for 4 h (Santos *et al.* 2007), resulting in cashew peduncle bagasse ash (CPBA).

Compositional analysis

The composition of CPBA was determined by energy dispersive X-ray analysis (EDX) using a Philips XL-30 scanning electron microscope. The X-ray diffraction (XRD) patterns for the mineralogical analysis were obtained at room temperature (300 K) using a Philips powder diffractometer (model X'Pert PRO). Bragg–Bretano geometry was used with Cu-ka radiation. The tube was operated at 40 kV and 40 mA. The diffraction data were collected over the range of $10^o \leq 2\theta \leq 60^o$ with 0.02º steps and an integration time of 2 s per point. Crystallinity (%) was obtained from the percentile ratio between the crystalline area and total area on the X-ray diffractogram (Hayakawa *et al.* 1997). X´Pert HighScore software was used to identify crystalline phases, and Rietveld's method (Rietica software) was used for structure refinement and consequent quantitative analysis.

The insoluble fraction of the ash was determined by a gravimetric method. A 0.5 mg amount of CPBAA was suspended in 1 mL Milli-Q water, stirred for at least 5 min, and centrifuged (16,000 rcf, 5 min), and the supernatant (soluble compounds) was withdrawn with a micropipette. The precipitate was re-suspended in 1 mL Milli-Q water, stirred for at least 2 min and centrifuged (16,000 rcf, 5 min), and the supernatant was again removed. This process was repeated three times, guaranteeing the total recovery of the soluble constituents. The precipitate from the supernatant extraction was then dried in an oven at 36 ºC for 48 h, and its mass determined with an analytical balance. The insoluble fraction was obtained (mean value of five repetitions) from the percentile ratio between the mass of the residue and the mass of the CPBA

Fungicidal assay

Fusarium oxysporum (URM-2489), *F. moniliforme* (URM-3226), *F. decemcellulare* (URM-3006) and *F. lateritium* (URM-2491) were obtained from the culture collections of the Department of Mycology, Federal University of Pernambuco, Brazil. The assay of CPBA for antifungal activity was performed according to a modified assay based on the method of Cunico *et al.* (2004). CPBA (0.5 mg) was dissolved in 1 mL of 0.15 M NaCl and centrifuged. The soluble fraction (CPBA solution) was separated from precipitated residues. The assay was also performed with $KHCO_3$ (0.20 mg) dissolved in 1 mL of 0.15 M NaCl (due to its use as a fungicide); 0.15 M NaCl served as the negative control or blank test, and 10 ppm Cercobin® 700 PM (a benzimidazol fungicide manufactured by IHARABRAS S.A., Brazil) as the positive control. The $KHCO_3$ concentration used in the assay (0.20 mg/ml) was the same as that found in the CPBA solution (after compositional analysis). The Cercobin® concentration used was that considered effective against *Fusarium* species (according to the manufacturer). Each experiment was carried out in 100 mm × 15 mm Petri dishes containing 10 mL of sterile potato-dextrose-agar (PDA) medium (15 g of agar, 20 g of dextrose, and 200 g of cooked potato). An aliquot of each sample or control was filtered using a 0.45-μm sterile syringe filter (Minisart®). Next, the sample (50 μL) was spread on solidified PDA medium in Petri dishes and a fungal mycelium disk (0.625 cm in diameter) was placed in the center of the Petri dish. The plates were incubated at 25 ºC for 72 h, and the colony growth zone diameter was determined for each solution. Antifungal activity was determined based on percent growth inhibition, according to the following formula (Rojas & Rondón 1995):

$$\text{Growth inhibition}(\%) = \frac{GZD_{nc} - GZD}{GZD_{nc}} \times 100$$

where GZD_{nc} is the growth zone diameter of the negative control (NaCl solution), and GZD is growth zone diameter of the Cercobin®, CPBA, and $KHCO_3$ solutions. A schematic representation of the experimental procedure is shown in Fig. 2.

