Research Paper

Potential use of Negramina (*Siparuna guianensis* Aubl.) essential oil to control wax moths and its selectivity in relation to honey bees

Taciano P. Ferreira¹, Eugenio E. Oliveira²*, Paulo H. Tschoeke³, Rodrigo G. Pinheiro⁴, Ana Maria S. Maira⁴, Raimundo Wagner S. Aguiar⁴

¹ Departamento de Química Ambiental, Universidade Federal de Tocantins, Gurupi, TO 77413-070, Brazil
² Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, MG 36570-900, Brazil
³ Departamento de Agronomia, Universidade Federal de Tocantins, Gurupi, TO 77413-070, Brazil
⁴ Departamento de Biotecnologia, Universidade Federal de Tocantins, Gurupi, TO 77413-070, Brazil

**ARTICLE INFO**

**Keywords:**
- *Galleria mellonella*
- *Achroia grisella*
- *Apis mellifera*
- Botanical insecticides
- Repellency
- Stored honey bee combs

**ABSTRACT**

The utilization of synthetic insecticides is one of the most prevalent strategies used to control wax moth infestations, especially when the strength of bee colonies is weak. However, toxicity to bees and contamination of their products has been considered to be consequences of insecticide residues, increasing the risk of hazards to human health and to the environment. Here, we evaluated whether the application of Negramina (*Siparuna guianensis* Aubl.), essential oil would be selective against the honey bees *Apis mellifera* L. without compromising the control of the wax moths *Galleria mellonella* L. and *Achroia grisella* F. The Negramina essential oil was chemically characterized and tested for insecticidal and repellent activities against *A. mellifera* as well as against both moth pests. The chemical composition of the essential oil revealed β-myrcene (79.7%) and 2-undecanone (14.6%) as the oil’s main constituents. While *G. mellonella* and *A. grisella* were similarly susceptible to Negramina oil, the forager bees were five- to 10-fold more tolerant to the actions of the essential oil. Furthermore, the Negramina oil (0.30 μg of essential oil/cm²) did not repel honey bee foragers but did exhibit repellent activities (0.08 μg of essential oil/cm²) against the larvae and adults of both wax moth species. By exhibiting desirable levels of selectivity against *A. mellifera* and providing relevant control levels against wax moths, the application of Negramina essential oil represents a desirable tool to replace the use of synthetic insecticides against wax moths in weak honey bee colonies as well as in stored honey bee combs.

**1. Introduction**

Although the application of inorganic pesticides has ancient roots, the environmental and human health hazards caused by the intense and indiscriminate use of synthetic pesticides have become a great public concern (Blancke et al., 2017; Clark, 2017; Guedes et al., 2016). For instance, the severe reductions in population number of managed bee hives around the world have gained public attention as a potential consequence of (among other factors, such as habitat fragmentation, pathogens, parasites, and poor nutrition) bee exposure to agricultural insecticides or even chemical products used to prevent the insect pest infestation of the bee hives (Gill et al., 2012; Goulson et al., 2015; Klein et al., 2017; Potts et al., 2010; Tomé et al., 2017).

Among the diverse groups of animals that may inhabit the bee hive environment, especially when the strength of bee colonies is weak, the lesser wax moth *Achroia grisella* Fabricius 1794 (Lepidoptera: Pyralidae) and the greater wax moth *Galleria mellonella* Linnaeus, 1758 (Lepidoptera: Pyralidae) are severe insect pests, as their larvae feed on combs, wax, and honey (Burges, 1978; Chatzivasileiadis and Sabelis, 1997; Elbehery et al., 2016; Hyrsl et al., 2007; Owayss and Abd-Elgayed, 2007; Sanad and Mohanny, 2015). These wax moth pests have been targeted by several fumigant insecticides (e.g., sulphur dioxide, acetic acid, formic acid, paradichlorobenzene (PDCB), methyl bromide and phosphine) that are well known to harm the bee populations and to contaminate the honey and other bee products (Burges, 1978, Charrière and Imdorf, 1997), which has led to an increased need to develop effective tools to control wax moths that pose little threat to the environment and human health.

