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Interference of aqueous and ethanolic solutions of *Adiantum latifolium* Lam. (Pteridaceae) leaves on in vitro *Ceratocystis cacaofunesta* mycelial growth

Interferência de soluções aquosas e alcoólicas de folhas de **Adiantum latifolium** *Lam. (Pteridaceae) no crescimento micelial de* **Ceratocystis cacaofunesta** *in vitro*

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ABSTRACT: *Ceratocystis cacaofunesta* is the etiologic agent of "Ceratocystis wilt of cacao", an irreversible disease that affects the vascular system of the plant. The management of the disease is difficult and economic and alternative solutions are needed. The medicinal plants compounds are known to have antimicrobial activity, and they could be an alternative choice in the *C. cacaofunesta* control. Considering this, this work aimed to verify the in vitro antifungal activity of aqueous and alcoholic solutions of *Adiantum latifolium* leaves on *C. cacaofunesta*. Plant material was collected at Atlantic Forest biome in cacao cultivation area in South of Bahia state. Aqueous and ethanolic solutions were made by boiling and maceration in 70% ethanol, respectively. After filtration, they were added to culture medium at 1, 5 and 10% dilution. A 7 mm disc colony of *C. cacaofunesta* was inoculated in the middle of the well containing Sabouraud dextrose agar (SDA) and the mycelial growth was observed. Controls consisted on SDA with sterile water or 70% ethanol at the same dilution of treatments, and Tebuconazole at 4 $\mu g.mL^{-1}$. Neither aqueous nor ethanolic solutions inhibited the mycelial growth. However, aqueous solution presence induced a higher mycelial growth rate. Conversely, aqueous solution treatment induced mycelial growth. Tebuconazole showed important mycelial growth inhibition and it could be considered in *C. cacaofunesta* propagation control in areas where genetic selection or handling management still fail.

KEYWORDS: maidenhair fern; Cocoa; mold; Atlantic Forest.

RESUMO: A espécie *Ceratocystis cacaofunesta* é o agente etiológico do mal-do-facão, patogenia caracterizada por danos irreversíveis no sistema vascular da planta. O controle da doença é difícil e a busca por soluções alternativas e econômicas é necessária. Sabe-se que os compostos das plantas medicinais possuem atividade microbiana e podem ser uma opção alternativa no controle de *C. cacaofunesta*. Baseado nisso, esse trabalho se propôs a verificar *in vitro* o potencial antifúngico das soluções aquosa e alcóolica de *Adiantum latifolium* sobre *C. cacaofunesta*. O material vegetal foi coletado no bioma Mata Atlântica em área de plantio de cacau, no sul da Bahia. Solução aquosa foi obtida por decocção e solução etanólica por maceração em etanol 70%. As soluções foram filtradas e adicionadas ao meio de cultura em diluições de 1, 5 e 10%. Inocularam-se fragmentos de 7 mm de colônia de *C. cacaofunesta* no centro do meio de cultura contendo ágar Sabouraud dextrose (ASD) e se observou o crescimento do disco micelial. Os controles consistiram em SDA com água estéril ou etanol a 70% na mesma diluição de tratamentos e o antifúngico Tebuconazol a 4 $\mu g.mL^{-1}$. Nenhuma concentração das soluções aquosa e alcóolica inibiu o crescimento micelial. Entretanto, a presença de solução aquosa induziu maior crescimento micelial. O antifúngico Tebuconazol apresentou efeito redutor importante do crescimento micelial e pode ser uma alternativa no controle da propagação do *C. cacaofunesta* em locais onde a seleção genética e o manejo adequado de instrumentos no momento da poda apresentam falhas.

PALAVRAS-CHAVE: avenca; cacaueiro; mal-do-facão; Mata Atlântica.

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Ceratocystis wilt is a fungal disease characterized by wilting and death of cacao (*Theobroma cacao* L.) (OLIVEIRA; LUZ, 2005). Along with witches' broom (*Moniliophthora* (syn. *Crinipellis*) *perniciosa*) and frosty pod rot (*Moniliophthora roreri*) constitutes the main important agent of production losses in North-eastern Brazilian cacao crops (OLIVEIRA; LUZ, 2005; ENGELBRECHT et al., 2007).

The etiologic agent in Brazilian crops is *Ceratocystis cacaofunesta* Engelbr. & T.C. Harr. (BASTOS; EVANS, 1978; BAKER et al., 2003; ALMEIDA et al., 2005; ENGELBRECHT; HARRINGTON, 2005) a species belonging to *Ceratocystis fimbriata* complex (ENGELBRECHT et al., 2007; FERREIRA et al., 2010; CAB INTERNATIONAL, 2019).

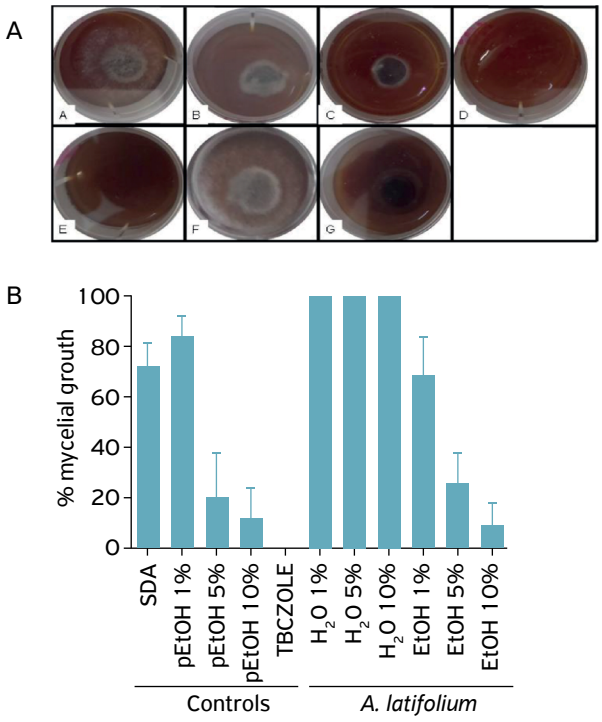
Fungicides, hygienic techniques (OLIVEIRA; LUZ, 2005) and genetic resistance selection of cacao trees (SILVA et al., 2012) have been considered in Ceratocystis wilt management; however, the short period between appearance of the disease and plant death turns this disease control difficult (SILVA et al., 2012) and research in this area are still needed.

Fungicides have been used to avoid dispersion of Ceratocystis agent and substances reported to have effect on species of this genus are Benzimidazole class such as Carbendazim, Methyl Thiophanate, Benomyl (PEREIRA; SANTOS, 1986) and Triazoles such as Tebuconazole (TOCHETTO et al., 2017). Nevertheless, resistance to these drugs (NISHIJIMA; SMALLEY, 1979) has already been reported. Searching for new possibilities to handle with Ceratocystis, medicinal plant metabolites have been considered as excellent options due to easy access, low cost and less or no toxicity to the environment (STANGARLIN et al., 1999; FIORI et al., 2000; VENTUROSO et al., 2011). In this context, ferns have been considered useful medicinal plants (PAN et al., 2011; CAO et al., 2017). Specially, the *Adiantum* genus has been traditionally used in Brazil to treat respiratory diseases (HARAGUCHI; CARVALHO, 2010) being the hypoglycemic and anti-inflammatory (IBRAHEIM et al., 2011) effects of *A. latifollium* proved in the literature. In addition, species of the same genus, the *A. capillus-veneris*, has been indicated as alternative treatment in the control of phytopathogens with promising results (FORMIGHIERI et al., 2010). Therefore, in this study, we verified the in vitro antifungal potential of *Adiantum latifolium* on *C. cacaofunesta* mycelial growth.

Leaves of *A. latifolium* were obtained from a *cocoa-cabruca* or *cabruca* agroforestry system (AGUIAR et al., 2003) at: -14.7889003753662 long: -39.0494003295898, botany identified and registered under voucher number HUESC12.156. After dryness (60°C for 72 hours), leaves were powdered to produce aqueous and ethanolic solutions. For aqueous solution, 26 g of vegetal powder were boiled in 100 mL of distilled water for 3 minutes. The decoction was filtered in Whatman number 2 filter followed by filtration on 0,20 µm diameter Sartorius membrane. For ethanolic solution, 26 g of powdered vegetal were imbibed in 100 mL of 70% ethanol protected from light for 72 hours. The ethanolic solution was also filtered and stored in aliquots until use.