All assays were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). An Anova test (Tukey post hoc) was performed to determine significant differences between groups (Triola 2008). The significance level used was 0.01 ($P \leq 0.01$). All statistical analyses were performed using OriginPro 7.5 software (OriginLab Corporation).

RESULTS AND DISCUSSION

Compositional analysis

EDX measurements of the CPBA powder indicated the presence of carbon (C), oxygen (O), phosphorous (P), potassium (K), magnesium (Mg), and sulfur (S) in the ash (Fig. 3). The XRD diffractogram of CPBA is shown in Fig. 4. Crystallinity was around 73%, indicating the presence of amorphous phases (27%). The crystallographic analysis indicated the presence of potassium bicarbonate - $KHCO_3$ (39.54%), potassium sulfate - K_2SO_4 (24.87%) and struvite-K - $MgKPO_4 \cdot 6H_2O$ (8.59%). The degree of adjustment refinement (χ^2) was 1.15 with residues Rp = 4.50 and Rwp = 1.95. The space group identified for $KHCO_3$ was the C/2m monoclinic. The space groups identified for K_2SO_4 were the orthorhombic Pmcn (α-K_2SO_4) and the Pman (β-K_2SO_4). The struvite--K ($MgKPO_4$ hexahydrate) of the ash is a phosphate compound analogous to struvite ($NH_4MgPO_4 \cdot 6H_2O$). The insoluble fraction was around 27%. Because the crystalline phases determined by XRD are soluble, the insoluble compounds are then the amorphous phases of CPBA. The compositional analysis showed that a solution of 0.5 mg of CPBA per 1 mL of 0.15M NaCl (concentration used in the assay for antifungal activity) should correspond to approximately 0.20 mg of $KHCO_3$, 0.12 mg of K_2SO_4, and 0.04 mg of $MgKPO_4 \cdot 6H_2O$.

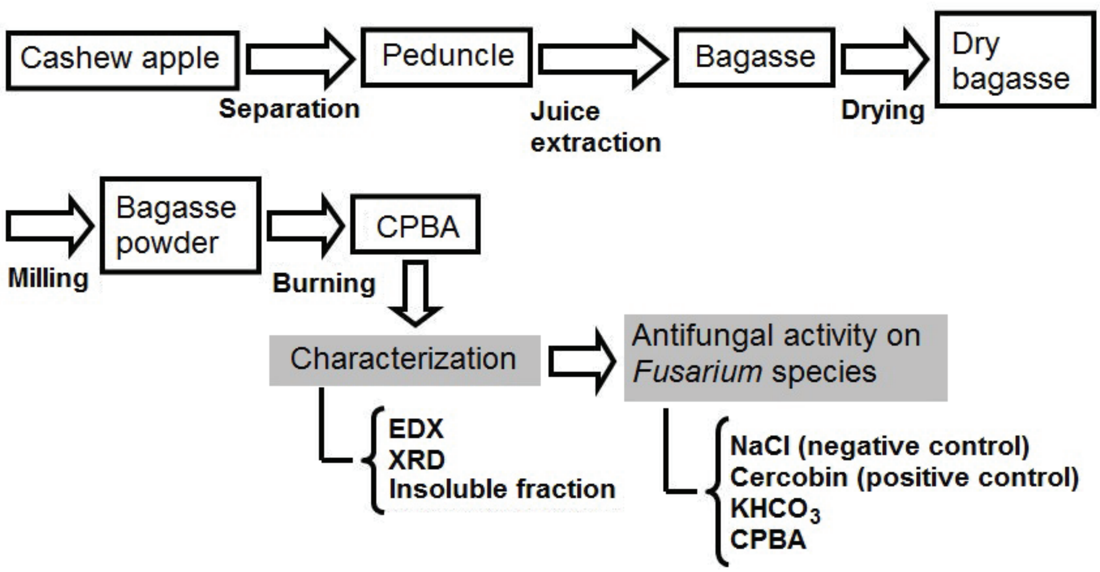

Figure 2. Schematic representation of the preparation, characterization, and antifungal activity of the cashew peduncle bagasse ash.

