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Extracts of *Ageratum conyzoides*, *Coriandrum sativum* and *Mentha piperita* inhibit the growth of the symbiotic fungus of leaf-cutting ants

W.C.C. Morais[a], M.A.P. Lima[b], J.C. Zanuncio[b,*], M.A. Oliveira[c], M.A.L. Bragança[d], J.E. Serrão[e], T.M.C. Della Lucia[b]

[a] Departamento de Entomologia, Universidade Federal de Viçosa, Av. P. H. Rolfs s/n, Centro, Viçosa 36570000, Minas Gerais, Brazil
[b] Departamento de Biologia Animal, Universidade Federal de Viçosa, Av. P. H. Rolfs s/n, Centro, Viçosa 36570000, Minas Gerais, Brazil
[c] Universidade Federal de Viçosa, Campus de Florestal, Campus Universitário, Florestal 35690000, Minas Gerais, Brazil
[d] Curso de Ciências Biológicas, Universidade Federal do Tocantins, Campus de Porto Nacional, Rua 3, Quadra 17, Porto Nacional 77500000, Tocantins, Brazil
[e] Departamento de Biologia Geral, Universidade Federal de Viçosa, Av. P. H. Rolfs s/n, Centro, Viçosa 36570000, Minas Gerais, Brazil

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ABSTRACT

Leaf-cutting ants live in symbiosis with the fungus *Leucoagaricus gongylophorus* (Singer) Möller that grows in their nests. This fungus is the main nutritional source for these ants that provide conditions for its development. Although plant extracts of *Ageratum conyzoides* L., *Coriandrum sativum* L. and *Mentha piperita* L. are known to cause mortality in ants in the laboratory, their effects on *L. gongylophorus* are still unknown. The aim of the present study was to determine the effects of the *A. conyzoides*, *C. sativum* and *M. piperita* extracts on *L. gongylophorus*. The biomass of the fungus grown by the leaf-cutting ants was assessed in culture medium with three concentrations (25, 50, and 100 mg/mL) of *A. conyzoides*, *C. sativum* and *M. piperita* extracts. The results showed that all the three extracts inhibited the growth of *L. gongylophorus*. At concentrations of 25, 50, and 100 mg/mL, the *A. conyzoides* extract exhibited 81, 93, and 100% reduction in the fungal biomass; the *C. sativum* extract showed 23, 27, and 100% reduction in the fungal biomass; and the *M. piperita* extract demonstrated 96, 99, and 100% reduction in the fungal biomass, respectively. Furthermore, the secondary metabolic compounds of these plants were found to have fungistatic and fungicidal properties, similar to that observed in other fungal species. In conclusion, the extracts of *A. conyzoides*, *C. sativum* and *M. piperita* inhibited the growth of *L. gongylophorus* in the laboratory, and should be further studied for their potential use in baits to control leaf-cutting ants.

1. Introduction

Leaf-cutting ants (*Atta* spp. and *Acromyrmex* spp.) live in symbiosis with the fungus *Leucoagaricus gongylophorus* (Singer) Möller that grows in their nests and acts as their main food source (Quinlan and Cherrett, 1979; Silva et al., 2003). These ants cut a variety of fresh plants, including agricultural and forest plants, for *L. gongylophorus* development. Because of the damage caused by these ants to crops, they are considered as one of the most important pests in Brazil (Zanetti et al., 2003; Zanuncio et al., 2004; Nickele et al., 2012).

To control leaf-cutting ants, the use of compounds that are toxic to *L. gongylophorus* may be helpful, because elimination of this fungus is known to cause death of these ants (Boulogne et al., 2012). Thus, numerous studies have been carried out to determine substances that are toxic to this symbiotic fungus (Zanetti et al., 2008). In particular, to reduce the use of synthetic pesticides, which are commonly used for the control of leaf-cutting ants (Zanuncio et al., 2004; Zanetti et al., 2014), many researchers have been focused on finding plant substances with fungicidal activity against these ants (Hebling et al., 2000; Bigi et al., 2004; Bueno et al., 2005; Leite et al., 2005). Essential oils and plant extracts have the potential to be used in the field to control these insects (Alonso and Santos, 2013; Castano-Quintana et al., 2013). In addition, plants that are toxic to both the leaf-cutting ants and the fungus can improve the control of this pest. In previous studies, extracts of *Ageratum conyzoides* L. and *Mentha piperita* L., applied topically onto the leaf-cutting ants or incorporated into their artificial diet, were observed to be toxic to the ants (Ribeiro et al., 2008); likewise, the extract of *Coriandrum*

* Corresponding author. Tel.: +55 31 3899 2924.
E-mail addresses: wagnercalixto@florestal.eng.br (W.C.C. Morais), maugusta@ufv.br (M.A.P. Lima), zanuncio@ufv.br, zanuncio@ufv.br (J.C. Zanuncio), marco.oliveira@ufv.br (M.A. Oliveira), marcosbr@mail.uft.edu.br (M.A.L. Bragança), jeserrao@ufv.br (J.E. Serrão), tdlucia@ufv.br (T.M.C.D. Lucia).

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sativum L. was also found to exhibit similar effects (M.M. Ribeiro, personal communication). However, the effects of these plants on *L. gongylophorus* are still unknown. Thus, the aim of the present study was to determine the toxic effects of *A. conyzoides*, *C. sativum*, and *M. piperita* hexane extracts on the symbiotic fungus *L. gongylophorus* of leaf-cutting ants.

2. Materials and methods

2.1. Preparation of the plant extracts

The leaves of *A. conyzoides*, *C. sativum* and *M. piperita* were collected from plants in their vegetative stages in Viçosa, Minas Gerais State, Brazil. A total of 500 g of leaves from various samples of each plant species were dried at 40 °C for 24 h, crushed and transferred to 2000-mL Erlenmeyer flasks for extraction with hexane for 48 h. The hexane solution, after filtration, was concentrated in a rotary evaporator under low pressure and low temperature (<50 °C) and stored in a refrigerator for biological testing (Moreira et al., 2007).

2.2. Fungus

L. gongylophorus was isolated from three laboratory colonies of the leafcutter ant *Atta sexdens rubropilosa* Forel (Hymenoptera:Formicidae). Mycelium fragments of this fungus were transferred to sterile Petri dishes containing culture medium (Pagnocca et al., 1990) and kept in an incubator at 26 ± 2 °C and 70% humidity.

2.3. Examination of the effects of the plant extracts on L. gongylophorus

The experimental design was completely randomized and the treatments included three concentrations of each plant extract (25, 50, and 100 mg of each plant extract per mL of dichloromethane) and the control. A total of 1 mL of each extract was mixed with 9 mL of the culture medium in a laminar flow hood (Pagnocca et al., 1990). This mixture was poured into Petri dishes (100 mm × 20 mm) comprising three 1-cm-diameter discs containing *L. gongylophorus* grown in laboratory. The control treatments had (1) fungal discs in Petri dishes containing only the culture medium and (2) fungal discs in Petri dishes containing the culture medium with the solvent dichloromethane. A total of 10 replications were prepared for each treatment. All the plates were sealed with a plastic wrap and incubated at 26 ± 2 °C and 70% relative humidity for 30 days in dark. Subsequently, the mycelia of *L. gongylophorus* were collected, dried at 40 °C for 48 h, and weighed on a precision balance. The data obtained were subjected to ANOVA and the means were compared by Tukey test at a significance level of 5% by using the STATISTICA (Statsoft, 2007).

3. Results

The three plant extracts examined were found to decrease the growth of *L. gongylophorus* ($P < 0.01$). The 25 mg/mL of *M. piperita* and *A. conyzoides* extracts could reduce the fungal biomass (Fig. 1A and C), whereas both 25 and 50 mg/mL of *C. sativum* extract exhibited no effect (Fig. 1B). However, all the extracts killed *L. gongylophorus* at the concentration of 100 mg/mL, because we found 100% of biomass reduction. On the other hand, the solvent, dichloromethane, added to the culture medium did not affect the growth of the fungus (Fig. 1).

