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Biological activity of sugarcane pyroligneous acid against *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) larvae

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***Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) annually cause enormous loss to the producers and their combat has become a worldwide challenge mainly due to several reports of pesticides resistance. Today, one of the best alternatives used in this combat is the application of natural insecticides such as neem oil and pyroligneous acid. This study demonstrates a method to obtain a hexane fraction from sugarcane pyroligneous acid, which can be easily applied, as well as its effectiveness against *S. frugiperda*. The hexane fraction exhibited LC_{50} of 2206,41 ppm after 24 h of exposure with a linear dose-response, indicating that the fraction can be used as a bio-insecticide against *S. frugiperda*.**

Key words: Wood vinegar, insecticide, *Saccharum officinarum*, fall armyworm, pyroligneous extract.

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), is a polyphagous species of tropical and semitropical zones (Sparks, 1979) that causes severe damage to crops (Kamaraj et al., 2008). The control of this pest is usually made with the use of synthetic insecticides (Tavares et al., 2010a; Wititsiri, 2011) which, although effective, can cause various problems, such as the presence of residues in food, poisoning of applicators, and outbreaks of secondary pests (Roel et al., 2000; Berlitz et al., 2012). Furthermore, the continued use of these pesticides may aid in development of a resistant pest population (Munoz et al., 2013).

An alternative for combating crop pests is the use of natural products such as neem (Correia et al., 2009; Tavares et al., 2010b), plant extracts (Roel et al., 2000; Tavares et al., 2009, 2011, 2013), and pyroligneous acid (Azevedo et al., 2007; Tavares et al., 2010a). The latter one is produced from the condensation of smoke from wood carbonization (Mendonça et al., 2006). This product is mainly characterized by low environmental impact and is a good alternative in organic agriculture. The pyroligneous acid, also known as wood vinegar, has been demonstrated to have low mammalian toxicity, lack neurotoxicity, have low persistence in the environment, and have high biodegradability (Céspedes et al., 2000).

The pyroligneous acid is normally composed of sugars, carboxylic acids, phenols (Fengel and Wegener, 1984; Kim et al., 2008), aldehydes, ketones, esters, furans and pyrans derivatives, nitrogen compounds, and other com-

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pounds (Ninomiya et al., 2004), and the major part is constituted by water (85%) and acetic acid (5.1%) (Zanetti et al., 2003). Studies on the pyroligneous acid have reported bactericidal, fungicidal, and termiticidal activity (Mu et al., 2003).

The extract is usually obtained from species used to make charcoal (Cai et al., 2012). In Brazil, the species most used for charcoal production is the *Pinus elliottii* var. *elliottii* Engelm. (Pinaceae) (Porto et al., 2007). One of the viable alternatives for obtaining this extract is from sugarcane bagasse (*Saccharum officinarum* L., Poaceae); this residue has been routinely burned as an energy source (Zandersons et al., 1999), and hence, by a simple condensation of the smoke released, the extract can be obtained, which can be processed and used as a bio-insecticide. This process could avoid the annual emission of millions of tons of gases into the atmosphere (Alves et al., 2010), and produce a product with low environmental impact. The aim of this study was to obtain and process sugarcane pyroligneous acid and evaluate it against *S. frugiperda* larvae.

MATERIALS AND METHODS

Plant material and extract fractionation

The bagasse of *S. officinarum* was collected from Santa Olinda S/A, located in the municipal of Sidrolândia - Mato Grosso do Sul State, Brazil. The pyroligneous acid was obtained from the Laboratory of Pharmacognosy - (Federal University of Mato Grosso do Sul - UFMS) in Campo Grande, Mato Grosso do Sul State, Brazil. For that purpose, the bagasse was placed in an Erlenmeyer flask and completely burned in a muffle at 300°C, and then the smoke was condensed and the extract was collected. The crude extract had its pH neutralized and then was partitioned with hexane; this hexane fraction of the pyroligneous acid (HF-PA) was used in the biological assay.

