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Essential oil of *Lippia sidoides* and its major compound thymol: Toxicity and walking response of populations of *Sitophilus zeamais* (Coleoptera: Curculionidae)

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ABSTRACT

The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), is the main pest of stored grains across tropical regions. An alternative strategy to the use of synthetic insecticides for the management of *S. zeamais* is the development of botanical insecticides based in the essential oils (EOs) from aromatic plants. In the present study, we evaluated the lethal and sublethal effects of the EO of *Lippia sidoides* (Cham.) and its major compounds (thymol and ρ-cymene) on different populations of *S. zeamais*. For this, we determined toxicity by fumigation of treatments of five populations from different Brazilian regions and assessed the lethal time and walking behavior for the most tolerant and susceptible populations. The lethal concentration required to kill 50% of *S. zeamais* populations ranged from 35.48 to 118.29 μL L^{-1} air for EO of *L. sidoides*, 65.00–91.23 μL L^{-1} air for thymol and 801.24 to 2188,83 μL L^{-1} air for ρ-cymene. Population from Jacarezinho was the most tolerant to treatments, while population of Rio Branco was the most susceptible one. The survival of *S. zeamais* populations was significantly affected by treatments and ρ-cymene showed the faster action on both Jacarezinho and Rio Branco populations (LT_{50} = 0.3 h). The walking behavior of maize weevil showed that the EO of *L. sidoides* and thymol present repellent effect, however, ρ-cymene present attractive effect. Therefore, the EO of *L. sidoides* and its major compound thymol are promising source to develop bioinsecticides for the management of *S. zeamais* populations with different levels of resistance.

1. Introduction

In recent years, resistance to insecticides and concerns about the risks of synthetic insecticides to non-target organisms, environment and human health have driven the search for alternative methods of controlling insect pests. Essential oils (EOs) from aromatic plants have been considered a safe alternative strategy to manage insect pests, including those of stored products (Isman, 2006; Santos et al., 2011).

The EOs from plants are complex mixtures with large numbers of volatile organic compounds (mono- and sesquiterpenes) that can be extracted from seeds, stems, leaves and flowers and which can exhibit bioactivity on a variety of insects. The efficiency of EO for insect control is, in large part, due to its complexity and the potential for additive or synergistic effects among its compounds. Advantages of using EO compounds in pest management compared to synthetic insecticides include a potential decrease of resistance development due to the mixing of compounds acting at various sites of action and the low persistence in the environment due to high volatile of compounds, and therefore lower toxicity to non-target organisms (Correa et al., 2015; Peixoto et al., 2015; Tak et al., 2016a).

The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), is considered the most destructive insect pest of stored

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grains due to qualitative and quantitative losses (Correa et al., 2015). Infestations by *S. zeamais* can substantially reduce the weight and increase the moisture of grains, providing favorable conditions for the growth of microorganisms, leading to even higher levels of post-harvest losses (Ribeiro et al., 2015). These grain infestations can occur both in the field, before harvesting, as well as in storage (Wale and Assegie, 2015).

The management of insect pests of stored products depended on insecticides and synthetic fumigants, mainly phosphine and methyl bromide (Paes et al., 2012; Zhang et al., 2016). However, inadequate application and intensive use of these insecticides at grain storage sites over the years have led to several problems, including the development of resistance and residues of synthetic insecticides in food (Kumar et al., 2011; Lu et al., 2013). Thus, it is necessary to develop new management tools and insecticidal compounds of low risk (Moreau and Isman, 2012).

The shrub *Lippia sidoides* Cham. (Verbenaceae) is a native plant from the semiarid of Brazilian northeast that has bioactivity against a range of organisms. In this study, we evaluated the lethal and sublethal effects of EO of *L. sidoides* and its major compound (thymol and ρ-cymene) on different populations of *S. zeamais* by fumigation exposure route.

2. Material and methods

2.1. S. zeamais populations

The Brazilian populations of *S. zeamais* used in this study was collected from different regions: north (municipality de Rio Branco, Acre), northeast (Aracaju, Sergipe), south (Jacarezinho, Paraná); southeast (Sete Lagoas, Minas Gerais) and midwest (Maracaju, Mato Grosso do Sul). All populations were reared at the Clínica Fitossanitária of the Federal University of Sergipe, located in the municipality of São Cristóvão, Sergipe, Brazil.

Populations of *S. zeamais* were reared in plastic container (1 L) with corn kernels previously washed and kept in freezer (−10 °C) for 30 days. Plastic containers were kept under controlled conditions in a biochemical oxygen demand (B.O.D) incubator chamber (26 ± 1 °C, 50 ± 2% RH and 12-h photoperiod).

2.2. EO of L. sidoides and its major compounds: obtaining and chemical analysis

The EO of *L. sidoides* was supplied by the company Produtos Naturais Ltda. (Horizonte, Ceará, Brazil). The chemical composition of EO of *L. sidoides* was performed as described in Oliveira et al. (2017). Tymol and ρ-cymene constituted the greatest proportion (> 10%) of compounds in the EO of *L. sidoides*, and were acquired from Sigma-Aldrich® company.

2.3. Bioassays

Initially, lethal concentrations of treatments (EO of *L. sidoides*, thymol and ρ-cymene) were determined for the five populations of *S. zeamais* via fumigation exposure. The two populations that presented the largest (tolerant) and smallest (susceptible) lethal concentration were then chosen to determine: *(i)* lethal time to cause mortality in 50% of individuals, and *(ii)* walking behavior in half treated arenas. In all bioassays, non-sexed adults of *S. zeamais* were used. The acetone solvent (Panreac, UV-IR-HPLC-GPC PAI-ACS, 99.9% purity) was used as control.

2.3.1. Lethal concentration by fumigation exposure

The experimental design was completely randomized with four repetitions. Each experimental unit was composed by 10 insects in an acrylic pot (22.08 cm^3). The treatments were applied at different concentrations in order to obtain a mortality range of > 1% and < 99%. These concentrations were applied on a filter paper (Unifil, cod. 501.009; 1 cm^2) using a microsyringer (Hamilton®). The treated paper was kept in the lid of the pots and covered with an organza to avoid direct contact of the insects with the substances. The maize weevils were placed on the bottom of the pots; then, the pots were set at 180° and held in this position to allow the saturation and homogenization of the vapor of the substances. The pots were kept in a B.O.D. incubator chamber under controlled conditions (26 ± 1 °C, 70 ± 5% RH, 12 h photoperiod). Evaluation of mortality was performed 72 h after the beginning of the experiments.

2.3.2. Lethal time

To determine the lethal time (LT_{50}) of treatments for tolerant and susceptible populations of *S. zeamais*, the LC_{95} determined in the lethal concentrations bioassays were used. The experimental design was completely randomized with 10 repetitions per treatment and population, following the same procedures used in the fumigation bioassays. The mortality was evaluated 10 min after exposure, every 30 min up to 2 h, and every 2 h until the survival of the control group reached 80%.

2.3.3. Walking behavior

Walking bioassays in half treated arenas were performed to analyze whether the *S. zeamais* individuals from most tolerant and susceptible populations were able to avoid contact with the treated side of the arena. Bioassays were conducted in a Petri dish (6 × 1.5 cm) covered with a filter paper (Unifil, cod. 501.009). Filter papers discs were separated into two halves (treated and untreated) and fixed with double-face tape at the bottom of the arenas. Each half was treated separately with 0.1 mL solution of the treatments (1.0%) and fixed to the arena after evaporation of the solvent. In the untreated half, 0.1 mL of the solvent (acetone) was applied. The experimental design was completely randomized with 30 repetitions for each treatment and population (N = 180).

In the center of each arena was inserted an adult of *S. zeamais* and its movement was video-recorded during 10 min using Panasonic SD5 SuperDynamics camera (model WV-CP504), equipped with a Spacecom 1/3" 3–8 mm F1.2 lens. The walking behavior was analyzed using EthoVision® XT software - version 8.5 (Noldus Integration System, Sterling, VA) and Studio 9 software (Pinnacle Systems, mountain View, CA). The parameters evaluated were the average displacement (cm), walking speed ($cm.s^{-1}$) and time spent in each side of the arena.

2.4. Statistical analysis

The mortality rates of insects submitted to treatments in the lethal concentration bioassays were corrected in relation to the control group using Abbott's formula (1925). Corrected data were then submitted to Probit analyses to determine the doses-mortality curves, for each treatment and population. Curves whose probability of acceptance of the null hypothesis (that the data have Normal distribution) by the $\chi 2$ test was greater than 0.05 were accepted. Curves and the lethal concentrations (LC_{50} and LC_{90}) with their respective 95% confidence intervals (95% CI) were obtained using PROC PROBIT (SAS Institute, 2008). 95% CIs were used to compare the toxicity among populations.

Survival curves were obtained using Kaplan-Meier estimators in the software SigmaPlot 11.0. Through these survival curves, it was possible to estimate the times necessary to cause the mortality of 50% of individuals of each population (LT_{50}). Data from the walking bioassays were submitted to multivariate analysis of variance (populations, treatment and population x treatment) (PROC GLM, with MANOVA) and the means were compared by t-test (PROC TTEST).