The percentage reduction in *L. gongylophorus* biomass achieved by the three plant extracts ranged from 81 to 100% (Table 1). The lowest concentration (25 mg/mL) of *M. piperita* and *A. conyzoides* extracts inhibited 96% e 81% of *L. gongylophorus* growth, respectively (Table 1).

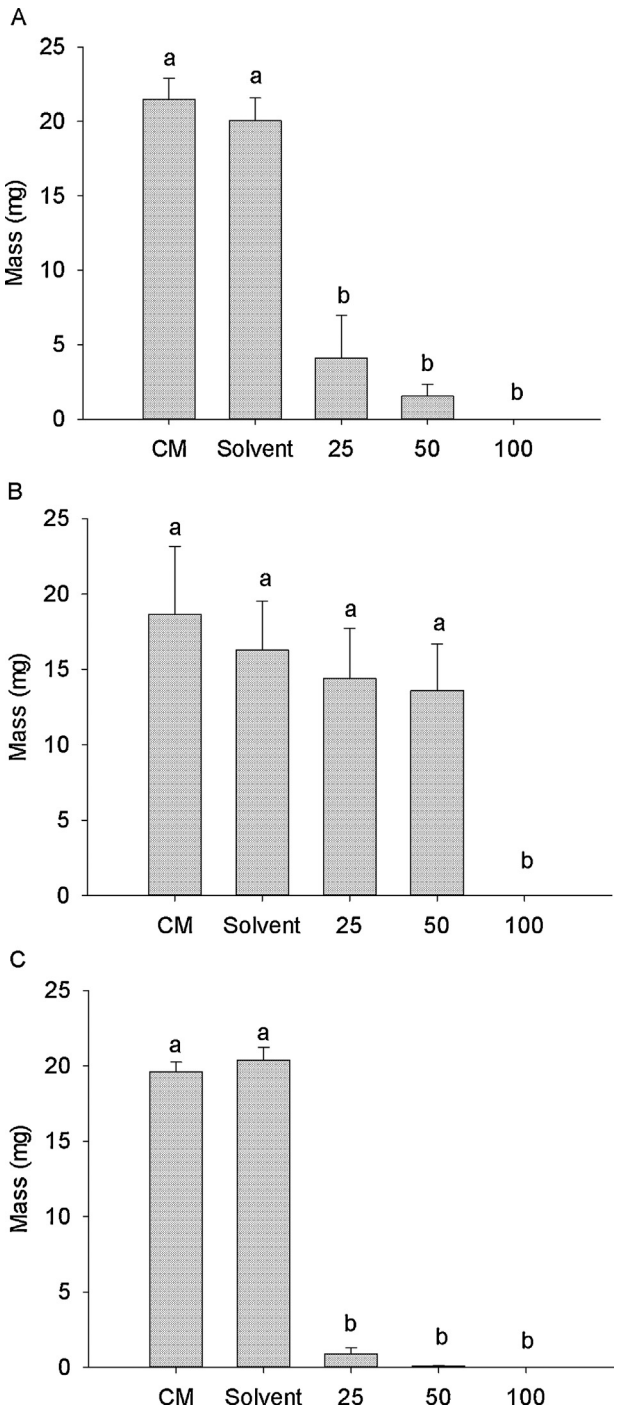

Fig. 1. Mean ± standard error of mycelium mass (mg) of *Leucoagaricus gongylophorus* in the culture medium (CM) with dichloromethane solvent (solvent) and *Ageratum conyzoides* (A), *Coriandrum sativum* (B) and *Mentha piperita* (C) extracts at the concentrations of 25, 50 and 100 mg/mL. Bars with the same letter show means without differences between them ($\alpha = 5\%$).

4. Discussion

The observed fungistatic and fungicidal effects of *M. piperita* on *L. gongylophorus* are in agreement with the findings reported in earlier studies that indicated that plant extracts affected pathogenic fungi such as *Aspergillus flavus* Link (Montes-Belmont and Carvajal,