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Article

Identification of Some Chemical Components of the Essential Oil from Molasses Grass (*Melinis minutiflora* Beauv.) and their Activity Against Cattle-Tick (*Boophilus microplus*)

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O óleo essencial do capim-gordura (*Melinis minutiflora* Beauv.) apresentou mortalidade para 100% das larvas de carrapato-do-boi (*Boophilus microplus*) após 10 min de exposição. A análise do óleo essencial por CG/EM permitiu a identificação de seis componentes majoritários: ácido propiônico, ácido butirico, álcool feniletílico, hexanal, 1,8-cineol e 9-*E*-eicoseno. Dois dos componentes (1,8-cineol and *n*-hexanal) apresentaram também 100% de mortalidade às larvas de carrapato-do-boi em 10 min. Observou-se também a presença do 2,6-di-*t*-butil-4-metilfenol que foi atribuída a um contaminante do éter dietílico, comercialmente fornecido, e usado para extração do óleo essencial.

The essential oil of molasses grass (*Melinis minutiflora* Beauv.) has shown virtually 100% lethal effect on larvae of the cattle-tick (*Boophilus microplus*) within 10 min exposure. GC/MS analysis of the essential oil led to the identification of six major components: propionic acid, butyric acid, phenylethyl alcohol, hexanal, 1,8-cineole and 9 -*E*-eicosene. Two of those components (1,8-cineole and *n*-hexanal) have also shown individually 100% lethal effect on cattle-tick larvae within 10 min. The presence of 2,6-di-*t*-butyl-4-methylphenol was assigned to a contaminant from commercially supplied diethyl ether used for the essential oil extraction.

Keywords: *monoterpenes, molasses grass, acaricide*

Introduction

The effects of *Melinis minutiflora* Beauv. on *Boophilus microplus* infestation have been previously studied, and the grass has been considered of potential use for biological control of cattle-tick. Popularly known in tropical areas as molasses grass, *Melinis minutiflora* is very sensitive to frost but it is well adapted to tropical and subtropical regions. Leaves and stems of this graminoid are covered with trichomes (or glandular hairs) where a viscous fluid of characteristic odor is secreted. This oily material is reported to be responsible for the ability of molasses grass to reduce cattle-tick infestation either by repelling or killing tick larvae[1,2].

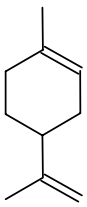
Previous field experiments with cattle-tick and molasses grass have indicated repellent rather than larvicidal

properties[3,4]. The lethal effect upon larvae was explained by Jesus[5] as being a consequence of exhaustion (mechanical effect caused by a viscous secretion which prevented the larvae from climbing the stem and reaching the leaves) and asphyxiation (when body of larvae become covered by plant secretion)[6]. A severe reduction in larval[7] and adult[8] tick population induced by molasses grass has been described. Recent *in vitro* and *in vivo* assays[9,10] permitted identification of the biological activity of molasses grass as being due to the trichomal essential oil, which is actually responsible for the repellent, acaricidal and ovicidal properties.

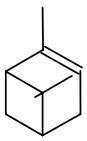
The secondary metabolites of trichomes of several plant species play a central role in plant protection against plant-feeding insects[11,12,13]. Monoterpenes occurring in trichomes have an insecticidal effect, particularly on termites[12,14]. Many monoterpenes, including limonene **1**, α-pinene **2** and myrcene **3** are involved in plant/animal interactions[13].

Additionally, secondary metabolites from higher plants have recently been used as pesticides or models for new synthetic pesticides, *e.g.*, toxaphene (insecticide and herbicide) and cinmethylin (herbicide). These chemicals were developed from plant derived natural products such as terpenoids that can be found in the essential oil secreted by the glandular trichomes of *Artemisia* (Compositae) or closely related genera[15]. Pine oil, a by-product of the sulfate wood pulping industry, has the terpenoid α-terpineol among its major constituents. α-Terpineol **4** has been reported to protect living trees within a 10 m radius from three bark beetle species attack[16]. Alfaro *et al.*[17] reported that pine oil is a feeding deterrent for *Pissodes strobi* (COLEOPTERA: CURCULIONIDAE). Fragrant volatile oils typically contain monoterpenes or their related monoterpenoids, including alcohols, ketones, aldehydes, carboxylic acids, and oxides[18]. These secondary plant substances are toxic to house fly, German cockroach, rice weevil, and western corn rootworm.

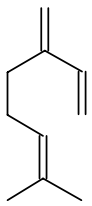
Sutherst and co-workers[19,20] have shown that tropical pasture legumes of the genus *Stylosanthes* produce a sticky secretion which immobilizes and kill cattle-ticks. These effects were attributed to a mixture of the monoterpenes α and β-pinene, **2** and **5**, two components of the essential oil of the grass.



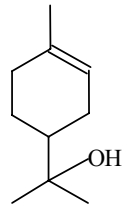
1
Limonene



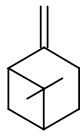
2
α-pinene



3
Myrcene



4
α-terpineol

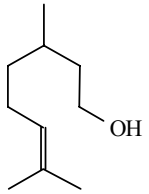


5
β-pinene

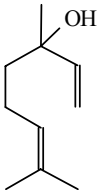
In a recent paper, we reported the biocidal activity of the essential oil from molasses grass on larvae of *Boophilus microplus*[21]. However, it is not clear so far which chemical components are active in the natural mixture. The present paper describes the GC/MS analysis of the essential oil and the acaricidal effect of two of its components. In addition the following monoterpenoids: citronellol **6**, linalool **7**, isopinocamphone **8** and camphor **9** were also tested for biological activity on *B. microplus*.

Material and Methods

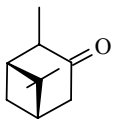
Plant material: all molasses grass samples were collected during the summer rain season at the Forest State Institute nursery in Belo Horizonte - MG, Brazil. Samples were cut at 10 cm high from the ground, packed into a plastic bag. Essential oil was obtained by steam distillation extraction. This procedure was based on the work of Hernández *et al.*[10] in which the authors reported the anti-tick activity of an oil fraction of the petroleum ether extract from molasses grass.



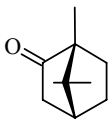
6
Citronellol



7
Linalool



8
Isopinocamphone



9
Camphor

Extraction of the essential oil: a 700 g sample of molasses grass cut into 10 cm (leaves and stems) pieces was steam distilled for 8 h. As no clear phase separation was observed, diethyl ether was used to extract the essential oil from the destillate. The extract was evaporated in a rotary evaporator at atmospheric pressure, at a controlled temperature of 35 °C and 0.07 g of essential oil was obtained. The yield was averaging 0.01% (lit.: 0.001%)[6]. The yellow oily residue was kept at 10 °C in a sealed glass tube.

To determine solvent residue, a blank extraction was carried out by taking one liter of the same batch of diethyl ether used for extraction and evaporating it in the rotary evaporator at atmospheric pressure and controlled temperature of 35 °C.

GC/MS - essential oil analysis: GC/MS data of major constituents of molasses grass essential oil were obtained using a HP 5890 Hewlett-Packard GC equipped with a fused silica capillary column 30 m x 0.25 mm, I.D. 0.25 μm, SE - 54 stationary phase. The temperature was programmed from 50 °C to 180 °C at 4 °C / min and then at 20 °C / min to 250 °C). The mass spectrometer, model HP 5971 was equipped with a mass quadrupole detector with helium as carrier gas, electronic impact ionization, 70 eV, ionization chamber at 180 °C and transfer line temperature of 280 °C.

Acaricidal activity test: the procedure described by Stone and Haydock[22] for LD_{50} evaluation on the acaricidal activity of commercial insecticides recommends wetting a squared filter paper (7.5 x 8.5 cm) with the substances under test in several dilutions. After drying the paper, approximately 100 larvae would be put on it. However, in the present case this procedure was inappropriate to both volatile substances and essential oil. The anti-tick activity test better used in this study was that recommended by Sutherst *et al.*[19], which originally consists of a clumped gauze of fine nylon containing the essential oil inside a larva-proof stainless steel gauze cylinder (1 cm x 1 cm). The cylinder was securely capped and held firmly in the middle of a 5 x 1.2 cm glass vial in which approximately 200 larvae were brushed. Some small modifications were made in the present case: a 1 x 15 cm filter paper band was wet with the essential oil / substances and placed into a glass test tube 15 cm long and 1.5 cm internal diameter (I.D.), at horizontal position, containing in the bottom a piece of cotton wool impregnated with PBS-phosphate buffer saline (pH 7.2) solution. Approximately 100 larvae were gently placed into the tube with a soft brush and the tube was capped with a rubber stopper.

The test for essential oil and pure substances were carried out by impregnating the paper band with 4 drops (≅ 80 mg) of each test substance in five repetitions, including control. A control test tube was prepared in the same way but no impregnating substance was used. Larval mortality was observed with a stereomicroscope every five minutes during the first hour and after 24 h of elapsed time. After 24 h all control larvae were still alive.

The acaricidal activity of the monoterpenes α and β-pinene was previously investigated by Sutherst and Wilson[20], along with biologically active analogues described in the literature[23,24,25,26,27].

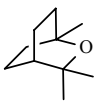
Solvent and reagents: diethyl ether used for essential oil extraction was pesticide residue analysis grade supplied by Grupo Quimica (Brazil).