Because botanical insecticides (particularly essential oils) supposedly do not pose the same risks as synthetic insecticides, the interest in and utilization of such control tools have become increasingly relevant in the control of insect pests (Dayan et al., 2009; Isman, 2016; Isman and Grieneisen, 2014; Regnault-Roger et al., 2012). Despite these essential oils are majorly composed by few molecules, it is noteworthy...
that these oils are complex mixtures of numerous molecules, and their biological activities may be shaped by the potential synergistic and antagonistic interactions among all these molecules and not only by major essential oil compounds (Bakkali et al., 2008; Koul et al., 2008). Regarding the potential of essential oils to control wax moths, only a few recent investigations have tested these plant-derived products against these insects (Elberbery et al., 2016; Owaysi and Abd-Elgayed, 2007; Sanad and Mohanny, 2015).

Despite having an extremely rich flora, the great vegetative diversity of the Neotropical region still is underexploited in terms of the discovery of biological substances. The aromatic medicinal plants *Siparuna guianensis* Aubl. (*Siparunaceae*), also known as Negramina or Capitiú in certain regions, is one of these underexploited Neotropical plants. In Brazil, for instance, these plants frequently occur in the Brazilian Northeast and Central-West regions (Renner and Haunser, 2005), and their derived products (e.g., leaves, bark, flowers or even essential oils) have been used in folk medicine (Bessa et al., 2015; Grenand et al., 2004; Valenti et al., 2010) and recent investigations, have shown the potential of the essential oil of Negramina to control mosquitoes and ticks (Aguiar et al., 2015; Diniz, 2014). Indeed, the economic and biotechnological potential of *S. guianensis* plants has risen consistently in the last few years, which has contributed to the increase the agronomical potential of these plants (Ferreira et al., 2017; Valente et al., 2011, 2014).

Thus, the present investigation was conducted with the aim to analyze the chemical composition of essential oils extracted from *S. guianensis* plants and to evaluate whether the application of these oils would be selective in relation to honey bees, *Apis mellifera* L., without compromising the control of the wax moths (i.e., *G. mellonella* and *A. grisella* F.).

2. Materials and methods

2.1. Plant material and steam distillation

Leaves of *S. guianensis* were collected in rural Gurupi County (*11°43′45″ latitude S, 49°04′07″ longitude W, Tocantins State, Brazil*). Samples of these plants were compared with material deposited (reference number 10.496) in the herbarium of the Universidade Federal do Tocantins (UFT, Porto Nacional-TO, Brazil). The present investigation was registered and approved by the Brazilian National Agencies (Patrimônio Genético - Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, n° 010580/2013-1). The essential oil was extracted from the *S. guianensis* leaves using the steam distillation method in a Clevenger apparatus as described by Aguiar et al. (2015) and stored at 4 °C until the insecticidal and repellent experiments were conducted.

2.2. Gas chromatography–mass spectrometry (GC–MS) analysis

The gas chromatographical (GC) analysis of *S. guianensis* essential oil was conducted using a Chemito 8510 GC instrument (Chemito Technologies Pvt. Ltd, Mumbai, India) equipped with a data processor. A BP-5 wide-bore capillary column (30 m-0.53 mm i.d., 1.0 mm film thickness) was used to separate the sample components (sample size 0.03 mL, measured using a Hamilton GC syringe with a 1.0 mL cap.). Hydrogen was used as the carrier gas at a flow rate of 5 mL/min and 20 p.s.i. inlet pressure. The GC column oven temperature went from 70 °C to 210 °C at a rate of 2.5 °C/min, with a final hold time of 5 min. Both injector and detector (FID) temperatures were maintained at 230 °C. Gas chromatography-mass spectrometry (GC–MS) analyses were carried out using a Trace DSQ MS (Thermo Electron Corporation) using a BP-5 capillary column (30 m × 0.25 mm × 0.25 μm) with helium as the carrier gas at a flow rate of 1 mL/min and a split ratio of 1:20. The column temperature went from 65 °C to 210 °C (10 min hold) at 3 °C/min. Mass spectra were recorded in the range 40–650 amu, operating at 70 eV, and the ion source temperature was maintained at 200 °C. The constituents of the oil were identified using standard reference compounds and by matching the mass spectra fragmentation pattern with the NIST Mass Spectra Library stored in the GC–MS database.

