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In vitro repellent effect of tea tree (*Melaleuca alternifolia*) and andiroba (*Carapa guianensis*) oils on *Haemotobia irritans* and *Chrysomya megacephala* flies

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Abstract. This study aimed to evaluate the repellent effect of tea tree (*Melaleuca alternifolia*) and andiroba (*Carapa guianensis*) essential oils on two species of flies (*Haemotobia irritans* and *Chrysomya megacephala*). For the *in vitro* studies, free-living adult flies were captured and reared in the laboratory. To verify the repellency effect, an apparatus was constructed where *H. irritans* and *C. megacephala* were exposed to andiroba and tea tree oils (5.0%), as well as to a known repellent (citronella, 5.0%) to validate the test. The study demonstrated that all three oils used showed *in vitro* repellent effect against both species of flies. It is possible to conclude that the essential oils (tea tree and andiroba) have repellent effect on these species of flies used in this study.

INTRODUCTION

Among the insects, flies are the most important ones related to human and animal health. There are many species of flies that may act as vectors transmitting viruses, bacteria, protozoa and helminthes, in addition to their possible parasitism as adults or larvae (Thyssen *et al.*, 2004, Béjar *et al.*, 2006).

The *Haemotobia irritans* is a blood-sucking fly that causes great economic losses in animal production (Campbell *et al.*, 2001). This insect causes animal discomfort and stress due to its painful sting which leads to irritability and, consequently, reduced food intake (Guimarães, 1984; Palavesam *et al.*, 2012). The *Chrysomya megacephala* is a fly of medical-sanitary importance, considered a vector of pathogenic microorganisms and the etiologic agent of secondary myiasis in humans and animals (Hall & Wall, 1995). This species can also be used as indicator of decomposition time in forensic studies (Wells & Kurahashi, 1994). Besides the economic losses related to production already mentioned, the presence of these flies increases costs of production, mainly the cost of drug treatment and labor.

Fly control involves a variety of chemicals that must be used in a continuous and excessive way, which may lead to insect resistance, in addition to the contamination of the environment (Barros, 2001; Freitas, 2008). Therefore, during the last few years, some farmers have appealed to natural substances, such as essential oils (Vieira & Cavalcante, 1999; Klauck *et al.*, 2014).

Recently our research group found out that the andiroba and tea tree oils show insecticidal and repellent effect against horn flies in cows (Klauck *et al.*, 2014). The *Melaleuca alternifolia* oil comes from a plant that belongs to the family Myrtaceae, a native species from Australia (Vieira *et al.*, 2004), that also has antibacterial and antifungal activities demonstrated *in vitro* tests against various pathogens (Castro *et al*., 2005). According to researchers, the active principle is related to its main compound, terpinen-4-ol (Vieira *et al*., 2004). On the other hand, *Carapa guianensis* is a large tree found throughout the Amazon Basin, and belongs to the Meliaceae family (Boufleuer, 2004), its oil has repellent and larvicidal effect against mosquitoes (Miot *et al*., 2004; Emerick, 2005) and adult bees (Santos *et al*., 2012).

In our previous study (Klauck *et al*., 2014), the repellent effect of andiroba and tea tree oils lasted only 48 hours when sprayed on cows, however more studies are needed to corroborate these findings. Therefore, the aim of this study was to evaluate for the first time the *in vitro* repellent effect of andiroba and tea tree oils against flies.

MATERIALS AND METHODS

Plant material

Andiroba oil (*C. guianensis*) RF3150 was purchased from Beraca Sabará Químicos e Ingredientes S/A (São Paulo, Brazil). The tea tree oil (*M. alternifolia*) was purchased from Chemical Importer Delaware Ltda, Brazil. To obtain the desired concentrations both essential oils were diluted in triton solution, as described previously (Chagas *et al*., 2003; Farias *et al*., 2007).