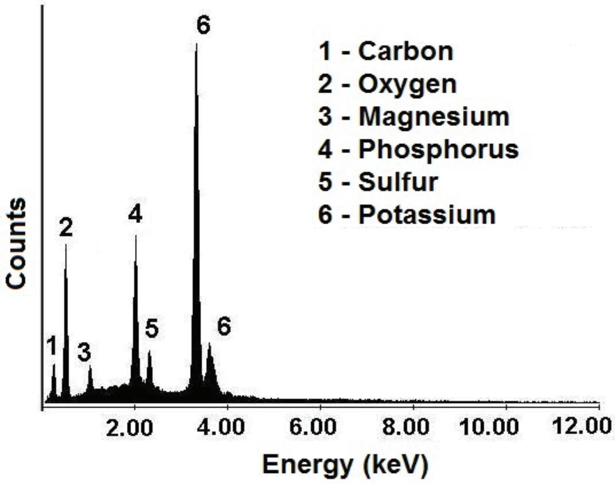

Figure 3. Energy dispersive x-ray analysis of the cashew peduncle bagasse ash, showing its main chemical elements: C, O, Mg, P, S and K.

The compositional analysis of the CPBA powder indicated the presence of the main crystalline phases reported by Santos *et al.* (2007), but in different concentrations. We did not detect the presence of $Mg_3(Si_2O_5)(OH)_4$ and $NaAl(HPO_4)_2$, as in the study of Santos *et al*. (2007). These differences can be associated with variations in the peduncle composition and in the preparation process of the ash (particles size after milling, heating/cooling rate and oxygen concentration during the burning, etc.). New experiments should be carried out to evaluate the influence of these factors on ash composition.

$KHCO_3$ is a crystalline solid synthetic, produced by the chemical reaction of potassium carbonate and carbon dioxide via electrolysis. K_2SO_4 has a higher manufacturing cost compared to KCl, another compound largely used to produce K fertilizers (FAO 1984). The use of $MgNH_4PO_4 \cdot 6H_2O$ is limited due to the costs of its production. Thus, obtaining these compounds using low-cost processes (burning) and a by-product of the cashew industry (bagasse) could be an economically viable alternative for the use of these residues.

Fungicidal assay

The antifungal activity (percent growth inhibition) was evaluated by the decrease in GZD, as compared to the NaCl solution (negative control), as shown in Fig. 5. Cercobin® solution (positive control) inhibited the growth (GZD decrease) of all the *Fusarium* species studied ($P<0.01$). CPBA solution had a statistically significant antifungal action ($P<0.01$) against *F. oxysporum*, *F. moniliforme,* and *F. lateritium* species, when compared to the negative control. CPBA showed a growth zone decrease larger than the Cercobin® solution ($P<0.01$). The $KHCO_3$ solution also showed antifungal activity ($P<0.01$) against *F. oxysporum*, *F. moniliforme* and *F. lateritium*. The growth inhibition zone of the $KHCO_3$ solution was smaller than that of Cercobin® and CPBA solutions ($P<0.01$). The CPBA and $KHCO_3$ solutions did not show statistically significant differences in mean growth inhibition ($P>0.01$) of *F. decemcellulare* as compared to the negative control, which demonstrated no antifungal activity against this species. Thus, they did not have an antifungal effect on this *Fusarium* species.

Thiophanate-methyl (active ingredient of Cercobin®) is a strong systemic antifungal agent (toxic), reported to be efficient in the control of *Fusarium* species (Simone & Chese 1989, Zidan *et al.* 2000). The results of the experiments confirmed its antifungal action in all the *Fusarium* species studied (percent growth inhibition greater than 25%).