2.3. Insect rearing

2.3.1. Wax moths *A. grisella* and *G. mellonella*

Larvae of *A. grisella* and *G. mellonella* were obtained from infested bee colonies collected from a regional beekeeper (11°73′34″ latitude S, 49°03′39″ longitude W). Each species colony was established with a minimum of 400 individuals, which assured representative genetic variability. The wax moths were reared according to methods previously described elsewhere (Ellis et al., 2013; Jones et al., 2002; Parra, 2002). Briefly, larvae of *A. grisella* and *G. mellonella* were fed with artificial diets developed by (Jones et al., 2002). These larvae were placed into plastic containers (width: 15 cm; length: 12 cm; height: 10 cm) with artificial diets and fed up to the pupal stage. The plastic boxes were cleaned and supplies were replenished at 2-day intervals. Once the adult moths emerged, they were transferred to plastic boxes (width: 55.5 cm; length: 40.3 cm; height: 36.5 cm) within which oily papers (for the plastic boxes used to rear *A. grisella*) or artificial diets (for the plastic boxes used to rear *G. mellonella*) had been placed to serve as oviposition sites. The *A. grisella* eggs collected on the oily papers were transferred to artificial diets, where the newly emerged larvae fed. These newly emerged larval colonies were placed into climate chambers at 25 °C, under a 12 h light regime in an insecticide-free environment. The artificial diets containing *G. mellonella* eggs were collected and transferred to climate chambers with the same environmental conditions described above.

2.3.2. Foragers of *A. mellifera*

The *A. mellifera* foragers were generously provided by Mr. Orlando Borges (Associação Gurupiense de Apicultores, Gurupi-TO, Brazil). Newly emerged bees (i.e., < 48 h) of four bee colonies were used in the survival or repellency experiments. We used new emerged bees (i.e., < 48 h), because they are the insects that perform the maintenance and protection of the hive against infestation by wax moths (Burges, 1978; Johnson, 2015; Sanad and Mohanny, 2015). The bees were obtained from healthy colonies without any infestation of wax moths and were transferred to the laboratory, where they were kept under controlled conditions similar to those found in their respective colonies (34 °C under complete darkness). Briefly, we select frames containing enough capped brood that will emerge in one to three days (Human et al., 2013; Williams et al., 2013), removed all the adult bees from the frames and placed the frames in adequate frame cages before transferring to the laboratory. The bee collection occurred at 48 h intervals. Smaller period intervals were used when we could collect sufficient amount of bees to be used in the survival or repellency experiments.

2.4. Toxicity and repellency bioassays

2.4.1. Concentration-mortality bioassays with the wax moths and honey bees

Lethal concentrations (LC50) of Negramina essential oils were estimated for second instar larvae of *A. grisella* and *G. mellonella* using a concentration range that varied from 0.001 μg – 3 μg of essential oil/cm². The essential oil was dissolved in solutions containing DMSO (1.6%) and applied to filter papers (Whatman no. 1), which were left to dry for 30 min. These filter papers were used to cover plastic containers (300 mL of volumetric capacity), where groups of 25 larvae were placed and exposed to the essential oil for 6 h. During the exposure to the essential oil (by direct contact to dry residues and to evaporating products), the plastic containers were kept under controlled conditions (i.e., 30 ± 2 °C and 60 ± 10% relative humidity). The control
دریافت فوری متن کامل مقاله

امکان دانلود نسخه تمام متن مقالات انگلیسی
امکان دانلود نسخه ترجمه شده مقالات
پذیرش سفارش ترجمه تخصصی
امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
امکان دانلود رایگان ۲ صفحه اول هر مقاله
امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
دانلود فوری مقاله پس از پرداخت آنلاین
پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات