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Biochemical parameters of *Spodoptera frugiperda* (J. E. Smith) treated with citronella oil (*Cymbopogon winterianus* Jowitt ex Bor) and its influence on reproduction

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ABSTRACT

Spodoptera frugiperda is the principal corn pest in Brazil. Searches for new control methods that minimize the adverse effects of synthetic insecticides have initiated a resurgence of the use of botanical insecticides. Citronella oil (a product of *Cymbopogon winterianus*) is an effective repellent and insecticide. Thus, biochemical profile changes in oil-treated larvae and its influence on reproduction were assessed. Corn leaves dipped in a 50 mg/mL concentration were offered to third instar larvae for 24 h and assessed in sixth instar to estimate protein, lipid, sugar, and glycogen levels. Adult testes and ovarioles were collected for histological and histochemical analysis 24 h after emergence. Number of eggs and hatching rate were also measured. Oil-treated larvae showed an increase in glycogen and a decrease in protein, lipid, and totals sugar content. Control testes exhibited connective tissue lining and cysts with abundant spermatozoids. However, intense peripheral vacuolation and neutral carbohydrates reduction occurred in oil-treated individuals. Control ovarioles showed normal morphologic characteristics. On the other hand, oil-treatment ovarioles showed follicular cell stratification and removal, reduced nurse cell development, reduced yolk quantity, a thinner conjunctiva sheath, and a reduction in proteins and neutral carbohydrates. Eggs derived from oil-treated pairs were unviable. Therefore, sub-lethal doses of citronella oil alters the biochemical profile of *S. frugiperda* larvae, causing damage to their reproductive histophysiology and results in diminished reproduction or reproductive failure.

1. Introduction

Characterized as a polyphagous pest, the armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), feeds on over 80 species of plants, affecting several economically important crops in different countries. In Brazil, this is the main pest of corn (Capinera, 2001; Cruz, 1995; Pogue, 2002). Its control is generally done with the use of synthetic insecticides which, despite their effectiveness, promote several agroecosystem problems and leave toxic residues in food (Cruz, 1995; Roel et al., 2000).

Integrated Pest Management (IPM) encourages the use of botanical pesticides as a viable and advantageous alternative in pest control (Bogorni and Vendramim, 2005; Isman, 2006; Roel et al., 2000). Java citronella, a type of citronella oil extracted from *Cymbopogon winterianus* Jowitt ex Bor (Poaceae), is an important product in Brazil (Martins, 2006) that has attracted agricultural interest as a potential insect repellant and insecticide. For example, Labinas and Crocomo (2002) showed that citronella oil caused feeding deterrence and mortality in *S. frugiperda* larvae through intake and/or topical contact tests.

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Synthetic or natural insecticides typically impair physiological processes in the target insect and impair their survival or reproductive capacity (Orr and Downer, 1982). Sharma et al. (2011) demonstrated the success of using changes in physiological parameters to assess and predict the toxicity and potential efficacy of phytochemicals in the control of mosquitos. Alterations in *Anopheles stephensi* (Liston) (Diptera: Culicidae) and *Culex quinquenfasciatus* (Say) (Diptera: Culicidae) biochemical levels when treated with *Artemisia annua* L. (Asteraceae) and *Azadirachta indica* A. Juss. (Meliaceae) extracts. The extracts caused reduction in feeding and digestion of food, interrupted protein synthesis and induce a physiological stress when levels of carbohydrate and lipid were increase, disrupting the metabolic activity of the larvae.

Some botanical insecticides are effective by acting at sub-lethal doses by influencing an insects' reproductive system. Alves et al. (2014) reported histopathological changes in larvae and adults testes, and in *S. frugiperda* adult ovarioles when fed with corn leaves treated with extracts from *Piper hispidinervum* Jacq. (Piperaceae). Medina et al. (2004) observed oogenesis inhibition in *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) treated with Azadirachtin, an extract from *A. indica*.

Insecticide efficacy and mode of action are dependent on the concentration used. Death typically occurs at higher concentrations and less intense and more durable effects occur in lower concentrations (Roel, 2001). This study aims to evaluate changes caused by a sub-lethal dose of citronella oil *on S. frugiperda* biochemical parameters and reproduction.

2. Materials and methods

This research was conducted at the Histology Laboratory, Department of Morphology and Animal Physiology (DMFA), and at the Center for Research Support (Cenapesq), Rural Federal University of Pernambuco (UFRPE), Recife, Brazil.

Insects were obtained from Histology Laboratory (UFRPE) breeding stock, where larvae were fed an artificial diet, modified for *Spodoptera*, according to Busato et al. (2006). Adults were fed with a 10% honey solution and were kept in Biochemical Oxygen Demand (BOD) incubators at 25 °C ± 0.2, 70% RH, and 12 h photophase.