K_2SO_4 and $MgKPO_4 \cdot 6H_2O$ K_2SO_4, present in CPBA, are not reported to be anti-fungal substances. Besides,

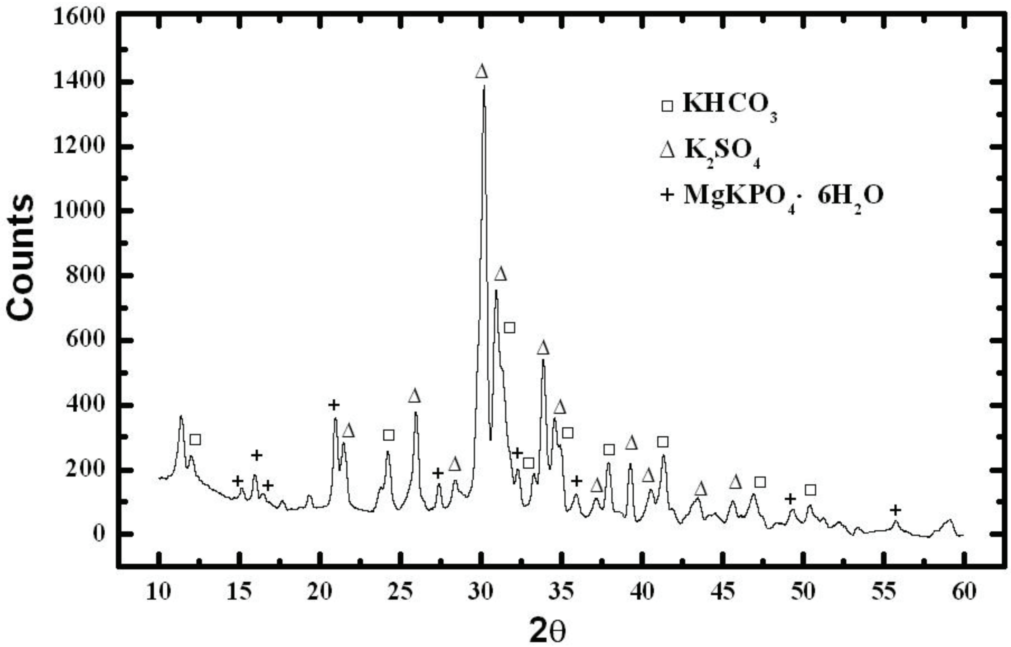

Figure 4. X-ray diffraction of the cashew peduncle bagasse ash, showing its crystalline phases: $KHCO_3$, K_2SO_4 and $MgKPO_4 \cdot 6H_2O$.