Oil characterization

The gas chromatography (GC) analyses were carried out using an Agilent Technologies 6890N GC-FID system, equipped with DB-5 capillary column (30mm x 0.25mm; film thickness of 0.25mm) connected to a flame ionization detector (FID). The injector and detector temperatures were set at 280°C. The carrier gas was helium, at a flow rate of 1.3 mL/min. The thermal programmer was 50-300°C at a rate of 5°C/min. Two replicates of oil samples were processed in the same way. Relative component concentration was calculated based on GC peak areas without using correction factors. The injection volume of the *C. guianensis* (andiroba) and *M. alternifolia* (tea tree) essential oils was 1 µL (Boligon *et al*., 2013). Gas chromatography mass (GC-MS) analyses were performed on an Agilent Technologies AutoSystem XL GC-MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (250°C). The transfer line temperature was 280°C. Helium was used as carrier gas (1.5mL/min) and the capillary columns used were a HP 5MS (30m x 0.25mm; film thickness of 0.25mm) and a HP Innowax (30m x 0.32mm i.d., film thickness of 0.5mm). The temperature program was the same as that used for the GC analyses. The injected volume of the essentials oils was 1 µL. Component identification of both oils was performed on the basis of retention index (RI), determined with reference of the homologous series of *n*-alkanes, C_7-C_{30}, under identical experimental conditions, comparing with the mass spectra library search (NIST and Wiley), and with the mass spectra literature date (Adams, 1995). The relative amounts of individual components were calculated based on the CG peak area (FID response).

Repellency test using *H. irritans* and *C. megacephala*

In vitro repellency tests were performed using an apparatus with different compartments. In compartments 1 and 2, cotton soaked with 2 mL of the essential oils or citronella (positive repellent control) was used; in compartments 3 and 4 had cottons soaked with distilled water and triton (2 mL). There were interconnections between compartments with transparent pipes of 1.8 cm diameter, which allowed free fly movement (Figure 1).

For the study, two species of flies were used: *H. irritans* captured using a net from a dairy farm with high infestation, and *C. megacephala* reared in the laboratory according to the methodology described by Da Silva *et al*. (2008). Flies selected for the test (90 specimens of each) were separated into 18 groups of 10 insects each. Later flies were exposed to andiroba, and tea tree oils, and citronella oil (positive repellent control) at 5.0%. All tests were performed in triplicate.

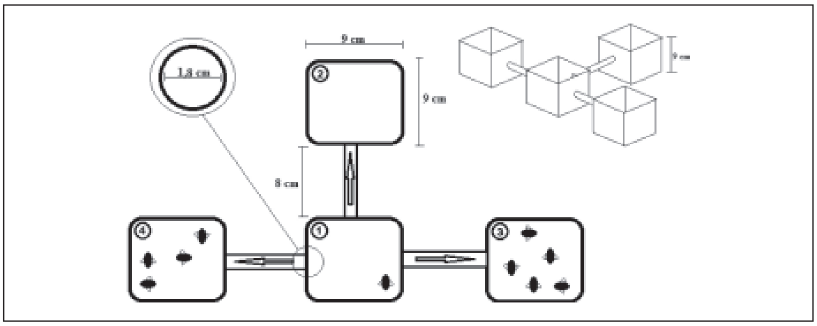

Figure 1. Apparatus to test oil repellency effect against flies. Cotton soaked with essential oils or citronella were placed in compartments 1 and 2, and cotton soaked only with distilled water was placed in compartments 3 and 4.

The test began when the flies were placed in compartment 1, along with the cotton containing the test solution. Then, the compartments 2, 3 and 4 were opened to allow free fly movement. To validate the apparatus, a previous study was performed with citronella oil, a product known for its insecticidal and repellent effect (Raja *et al.*, 2001). To assess the repellency effect, after treatment all flies were counted in each compartment at pre-determined intervals (30, 60, 120, 180, and 240 minutes).

2.4 Statistical analysis

The results showed normal distribution, and Fisher's test was used to compare each compartment in the apparatus. It was considered significantly different when $P<0.05$.

RESULTS

Qualitative and quantitative analyses of *M. alternifolia* (tea tree) and *C. guianensis* (andiroba) essential oils are shown in Figure 2. The three main components of andiroba oil were α-humulene, bicyclogermacrene, and germacrene-D (53.34%). The two main components of tea tree oil were terpinen-4-ol, and γ-terpinene (62.13%).

