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Target and non-target toxicity of botanical insecticide derived from *Couroupita guianensis* L. flower against generalist herbivore, *Spodoptera litura* Fab. and an earthworm, *Eisenia foetida* Savigny

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ABSTRACT

Botanical insecticides may provide alternatives to synthetic insecticides for controlling *Spodoptera litura* (F.) and they are target specific, biodegradable, and harmless to mammals. Eight natural chemical compounds with larvicidal activity were identified from fraction F_6 of *C. guianensis* flower extract. Probit analysis of 95% confidence level exposed an LC_{50} of 223 ppm against *S. litura* third instar larvae. The growth and development of *S. litura* was affected in sub-lethal concentrations of fraction F_6 (50, 100, 150 and 200 ppm) compared to controls. Similarly nutritional indices values decreased significantly compared to controls. Fraction F_6 also damaged the gut epithelial layer and brush border membrane (BBM). This study also resolved the effects of toxicity to non-target earthworm treated with fraction F_6 and chemical pesticides (monotrophos and cypermethrin) and the results showed that fraction F_6 had no harmful effect on *E. fetida*. Further, fraction F_6 was eluted and sub fractions F_{6c} (50 ppm) showed high mortality against *S. litura* third instar larvae. Octacosane from fraction F_{6c} was established and confirmed using IR spectrum and HPLC. The time of retention of fraction F_{6c} was confirmed with the octacosane standard. Fraction F_6 of *C. guianensis* extract caused dose-dependent mortality towards *S. litura*. Octacosane in fraction F_{6c} was establish to be the prominent chemical compound associated with causing mortality but other compounds present in the fraction F_6 were shown to be associated with changes in development of *S. litura* at low dosages. *S. litura* at low dosage. Therefore, these findings suggest that octacosane may be one of the major insecticidal compounds affecting *S. litura* survival.

1. Introduction

In many areas, agricultural pests have established resistance to synthetic chemical insecticides. To overcome the increasing difficulties of handling pesticide resistance, it would be beneficial to replace many synthetic pesticides with botanical insecticides and its natural secondary metabolites were breakdown down very rapidly in the nature (Senthil-Nathan et al., 2006b; Senthil-Nathan et al., 2008a; Senthil-Nathan et al., 2009). Botanical insecticides

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offer alternatives to use of chemical insecticides because many of them are target specific, biodegradable, and have lower toxicity to mammals (Senthil-Nathan et al., 2006a, b, 2007; Maheswaran and Ignacimuthu, 2013).

Spodoptera litura Fab. (Lepidoptera: Noctuidae) is a serious polyphagous pest that causes severe damage during the early seedling stage of agricultural crops. *S. litura* has hundred and fifty host types including cotton, soybean, celery, tomato, chrysanthemum, groundnut, tobacco, crucifers and sunflower (Senthil-Nathan and Kalaivani, 2006; Chandrasekaran et al., 2012). In India, farmers used synthetic insecticides to control *S. litura*, (Sree et al., 2010) but this pest has established resistance to most number of the existing organochlorines, organophosphates, and carbamates (Subramanian et al., 2005). *S. litura* field populations have settled resistance ranged from 100- to 2700-fold against pyrethroids such as deltamethrin, cypermethrin, ethofenprox, ethofenprox + PAP and fenvalerate (Yonggyun et al., 1998). Thus it is urgent to find new ways to control lepidopteran pests.

Plants are the biochemical factories in nature able to produce a complex mixture of different chemical compounds (Kumari et al., 2009) such as alkaloids, phenolic compounds, flavonoids, coumarins, terpenes and terpenoids which encounters pest attack (Kabir et al., 2013). *Couroupita guianensis* (Aublet) (Ericales: Lecythidaceae) is native to northeastern South America that is commonly grown in Southern India and Malaysia. Its flowers are used in medicine (Revilla, 2002; Umachigi et al., 2007). Various parts of the tree contain glycosides, couroupitone, essential oils, phenolic substances, indirubcin, isatin and keto steroids (Rajamanickam et al., 2009; Devi et al., 2012). The extracts of *C. guianensis* have antifungal, antibiotic, antiseptic and analgesic properties (Tayade and Adivarekar, 2014). Additionally some extracts show larvicidal activity against *Culex quinquefasciatus* (Say) (Desal et al., 2003) and *Helicoverpa armigera* (Hübner) (Baskar et al., 2011).

Earthworms are important organisms of terrestrial ecosystems, acting as decomposers in improving soil structure (Vasantha-Srinivasan et al., 2016). Earthworms are known to accumulate toxicants and organisms feeding upon them adversely affected (Arillo and Melodia, 1991; Addison and Holmes, 1995). Earthworms are subtle indicators of soil quality (Hendawi et al., 2004). *Eisenia fetida* is broadly used earthworm species for evaluation of the ecological risks of toxic substances in the terrestrial ecosystem (Lankadurai et al., 2015). Since its culture were easily maintained and there are high literature data are available on the development and ecotoxicology evaluation (Owojori et al., 2009) this species has been selected for our study.

