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

**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 T ebucaronazole at 4 µg.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. T ebucaronazole 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.
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*) and frosty pod rot (*Moniliophthora roperi*) 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* Engler. & 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 Carbenazim, 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) being the hypoglycemic and antihypertensive action. In addition, species of the same genus, the *A. capillus-veneris* leaves aqueous and 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)

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

| %                | Ethanol 70% | SDA 4 µg.mL⁻¹ |
|------------------|-------------|---------------|
| Aqueous          | 100 ± 0     | 100 ± 0       | 100 ± 0       | 85.4 ± 13.3 | 18.8 ± 32.6 | 19.9 ± 11.5 | 71.7 ± 17.8 | 0 ± 0       |
|                  | a, B        | a, B          | a, B          | a, B         | b, A, c     | b, A, d     | b, A, e     | a           |
| Ethanolic        | 68.5 ± 27.2 | 24.7 ± 23.1   | 8.6 ± 14.8    | 13.3 ± 22.6  | 17.8 ± 30.6 | 24.7 ± 18.8 | 11.5 ± 18.5 | 0 ± 0       |
|                  | a, B, c     | b, B, d       | b, B, e       | a, B         | a, B        | a           |            |             |

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.

It is worth noting that the fungicide Tebuconazole (Folicur 200 EC) used in this study showed high inhibition on *Fusarium solani* and *Azadirachta indica* A. Juss extract on *Fusarium solani*. 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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