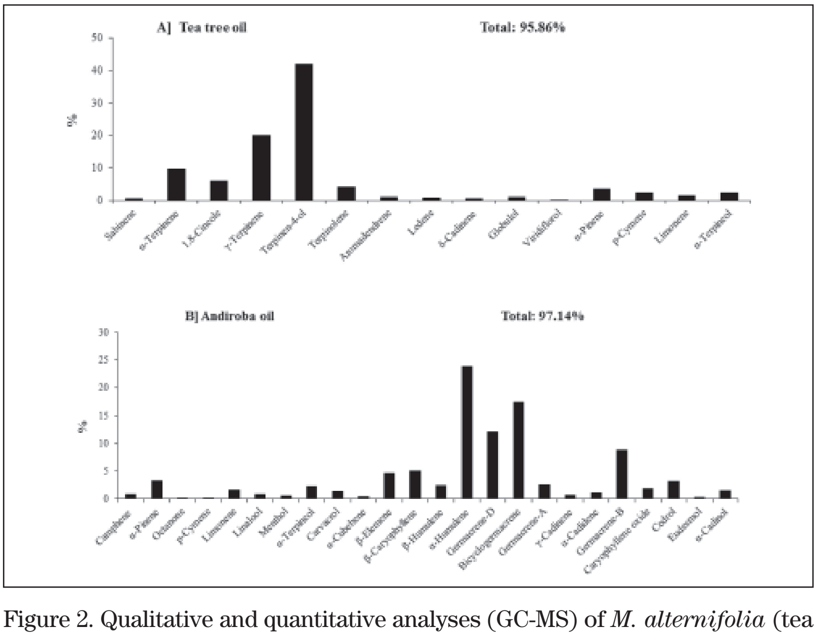

Figure 2. Qualitative and quantitative analyses (GC-MS) of *M. alternifolia* (tea tree) and *C. guianensis* (andiroba) essential oils.

The results from the repellency test of the essential oils against *H. irritans* and *C. megacephala* are shown in Figures 3 and 4. The new apparatus was useful when validating citronella oil (Figure 3A). The oils from andiroba and tea tree tested in this study also showed significant repellent activity, as shown in Figures 3 and 4 (B and C).

DISCUSSION

Efforts towards drug discovery and prudent use of chemicals are the mainstay for overcoming the worldwide problem of insect resistance. One resort for drug discovery is natural products crude or isolated from medicinal plants. In this work, the activity of tea tree and andiroba oils were evaluated against fly species, showing an important repellent effect in both species tested. *In vitro* repellency test showed encouraging results for both oils used, and citronella oil was 100% effective to repel *H. irritans* and *C. megacephala*. Raja *et al*. (2001) demonstrated the insecticidal and repellent activities against mosquitoes and flies when they studied the oils from *Mentha arvensis*, *Mentha piperata*, *Mentha spicata*, and *Cymbopogon nardus*. In the current study, the results obtained with the oils studied were similar for *H. irritans*, however our

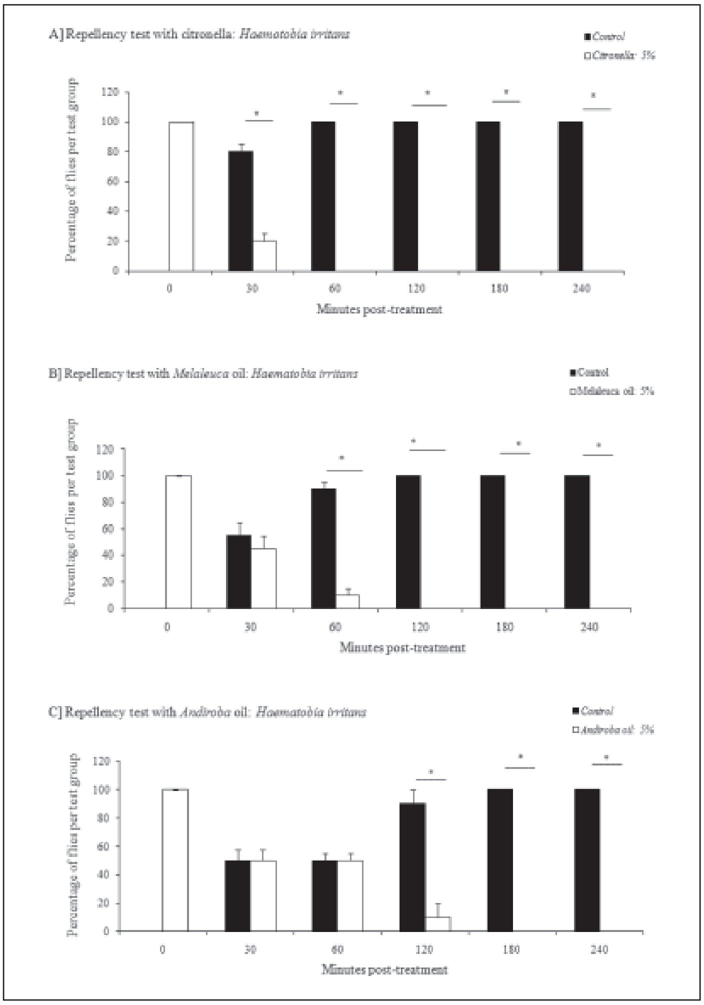

Figure 3. *In vitro* repellent activity of citronella (A), tea tree (B) and andiroba oils (C) against *H. irritans* (*P<0.05). In this experiment were used 10 flies per group.

