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Target and non-target response of *Swietenia Mahagoni* Jacq. chemical constituents against tobacco cutworm *Spodoptera litura* Fab. and earthworm, *Eudrilus eugeniae* Kinb

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HIGHLIGHTS

- Toxicological screening of *Swietenia mahagoni* Jacq. against the lepidopteran pest *Spodoptera litura.*
- The lethal concentration (LC_{50} and LC_{90}) was observed at the dosage of 31.04 and 86.82 ppm respectively.
- Sub-lethal concentrations (30 ppm) showed higher larval and pupal durations.
- Midgut histology showed that methanolic extracts significantly disturbs the gut epithelial layer, lumen and brush border membrane.
- Bio-rational plant product from *S. mahagoni* displays a significant effect to reduce lepidopteran pests.

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ABSTRACT

Toxicological screening of *Swietenia mahagoni* Jacq. (Meliaceae, West Indies Mahogany) against the lepidopteran pest *Spodoptera litura* was examined. Phytochemical screening through GC-MS analysis revealed nine peaks with prominent peak area % in Bis (2-ethylhexyl) phthalate (31.5%) was observed. The larvae exposed to discriminating dosage of 100 ppm deliver significant mortality rate compare to other treatment concentrations. The lethal concentrations (LC_{50} and LC_{90}) was observed at the dosage of 31.04 and 86.82 ppm respectively. Sub-lethal concentrations (30 ppm) showed higher larval and pupal durations. However, pupal weight and mean fecundity rate reduced significantly. Similarly, the adult longevity reduced significantly in dose dependent manner. Midgut histology studies showed that the methanolic extracts significantly disturbs the gut epithelial layer, lumen and brush border membrane compare to the control. The soil assay on a non-target beneficial organism, the soil indicator earthworm *Eudrilus eugeniae*, with extracts from *S. mahagoni* (200 mg/kg) showed no toxicity compared to Monocrotophos at the dosage of 10 ppm/kg. Current results suggest that this bio-rational plant product from *S. mahagoni* displays a significant effect to reduce lepidopteran pests with low toxicity to other beneficial species.

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1. Introduction

Chemical pesticides provide significant protection on agriculture crops, domesticated animals, and humans from pests and pathogens (Edwin et al., 2016a,b). Approximately five billion pounds of chemicals were applied in agriculture over the past

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decade worldwide (Ponsankar et al., 2016a). However, improper use in many developed nations results in insecticide resistance and increased environmental regulation of pesticides (Senthil-Nathan et al., 2006a,b; Vasantha-Srinivasan et al., 2017). The current trend is to examine alternatives to synthetic pesticides which provide effective pest control, while being more environmentally friendly. Progressively, biological control organisms and botanical insecticides have been established against the major agriculture pests (Senthil-Nathan et al., 2005a,b; Chandrasekaran et al., 2012; Selin-Rani et al., 2016a).

Botanical insecticides have been involved in the discovery and development of synthetic chemical products (Kalaivani et al., 2012). Identification of novel botanical chemistries provides a growing foundation which may produce chemistries with quicker degradation into benign compounds, while providing effective pest and pathogen suppression (Vasantha-Srinivasan et al., 2016). Phytochemicals derived from botanicals have historically demonstrated activity against a wide range of pests through acute toxicity, however better identification and quantification on the extracted chemistries and purified compounds are needed to advance botanical insecticides as suitable commercial alternatives to currently used chemical pesticides (Senthil-Nathan et al., 2007). Thus establishing standards for quality analyses and activity bioassays will increase the trend towards discovery and development of future botanical-based products for designing new, improved pesticides (Senthil-Nathan, 2013).

Swietenia mahagoni Jacq. (Meliaceae) commonly known as mahagoni native to West Indies. The tree is a large, deciduous and economically important timber tree (Anonymous, 1989; Mostafa et al., 2011). The tree is a moist zone plant which is planted broadly in Southern Asia and in the Pacific region (Schmidt and Joker, 2000; Akbar, 2009). Most of the areal parts of the plant, flowers, and leaves have been shown to contain phytochemicals with medical usese, ie. —human immunity, anti-microbial, anti-oxidant, anti-inflammatory and anti-diabetic effects (Naveen et al., 2014). Historical reports on the uses of *S. mahagoni* extracts report safe usage across traditional remedies, however many of these studies are not backed up by scientific studies (Hajra et al., 2012). As an insecticide or repellent the leaf extracts of these species have demonstrated insect repellent and larvicidal activity against mosquito species which transmit human and animal pathogens (Adhikari et al., 2012; Adhikari and Chandra, 2014). Chemical characterization of *S. mahagoni* species has identified more than forty limonoids belonging to the gendunin, mexicanolide, and phragmalin structural types (Chen et al., 2007).