For the experiments, larvae were fed with AG105 double hybrid corn leaves grown in a greenhouse with two plants per 5L pots containing mixtures of soil and humus (2:1), adding 12.1 g N, P, K (formulation 4:14:8).

Citronella oil was obtained from the Federal University of Paraíba, Bananeiras Campus. Chromatography was performed at the Chemical Ecology Laboratory, Department of Fundamental Chemistry, Federal University of Pernambuco (UFPE), Recife, Brazil. To determine its chemical contents, the citronella oil was diluted in hexane and analyzed by gas chromatography coupled with mass spectrometry in a quadrupole GC/MS Agilent 5975C Series system (Agilent Technologies, Palo Alto, USA) equipped with a DB-5 nonpolar column (Agilent J&W; 60 × 0.25 mm id, film thickness of 0.25 μm) in which a 1 μL aliquot was injected in split 1:20. The GC conditions were: helium as carrier gas; initial temperature of 60 °C for 3 min, adding 2,5 °C/min up to 240 °C and maintaining this temperature for 10 min. Mass spectrometry: 200 °C with recorded mass spectra of 70 eV (in EI mode) and scanning speed of 0,5scan^{-s} de m/z 20–350.

2.1. Bioassays

In order to perform bioassays, corn leaf pieces between 20 and 40 days old were immersed in solution containing essential citronella oil 50 mg essential oil + 98 mL distilled water + 2 mL dimethyl sulfoxide (DMSO), and for control group, corn leaf pieces were only immersed in a 98 mL distilled water and 2 mL DMSO solution. Leaf pieces were then dried at room temperature and offered to 10-day old (3rd instar) *S. frugiperda* larvae for 24 h. After this, larvae were fed on untreated corn leaves until they matured (6th instar). Each treatment consisted of 60 larvae individualized in 80 mL lidded plastic pots.

2.2. Biochemical tests

Sixth instar entire larvae were macerated in 5 mL of phosphate buffer solution and evaluated for the following.

2.2.1. Total proteins

200 μL macerate with the addition of 300 μL phosphate buffer were used and centrifuged at 2000 rpm for 2 min. For quantitation, the Bradford protein assay method (Bradford, 1976) was used and performed in a 595 nm spectrophotometer.

2.2.2. Glycogen, lipids, and sugar

Glycogen, sugar and totals lipid content were assessed following the method of Van Handel (1985a,b): 200 μL homogenate with addition of 200 μL sodium sulfate and 800 μL methanol and chloroform (1:1) were centrifuged at 2000 rpm for 2 min. The precipitate was used for glycogen analysis, and the supernatant was transferred to another test tube, where sugar and lipids were separated. Lipids were analyzed by spectrophotometry using the vanillin-phosphoric acid method (Van Handel, 1985a), while sugar and glycogen content were analyzed with the anthrone-sulfuric acid method (Van Handel 1985b). Absorbance was read at 625 nm.

Ten repetitions in duplicate were used per treatment. These ten values were averaged and the data were subjected to a t-test using the SAS program (SAS Institute, 2001).

2.3. Adult gonad histological analysis and immunohistochemistry

2.3.1. S. frugiperda

Adult ovarioles and testes were collected 24 h after emergence. These were fixed in 10% formalin for 24 h and dehydrated in increasing ethanol baths (70–100%) for 10 min each, then soaked in Historesin + alcohol (1:1) for 24 h and embedded in Leica© historesin. Sections of 5 μm and 7 μm thick were obtained with a LEICA RM2035 microtome. The obtained sections were submitted to morphological analysis with hematoxylin-eosin (H-E). It was also made histochemical analysis for: detection of neutral polysaccharides with periodic acid-Schiff (PAS), which, the results were analyzed according to the amount of PAS-positive granules, and staining intensity of it; and detection of total protein with Xylidine ponceau stain, according to the staining intensity. Histological immunohistochemistry analysis was performed using a LEICA DM500 light photomicroscope.

2.4. Oviposition and hatched larvae rate

For egg counting, ten newly emerged control treatment *S. frugiperda* adult couples and five newly emerged citronella oil treatment *S. frugiperda* adult couples were formed. Only five treatment couples were able to be made because of high mortality experienced by the larvaes and newly emerged adults treated with citronella oil on larval stage. Couples were placed in 10 × 15 cm PVC tube cages, covered with plastic film and internally coated with continuous paper, as oviposition substrate. The cages paper cover was changed every day for egg masses collection. Eggs laid and hatched larvae counting were made. Data was subjected to analy-