The current study deals with: (a) evaluation of the active compounds existing in the flower of *C. guianensis* and investigation of active compounds through gas chromatographic techniques, (b) assessment of the dose dependent toxicity of active compounds against *S. litura*, (c) measurement of the behavioral response of *S. litura* treated with active compounds of *C. guianensis*, (d) estimation of bioefficacy of active fractions on nutritional physiology of *S. litura* and (e) effects of active fractions on gut histological alterations in the *S. litura*, (e) ecotoxicological evaluation of active fraction and chemical pesticides against *E. fetida*.

2. Materials and methods

2.1. Plant collection

The *C. guianensis* flowers were collected from Vilvavananathar temple, Kadayam, Tirunelveli district, Tami lNadu (Fig. 1A). Freshly collected flowers were shadow dried at room temperature for five days. The dried flowers (250 g) were powdered mechanically

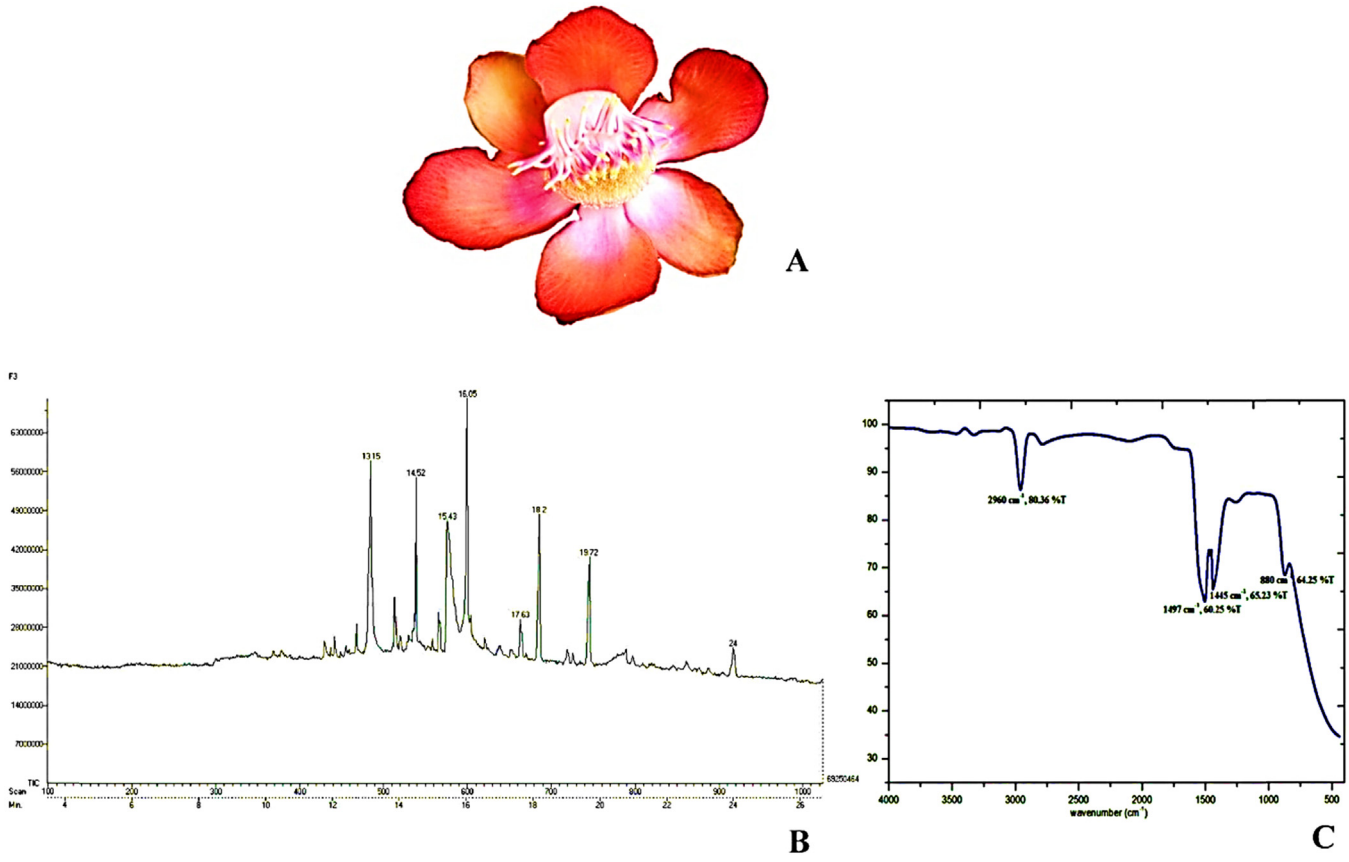

Fig. 1. Morphology of Flower *C. guianensis* (A); GC–MS analysis of *C. guianensis* fraction F_6 (B) and FTIR spectrum of fraction F_{6c} (C).