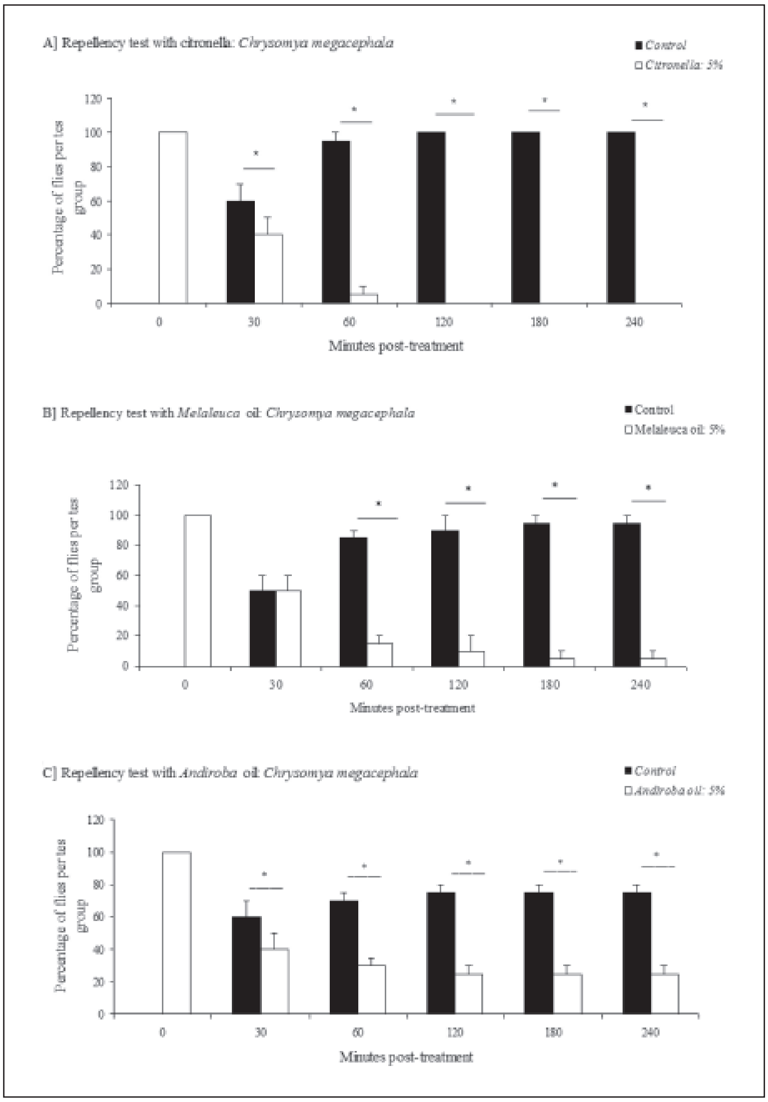

Figure 4. *In vitro* repellent activity of citronella (A), tea tree (B) and andiroba oils (C) against *Chrysomyia megacephala* (*P<0.05). In this experiment 10 flies were used per group.

results against *C. megacephala* showed lower efficacy when compared to the citronella oil, and the effect of tea tree oil repellency was of 95%, while the andiroba oil was 75%. An important result to be reported was the death of the flies that remained in the compartments with andiroba oil.

The insecticidal effect of tea tree oil can be related to its major components, that is, terpinen-4-ol and γ-terpinene. Terpinen-4-ol was tested against larvae of the oriental armyworm, *Mythimna separata*, and its toxic effect on the larvae was previously demonstrated (Ma & Zhang, 2004), a fact that may also have occurred in our study. The other component, γ-terpinene, also has proved insecticidal effect (Abbassy *et al.*, 2009).

In the literature, we found no specific studies with the three major components present in andiroba oil (α-humulene, bicyclogermacrene, and germacrene-D) that could be effective against flies. However, these components are present in other plants and they have proved insecticidal activities

(François *et al.*, 2009; Dell'Agli *et al.*, 2012). Important to note that other components present in these oils in lower concentration may also be responsible for the insecticidal and repellent effects, so further studies with these isolated components will be performed in the future by our research group.

A previous study from our research group had suggested that andiroba and tea tree oils have repellent effect when tested in cows (Klauck *et al.*, 2014). Based on new tests, under controlled conditions, we conclude that the essential oils mentioned above present repellent effect against these tested flies. It is important to note that the present work evaluated for the first time the repellent activity of these essential oils against *H. irritans* and *C. megacephala*. Therefore, this study suggests that these new natural products may be used for controlling flies.

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