Spodoptera litura Fab. (Lepidoptera: Noctuidae) is a polyphagous and economically significant pest (Senthil-Nathan and Kalaivani, 2005, 2006). As a generalist feeder is can survive on more than 150 host plants, being one of the most damaging agricultural pests in Asian countries (Edwin et al., 2016c). Chemical insecticides to control *S. litura* have failed due to the development of resistance to various classes of chemicals (Hu et al., 2007; Edwin et al., 2016c; Ponsankar et al., 2016b). Therefore, research was directed to discover potential botanical insecticides for use in the management of this pest (Selin-Rani et al., 2016b).

Earthworms are significant components of terrestrial ecosystems whose primary role as decomposers which improve soil structure and nutrition. They are regularly exposed to toxic chemical pesticides (Chen et al., 2014; Gopinathan et al., 2015). Worms are appropriate biological indicators related with faunal diversity and soil fertility (Vasantha-Srinivasan et al., 2016). *Eudrilus eugeniae* (Kinberg) dwells at the surface layer of soggy soils enriched with organic matter (Bouche, 1997). The earthworm family of Eudrillidae are easy to maintain and they have been used as indicator species across many ecotoxicology studies (Ponsankar et al., 2016a).

Thus the main purpose of this ecotoxicology study was to evaluate: (a) isolation and determination of major active compounds present in the methanolic leaf extracts of *S. mahagoni* through GC-MS analysis; (b) assessment of dose dependent toxicity of *S. mahagoni* against the lepidopteran pest; (c) detection of behavioral and developmental changes in *S. litura* with sub-lethal dosage of leaf extracts of *S. mahagoni*; (d) observation of histopathological changes in the mid gut tissues of *S. litura*; (e) non-target screening of *S. mahagoni* against the bio indicator earthworm *E. eugeniae*.

2. Methodology

2.1. Plant harvesting and crude extract preparation

The leaves of the healthy plant *S. mahagoni* were used as plant material. Fresh leaves were collected in and around Southern Western Ghats, Tirunelveli, India. The collected plant material were authenticated and voucher specimen (1145) of this collection has been submitted to herbarium of SPKCESS, Manonmaniam Sundaranar University, India. The leaves were washed and shadow dried at room temperature for one week until they become stiff, then pulverized to powder. Preparation of crude extract was extracted based on the adapted methodology of Thanigaivel et al. (2017). The dried leaf powder (500 g) were extracted in a Soxhlet apparatus using methanol (60–80 °C, AR grade Worli Road, Mumbai, Maharashtra 400030, India) until exhaustion. The excessive solvents from the leaf extract was separated in a vacuum rotary evaporator under reduced pressure of 22–26 mm Hg at 40 °C and the concentrate was further evaporated to complete dryness at room temperature. The crude extracts was stored in a brown bottle (4 °C) for GC-MS analysis and further toxicity assays.

2.2. Chemical characterization

The methanol extract of active fractions of *S. mahagoni* was subjected to GC–MS analysis (JEOL GCMATE II GC-MS-Agilent Technologies 6890 N Network GC system for gas chromatography). Two micro liter of active fractions was dissolved in HPLC grade methanol and subjected to GC and MS, JEOL GC mate equipped with secondary electron multiplier. The column (HP5) was fused silica 50 mm × 0.25 mm I.D. Analysis conditions were 20 min at 100 °C and 3 min at 235 °C for column temperature and 240 °C for injector temperature. Helium was the carrier gas and split ratio was 5:4. The sample (1 μl) was evaporated in a split less injector at 300 °C; run time was 22 min. The compounds were compared to chemical standards and identified with gas chromatography coupled with mass spectrometry. The molecular weight, molecular formula and structure of the compounds of the test materials were determined by interpretation of mass spectrum of GC-MS using the database of the National Institute Standard and Technology (NIST).

2.3. Insect culture

S. litura cultures were maintained at the laboratory, Sri Paramakalyani Centre for Excellence in Environmental Sciences (SPKCEES), Manonmaniam Sundaranar University, Tamil Nadu. The eggs of *S. litura* were surface sterilized in situ by dipping in 0.02% sodium hypochlorite for 5 min, then rinsed with distilled water for 2 min. Then they were allowed to hatch. Post hatching larvae were reared on castor leaves (*Ricinus communis*) to the pre-pupal stage. Pre-pupae were detached and provided with vermiculture clay as pupation sites. Emerging adult moths were transferred to cages and fed on a 10% (w/v) sucrose solution. Moths were transferred at a

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