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Toxicity and behavioral effect of 3β,24,25-trihydroxycycloartane and beddomei lactone on the rice leaffolder *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae)

Sengottayan Senthil-Nathan[a,b,*], Man-Young Choi[a,c], Hong-Yul Seo[a,d], Chae-Hoon Paik[a], Kandaswamy Kalaivani[a]

[a]*Plant Environment Division, Honam Agricultural Research Institute (HARI), National Institute of Crop Science (NICS), Rural Development Administration (RDA), #381 Songhak-dong, Iksan, Chonbuk 570-080, Republic of Korea*
[b]*Division of Biopesticides and Environmental Toxicology, Sri Paramakalyani Centre for Environmental Sciences (SPKCES), Manonmaniam Sundaranar University, Alwarkurichi 627 412, Tirunelveli, Tamil Nadu, India*
[c]*Agricultural Pest Division, National Institute of Agricultural Sciences and Technology (NIAST), RDA, Seodoon dong, Suwon, Republic of Korea*
[d]*National Institute of Biological Resources, Environmental Research Complex, Gyoungseo-dong, Seo-gu, Incheon 404-170, Republic of Korea*

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Abstract

Treatment of *Dysoxylum* pure triterpenes 3β,24,25-trihydroxycycloartane and beddomei lactone to the rice leaffolder (RLF), *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae), resulted in prolonged larval duration and reduced larval weight. In leaf cut choice assay and topical application experiments, beddomei lactone and 3β,24,25-trihydroxycycloartaneto showed strong antifeedant and growth inhibitor activity against fourth instar larvae of *C. medinalis*. Also average leaf consumption was decreased (89%) by the treatment of the two terpinoids when compared with controls. Number of eggs laid by the female (fecundity) was decreased and oviposition deterrence index was increased due to the treatment. This result further shows that the pure triterpenes of *Dysoxylum* act as both an antifeedant and chronic toxin to the rice leaffolder larvae.

Keywords: *Dysoxylum*; Triterpenes; Antifeedant; Deterrence; Toxicity; Leaffolder; Biology; Weight; Oviposition

1. Introduction

Botanical insecticides are naturally occurring toxins extracted from plants. There are several advantages including low cost, comparatively benign environmental effects, low biohazard, comparatively low mammalian toxicity, low phytotoxicity, and reduced likelihood of resistance to use botanical rather than synthetic insecticides. Presently most synthetic and chemical insecticides act as acute toxins, causing rapid insect mortality due to interference with vital physiological functions (Coat, 1994; Campiche et al., 2006). Plant-derived insecticides breakdown quickly in the environment, resulting in little risk of residues on food crops. Some materials can be used shortly before harvest (Ross, 2005). Most botanicals are rapid acting and those are primarily harmful to insect pests (Silva-Aguayo, 2006). Botanical insecticides have a broader spectrum of activity than most biopesticides and therefore may be used for several pest species (Chiasson et al., 2004).

Plants are like natural laboratories where a great number of chemicals are biosynthesized. In fact, they may be considered the most important source of chemical compounds. Primary plant metabolism synthesizes essential

Abbreviations: RLF, rice leaffolder; 3β, 24, 25-THCL, 3β, 24, 25-trihydroxycycloartane; BL, beddomei lactone; FDI, feeding deterrence index; ODI, oviposition deterrence index; EC, effective concentration.

*Corresponding author at: Division of Biopesticides and Environmental Toxicology, Sri Paramakalyani Centre for Environmental Sciences (SPKCES), Manonmaniam Sundaranar University, Alwarkurichi 627 412, Tirunelveli, Tamil Nadu, India. Fax: +91 4634 283270.
E-mail addresses: senthilkalaidr@hotmail.com, senthil@ms-university.org (S. Senthil-Nathan).

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compounds which are present in all plant species (Silva-Aguayo, 2006). On the contrary, the end products of secondary metabolism are neither essential nor universally present in all plants (Glander, 2005). Common among these metabolites are compounds with protective action against insects, such as alkaloids, non-proteic amino acids, steroids, phenols, flavonoids, glycosides, glucosinolates, quinones, tannins, and terpenoids (Swain, 1977; Silva-Aguayo, 2006).

Insecticidal secondary metabolites have been isolated from plants such as pyrethrins (order Asterales, fam. Asteraceae), nicotine (order Solanales, fam. Solanaceae), sabadilla (order Liliales, fam. Liliaceae), rotenone (order Fabales, fam. Fabaceae), ryanodine (order Violales, fam. Flacourtiaceae), E-beta-farnesene (order Asparagales, fam. Orchidaceae). Much research has been conducted on screening of the Meliaceae, for triterpenes (limonoids) (Ambrozin et al., 2006).

Compounds belonging to this group have exhibited a range of biological activities on insects, insecticidal, insect antifeedant, growth regulating, hormonal inhibition, and larvicidal (Schmutterer, 1990; Ascher, 1993; Ma et al., 2000; Shafeek et al., 2003). The triterpenes occurring in Meliaceae are also known as meliacins. Out of more than 300 known triterpenes, about one-third is derived from neem (*Azadirachta indica* A. Juss) and Chinaberry (*Melia azedarach* Lin.) alone (Roy and Saraf, 2006).

Apart from two important genera (neem and chinaberry) of Meliaceae several other species demonstrated biological effects on insects, i.e. *Khaya ivorensis* A. Chev. (Vanucci et al., 1992), *Dysoxylum spectabile*, Hooke f., (Russell et al., 1994), *Aglaia* sp. (Satasook et al., 1994), *Carapa guianensis* Aubl., *Cedrela fissilis* Vell. (Ambrozin et al., 2006), and *Munronia henryi* Harms (Qi et al., 2003).

Dysoxylum malabaricum Bedd. and *Dysoxylum beddomei* Hiern (Meliaceae) were critically endangered and economically important trees of Western Ghats, Southern India. The leaves of *Dysoxylum* sp. contain several triterpenes. Leaf extracts of *D. malabaricum* affect insects in a variety of ways, acting as an antifeedant, growth retardant, and larvicide (Govindachari et al., 1994, 1999; Senthil-Nathan et al., 2006a). Many other investigators have isolated triterpenes from *Dysoxylum* sp. (Singh et al., 1976; Hisham et al., 2001, 2004; Luo et al., 2002; Jayakumar et al., 2003) but their detailed bioactivity against crop pests remain unexplored.

The present research furthers the work done by Senthil-Nathan et al. (2006a) on crude extract of *D. malabaricum*. It investigates the effect of pure terpinoids identified from *Dysoxylum* sp. on the feeding, growth and reproduction of the rice leaffolder (RLF) *C. medinalis*.

2. Materials and methods

2.1. Laboratory mass culture of Cnaphalocrocis medinalis

We collected *Cnaphalocrocis medinalis* larvae from the paddy fields in and around Erode District, Tamil Nadu, India. We reared larvae in a greenhouse at 27±2 °C, in a 14:10 light:dark photoperiod and 85% relative humidity. We placed larvae on rice plants in earthenware pots covered with mesh sleeves. Pots were 18 cm tall with a 20 cm top diameter. Each pot held 15 plants and gave 63 (±3) tillers. We placed pots in a metal tray with about 10 cm of water (Senthil-Nathan, 2006). We initiated the culture with partly grown larvae from the field. Thereafter, we placed newly hatched larvae on ca. 60-day-old plants of the rice variety 'IR20'.

To maintain the culture, we placed 12 female and 13 male moths in an oviposition cage containing one potted plant. We fed the moths with 10% honey solution to enhance oviposition. After 2 days, we removed the potted plants from the oviposition cage. We clipped leaf portions containing eggs and placed on moist filter paper in Petri dishes. These eggs were used to establish the culture of *C. medinalis*.

2.2. Preparation of dysoxylum pure compounds

3β,24,25-trihydroxycycloartane (3β,24,25-THCL) and beddomei lactone (BL) (Fig. 1A and B) were isolated from *D. beddomei* and *D. malabaricum*, respectively, and were received from Dr. Jayakumar, M.G. College, Trivandrum, Kerala, India (Jayakumar et al., 2003). They were dissolved in isopropanol and different concentrations were prepared by dilution with isopropanol.

2.3. Effective concentrations (EC50)

We treated the 'IR20' leaf cuts (9 cm) with 1.5, 3, 6, and 12 ppm of 3β,24,25-THCL and BL. We treated control leaves with 1% isopropanol and air-dried. We allowed the leaves to dry at room temperature for 10 min and then placed in 15 cm diameter Petri dishes. We carried out the

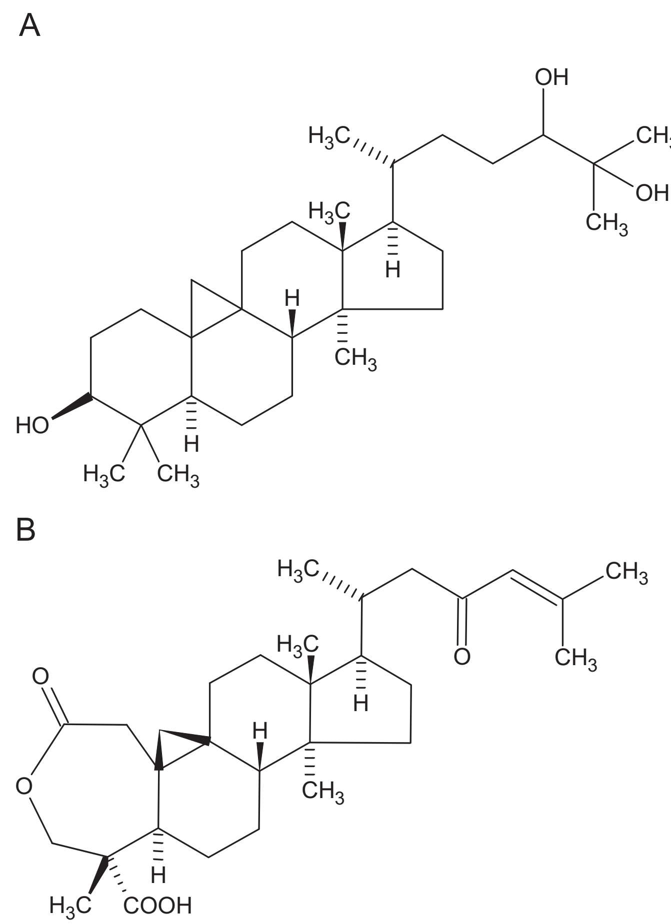

Fig. 1. Structure of *Dysoxylum* limonoids tested against *C. medinalis*: (A) 3β,24,25-trihydroxycycloartane and (B) beddomei lactone.