C. cacaofunesta was cultivated in Sabouraud dextrose agar (SDA) (130 g/L) at 25 to 30°C and protected from light until total growth to obtain inoculum. The in vitro antifungal test was performed in 6 or 24 well plates which gives a growth area of 9.60 (for tests) and 1.93 (for controls) cm^2 respectively. The *A. latifolium* aqueous and ethanolic solutions were incorporated in the SDA at 1, 5 and 10% proportion. Controls consisted on SDA, SDA with 70% ethanol at the same proportion of ethanolic solution (PEtOH) and ADS with 4µg.mL^{-1} of Tebuconazole (Folicur 200 ec, Bayer). Each well received 7 mm discs obtained from the border of fungus colony displaced in the middle of the well. Plates stayed at 25 to 30°C and protected from light until total mycelial growth (wall of the well limit) of control (100% mycelial growth).

The antifungal test did not show inhibition of *C. cacaofunesta* mycelial growth by *A. latifolium* leaves aqueous or ethanolic solutions. Conversely, the mycelial growth of *C. cacaofunesta* tended to be stimulated by the aqueous solution when compared to control (Fig. 1 and Table 1). The same effect was observed by AMARAL et al. (2005)



Results were expressed as mean ± standard deviation of three independent experiments. ANOVA followed by Tukey post-test; *p < 0,05.

Figure 1. *C. cacaofunesta* mycelial growth in Sabouraud dextrose agar (SDA) supplemented with aqueous or ethanolic solutions of *A. latifolium* leaves. (A) Mycelial growth in Petri dishes: A — SDA + 10% ethanolic solution; B — SDA + 5% ethanolic solution; C — SDA + 1% ethanolic solution; D — SDA + 10% aqueous solution without inoculum; E — SDA + 10% aqueous solution without inoculum; F — SDA without supplementation; G — SDA + Tebuconazole at 4 µg.mL^{-1}. (B) Mycelial growth percentage: pEtOH – pure ethanolic solution; EtOH – *A. latifolium* ethanolic solution; H_2O – *A. latifolium* aqueous solution.

Table 1. *Ceratocystis cacaofunesta* mycelial growth evaluation in Sabouraud dextrose agar supplemented with *A. latifolium* leaves aqueous and ethanolic solutions.

	%			Ethanol 70%			SDA	TBCZOLE 4 µg.mL^{-1}
	1	5	10	1	5	10		
Aqueous	100 ± 0 a, B	100 ± 0 a, B	100 ± 0 a, B	85,4 ± 13,3 a, B, c	18,8 ± 32,6 b, A, d	19,9 ± 11,5 b, A, e	71,7 ± 17,8 a	0 ± 0 A
Ethanolic	68,5 ± 27,2 a, B, c	24,7 ± 23,1 b, B, d	8,6 ± 14,8 b, B, e					

ANOVA followed by multiple comparison Tukey test ($p < 0,05$) (average ± standard deviation). SDA: Sabouraud dextrose agar; TBCZOLE: tebuconazole. Equal letters mean no significant difference between results.

for *Albizzia lebbeck* Benth extract on *Fusarium solani* and *Sclerotium rolfsii* and VENTUROSO et al. (2011) testing *Azadirachta indica* A. Juss extract on *F. solani*.

It is worth noting that the fungicide Tebuconazole (Folicur 200 EC) used in this study showed high inhibition on *C. cacaofunesta*. This fungicide is recommended for the control of *Fusarium subglutinans* in pineapple crops, *Colletotrichum gossypii* var. *cephalosporioides* of cotton and *Alternaria porri* in garlic plantation, but not for *C. cacaofunesta* control. Therefore, following the in vitro tests this fungicide may be an alternative to control *C. cacaofunesta* propagation in areas where cacao genetic selection or handling management still fail.

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