Bioassay procedures

The eggs of *S. frugiperda* were provided by the Laboratory of Entomology of the Anhanguera University (Uniderp) in Campo Grande, Mato Grosso do Sul State, Brazil. After the eggs hatched, the larvae were placed on artificial diet (Greene et al., 1976) with 12-12-h photoperiod, at 27 ± 3°C, and under 70 ± 5% of relative humidity. The tests were divided into three groups of 10 neonatal larvae for each concentration: Negative group, Artificial diet and acetone; positive group, artificial diet plus neem oil at concentration of 12000 ppm (Neem oil 95%, Serôdia®, Campo Grande, Mato Grosso do Sul State, Brazil) plus acetone; and test group, artificial diet plus the HF-PA (60, 120, 360, 600, 1200, 3600, 6000, 12000, and 36000 ppm) and acetone to dilute the extract.

Statistical analysis

The experiment was monitored at 24 h period, observing mortality in relation to concentrations of HF-PA, and the statistical program used was SPSS Statistics 20.0.0 to apply simple linear regression model ($P < 0.05$). To calculate the LC_{50}, Probit analysis from the logarithms of the concentrations was used.

RESULTS AND DISCUSSION

The yield of pyroligneous extract was approximately 40%. This extract was neutralized once acetic acid constituted approximately 5% of the extract (Zanetti et al., 2003) because this compound could interfere in the bioassay and degrade other compounds present. Another step was the fractionation of the extract; this step was aimed mainly to obtain an extract without water. The HF-PA showed a neutral pH and can be easily concentrated; these characteristics allowed easy development of new products and application of this extract.

The bioassay using HF-PA showed significant change, when compared with the negative control group. All the different concentrations of the extract caused significant mortality in 24 h, which was also observed in the positive control group (neem oil). The highest concentration evaluated (36000 ppm) resulted in 100% death, and the lowest concentration (60 ppm) caused only 10% of deaths (Figure 1). The LC_{50} of HF-PA was 2206,41 ppm. Our results suggested a linear dose-response (Figure 2), which was also confirmed by r^2 (0.83). Neonatal nymphs of *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) showed 66.59% mortality caused by neem oil (Natuneem®) and 67.45% of deaths caused by pyroligneous acid (Pironat®) (Azevedo et al., 2005). Neem oil (Natuneem®) and crude pyroligneous extract (Biopirol7M®) tested on eggs of *S. frugiperda* and *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae), confirmed mortality of eggs of different ages with different concentrations of the extract, and the extracts' satisfactory effect in controlling these pests (Tavares et al., 2010a, b). However, neem oil (Natuneem®) and commercial crude pyroligneous acid (Biopirol7M® and Pironat®), no significant mortality was observed when the extract was applied to adults of *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) (Efrom et al., 2011). These contradictions among different studies are related to the stages of the insects used in the experiments. Another possibility is related to the tolerance of the insect to insecticides in the course of their development (Yu, 1983; Schmutterer, 1990).

One of the apparent causes of the activity against the larvae is the synergistic effect among the compounds (Richards et al., 2010); this synergism occurs between different classes of compounds or structurally similar compounds, and the effects may range from antifeeding effect to toxicity activity (Dyer et al., 2003). The wide range of compound prevents resistance development (Rice, 1993). These secondary metabolites have different sites of action and molecular targets. They interact with important metabolic and enzymatic inhibitors (Céspedes et al., 2000; Torres et al., 2003) against microbial pathogens and invertebrates (Wink and Schimmer, 1999). Thus, for example, some secondary metabolites such as the nitrogenous compounds may act by blocking