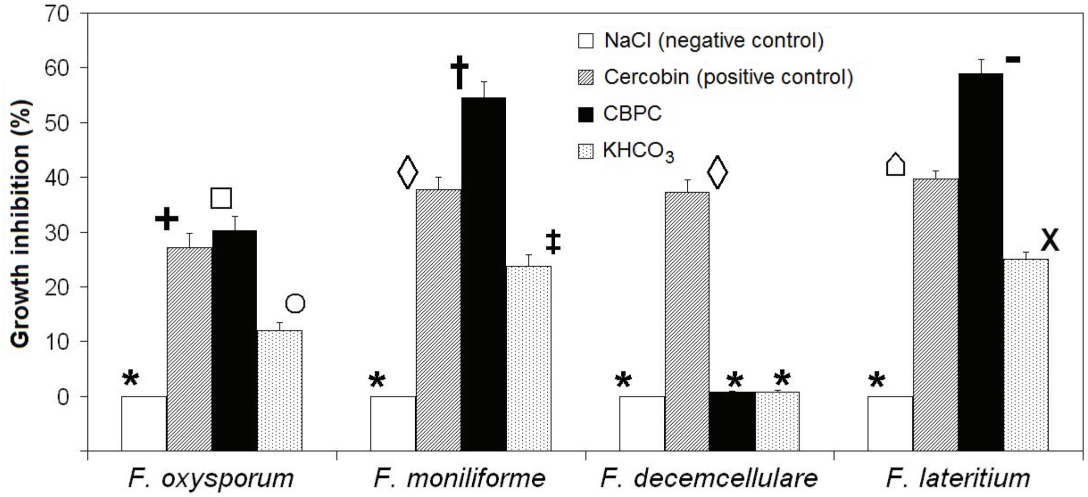

Figure 5. Percent growth inhibition (mean ± SD) for the antifungal activity assay against *F. oxysporum*, *F. moniliforme*, *F. decemcellulare* and *F. lateritium*. Different symbols above bars indicate statistically significant differences between means (P<0.01).

the assays of antifungal activity showed that CPBA and $KHCO_3$ solutions were respectively inhibitory and non--inhibitory in the same *Fusarium* species. Therefore, the antifungal activity of the CPBA solution was attributed mainly to $KHCO_3$. Bicarbonate ion (HCO_3^-) has been identified as a probable cause of mycelial growth inhibition in fungi. The bicarbonate causes the collapse of hyphal walls and shrinkage of conidia. Basically, $KHCO_3$ shows the following antifungal effects: buffers a high pH that is detrimental to spores; inhibits reproduction by creating an unfavorable environment for the emergence of germ tubes; elevated pH inhibits enzymes that are key to fungal growth and development; disrupts normal cell physiology; and causes mycelia to shrivel and dehydrate. The difference in the intensity of antifungal activity between CPBA and $KHCO_3$ solutions should be investigated. Increased pH also plays a significant role in antifungal activity (Deliopoulos *et al.* 2010). A possible action of combined K_2SO_4 and $MgKPO_4 \cdot 6H_2O$ K_2SO_4 can also be associated with this activity. Further studies involving the use of different concentrations of CPBA, $KHCO_3$, K_2SO_4 and $MgKPO_4 \cdot 6H_2O$ K_2SO_4 (separately and combined), pH levels, and microscopic analysis of the fungus (scanning electron and atomic force) should be performed to explain this effect.

Another important result of this study was the fungicidal action of CPBA (except against *F. decemcellulare*), as compared to Cercobin®. CPBA was shown to be a possible alternative to the use of thiophanate-methyl (a toxic antifungal agent) on *F. oxysporum*, *F. moniliforme*, and *F. lateritium*. *Fusarium oxysporum* causes vascular wilt diseases in a wide range of crops. Hosts of *F. oxysporum* include potato, sugarcane, garden bean, cowpea, prickly pear, cultivated zinnia, pansy, Assam rattlebox, Baby's breath, and *Musa* sp. (Armstrong & Armstrong 1981, Raabe *et al.* 1981). *Fusarium moniliforme* (=*F. verticillioides*) has been reported (Maiorano *et al*. 2009) to be the cause of ear, stalk, and root rots of corn. The rotting of the corn plant causes very serious crop losses. Chlorotic leaf distortion (CLD) is an unusual disorder of sweet potato caused by *F. lateritium (Hyun & Clark 1998). Therefore,* CPBA *could be a* non-toxic antifungal agent of great economic importance in the control of phytopathogens and the mycotoxicity caused by this *Fusarium* species.

CONCLUSIONS

In conclusion, we produced and characterized cashew (*Anacardium occidentale* L.) peduncle bagasse ash, and tested its antifungal activity on *Fusarium* species. The solution of this residue showed antifungal activity greater than that of thiophanate-methyl against *F. oxysporum*, *F. moniliforme* and *F. lateritium*. The ash produced from cashew peduncle bagasse has great potential to be a powerful non-toxic fungicidal agent against certain *Fusarium* species, and experiments using this agent on crops should be performed. In addition, due to its composition, this agro-industrial residue is a promising non-perishable source of potassium, sulfur, phosphorus and magnesium for fertilizers, and has other diverse applications because it contains potassium bicarbonate.

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