Except for the citronellol, supplied by Elo Vital (Brazil) and isopinocamphone synthesized in the laboratory by Prates[28], the pure substances used in the test were of analytical-reagent grade supplied by Aldrich (USA). Cooking olive oil was used for dilution in acaricidal test.

Results and Discussion

GC/MS analysis allowed us to identify six out of ten components from the essential oil. Propionic acid (43.0%) **10** and the monoterpenoid 1,8-cineole (10.6%) **11** were the major constituents identified (Table 1). Other components found were: hexanal **12**, phenylethyl alcohol **13,** butyric acid **14**, and 9 - *E* - eicosene **15**. The substance assignment was inferred by the match quality score (Table 1). By this criterion 1,8-cineole and phenylethyl alcohol were assigned with 93 and 94% certainty, respectively, which reflects goodness of fit from data available in the MS digital library. Finally, mass spectra were compared with those avaliable in literature.

The results of the acaricidal test presented in Table 2 showed the lethal effect of the essential oil and of single components contained in the oil against cattle-tick larvae. Virtually 100% of larvae were dead in 10 min. The monoterpenes α and β-pinene separately or combined had similar effects, but a 1 M solution of each monoterpene in olive oil was less effective than the pure compounds, and the 1 M solutions required 24 h to produce 100% mortality. The two available components of the molasses essential oil, 1,8-cineole and hexanal were lethal to 100% of the larvae in 5 min. Among other tested monoterpenes, citronellol was lethal in 5 min; linalool in 15 min, followed by isopinocamphone and camphor, with 45 min and one hour, respectively. The present results confirm data reported by Hernandez *et al.*[10], *i.e.*, that the essential oil of molasses grass can also lead to 100% mortality, within 10 min, even though those authors have not described the chemical nature of the oil.



11
1,8 - cineole

Table 1. GC/MS data for molasses grass essential oil.

Retention Time (RT)	Kovats' Index	Amount (%)*	Match Quality (%)	Identification
1.49	779	4.6	-	N.I.
1.54	781	43.0	50	propionic acid
1.77	788	3.3	-	butyric acid
1.84	790	5.0	-	N.I.
3.43	837	5.0	67	hexanal
4.70	876	5.2	-	N.I.
9.18	1010	10.6	93	1,8-cineole
9.70	1025	4.5	94	phenylethyl alcohol
35.30	1792	8.0	84	9 -*E* -eicosene

(*) Mass spectra showed an additionally significant amount (~ 10%) of 2,6-di-*t*-butyl-4-methylphenol (match quality 93%), which was identified as residual contamination from ethyl ether used as solvent.
N.I.: not identified.

Table 2. Time for 100% mortality of cattle-tick larvae (approximately 100) due to *Melinis minutiflora* essential oil and some pure compounds.

Substances / Essential Oil	Time
(+)α-Pinene	10 min
β-Pinene	10 min
α + β-Pinene (1:1)	10 min
(+)α-Pinene 1 M*	24 h
β-Pinene 1 M*	24 h
1,8-Cineole	5 min
Hexanal	5 min
Citronellol	5 min
Linalool	15 min
Isopinocamphone	45 min
(+)-Camphor	60 min
Melinis minutiflora essential oil	10 min

(*) Olive oil solution.

It should be mentioned that in the GC/MS the presence of the monoterpenes α and β-pinene was not observed. By contrast, these substances were found to be the main acaricidal constituents of *Stylosanthes* essential oil[20]. For this reason they were initially expected to be present in the *Melinis minutiflora* essential oil[28].

Conclusion

The anti-tick activity of molasses grass essential oil has been confirmed in the present study. GC/MS data has allowed the identification of six out of ten substances in the essential oil. The major components are propionic acid (43.0%) **10** and the monoterpenoid, 1,8-cineole (10.6%) **11.**

In view of the promising activity exhibited by monoterpenic substances (1,8-cineole, citronellol > α and β-pinene > linalool > isopinocamphone > camphor) against cattle-tick larvae, most of which are constituents of several essential oils, these compounds may be profitable as leads to develop safer insecticides *in natura* or in adequate formulation or even by further chemical transformation. Among non-monoterpenoids, hexanal, a molasses grass essential oil constituent, was also very active, while the major component (propionic acid) should be tested in the near future.

The present study confirms field observations concerning the molasses grass anti-tick activity, and gives experimental support to previously reported data[10] stating that the acaricidal action of its essential oil is not only of mechanical, but also of a chemical nature. Moreover, this work has also shown that some of its chemical components act directly as cattle-tick larvacide, and consequently must play a role on the observed influence of molasses in reducing tick populations in the field.

Acknowledgments

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