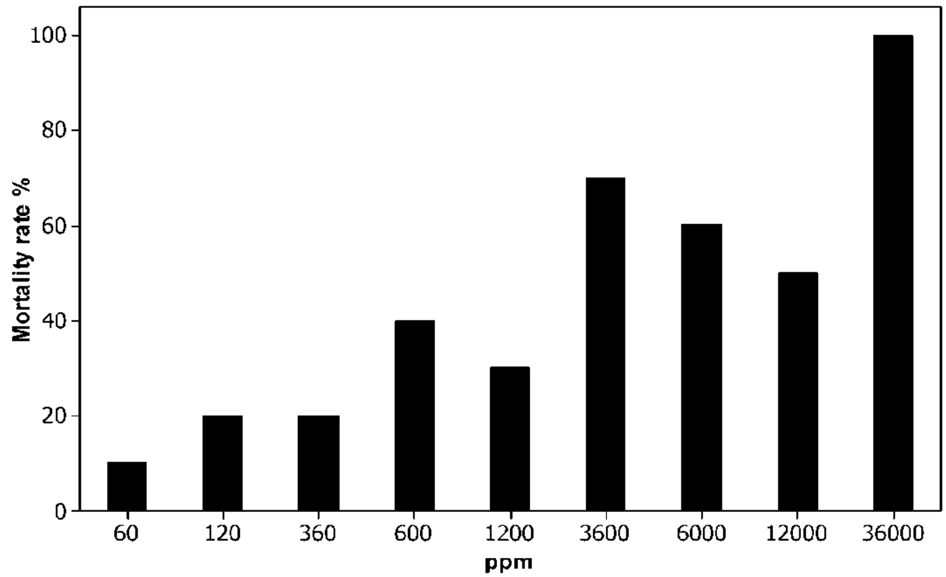

Figure 1. Distribution of percentages of mortality of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae caused by HF-PA.

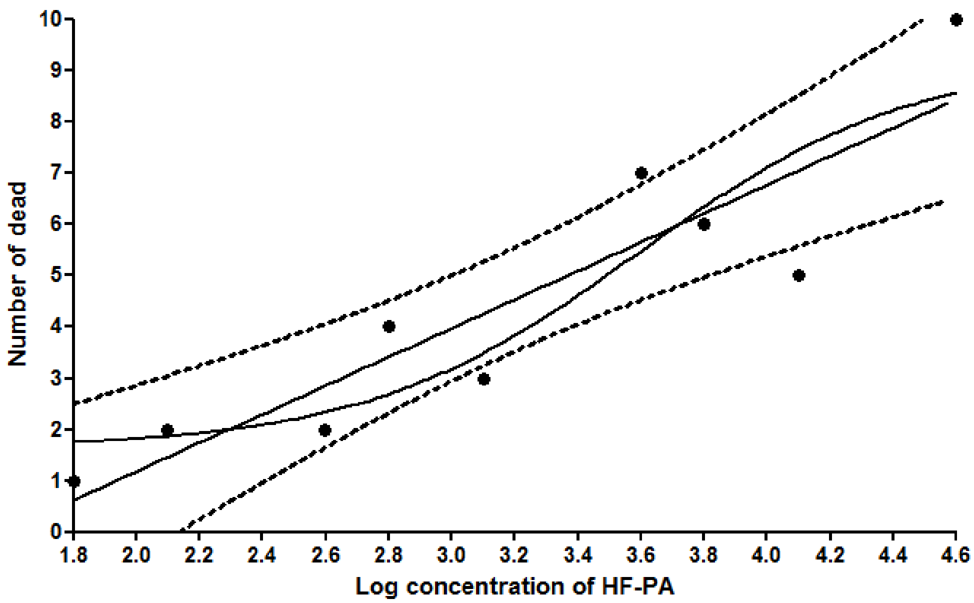

Figure 2. Graph regression analysis ($P < 0.05$) presenting the log of the dose against the number of dead *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae during 24 h.

the nervous system activity (Kagabu, 2008), or by inhibiting synthesis of deoxyribonucleic acid (DNA), and modifying the permeability of the membrane by changing the composition with respect to structural proteins (Schmeller et al., 1997). Another mechanism of action of PA-HF could be related to the presence of phenolic compounds, one of the main groups being investigated for pest control (Henn, 1997).

The pyroligneous extract used was toxic to *S. frugiperda* larvae and displayed a linear dose-response with an LC_{50} of 2206,41 ppm. Furthermore, the possibility of preparation of a low-cost fraction of the sugarcane pyroligneous acid was also demonstrated. These results open new possibilities for the fraction to be tested against different pests of crops and agriculture, providing useful application for the smoke produced by the burning of sugarcane bagasse.

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