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Bioactivity of essential oils and sesquiterpenes of *Chloroxylon swietenia* DC against *Helicoverpa armigera*

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Essential oils from the leaves and stems of *Chloroxylon swietenia* DC were obtained by hydrodistillation and the chemical composition was determined by GC and GC–MS. The major identified components in leaf oil were limonene, geijerene, pregeijerene, germacrene D and trans-β-ocimene, while stem oil essentially contained methyl eugenol, limonene and geijerene. Laboratory bioassays of the essential oils and isolated compounds were evaluated for insecticidal and antifeedant activities on *Helicoverpa armigera*. Toxicity was determined by topical application of the total extracts and the isolates at varying concentrations, where leaf oil, stem oil, geijerene and pregeijerene were found to be more toxic than germacrene D and limonene, with LD_{50} values of 22.3, 26.9, 39.4 and 45.8 µg/larva respectively. Further, geijerene and pregeijerene displayed maximum feeding deterrence as well with DC_{50} of 89.8 and

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99.6 μg/sq. cm. The results indicate that these natural products may find potential application as useful, biodegradable, environmentally safe insect control and crop protectant agents.

Keywords: *Chloroxylon swietenia*, essential oil, *Helicoverpa armigera*, insecticidal activity, sesquiterpenes.

It is well known that insect pests play a major role in damaging the crops and the need continues for efficacious control agents. The use of pesticides and insecticides is one means of preventing losses from insects. Due to the problems associated with the indiscriminate use of synthetic insecticides like insect resistance and impact on non-target organisms, many scientists throughout the world have concentrated on the search for active natural products derived from plants as ecologically safe alternatives, because globally there is growing awareness and desire to utilize natural and environment-friendly compounds for pest control. In this connection, essential oils, which are complex mixtures of individual compounds and have been shown to possess a broad spectrum of pest control properties, have been widely investigated for their larvicidal, toxic, repellent, ovicidal, antifeedant and antioviposition effects[1–4]. The cotton bollworm, *Helicoverpa armigera* is a polyphagous and major insect pest in India, which has been reported to attack more than 200 different cultivated crop plants, ultimately causing severe loss of production[5,6]. Currently, it has become difficult to control this pest because of widespread development of resistance to conventional insecticides[7,8]. Due to the failure of chemical pesticides and insecticides in control, there is a need for the development of pest-management strategies which involve the use of safe and natural compounds that are plant-derived. As a part of the screening programme for indigenous, phytochemically active botanicals that grow in South India, we have chosen *Chloroxylon swietenia*, because the plant displayed abundant insect-repellent activity. Based on the knowledge that is historically available, we isolate bioactive compounds of *C. swietenia* and evaluate their phytochemical profiles and their performance.

C. swietenia DC which belongs to the family Rutaceae, is a medicinal and aromatic tree of the dry deciduous forests, popularly known as East Indian satinwood. The potential use of essential oil of *C. swietenia* and geijerene and pregeijerene as crop protectant has not been investigated. Here we report the insecticidal and antifeedant activities of essential oils and isolate compounds of *C. swietenia* on *H. armigera*.

Leaves and stems of *C. swietenia*, collected from the forests of Kinnerasani region, Andhra Pradesh, were air-dried and hydrodistilled in a Clevenger apparatus for 4 h. The distilled oil was dried over anhydrous sodium sulphate and stored under nitrogen atmosphere until further use. A voucher specimen was deposited in the Department of Botany, Osmania University, Hyderabad (No. OUBOT 4784).

Gas chromatography (GC) was carried out on a Varian-gas chromatograph equipped with flame ionization detector and a BP-1 capillary column. Helium with a flow rate of 1.0 ml/min was employed as carrier gas. Temperature was programmed from 60 to 220°C at a ramp rate of 5°C/min, with a final hold time of 6 min. The injector and detector temperatures were maintained at 250 and 300°C respectively. The sample was injected with 1 : 20 split ratio.

Gas Chromatography–Mass Spectrometry (GC–MS) analysis was performed on an Agilent 6890 GC equipped with 5973 N mass selective detector and HP-5 capillary column. The oven temperature was programmed from 50 to 280°C at 4°C/min and held at this temperature for 5 min. Inlet and interface temperatures were 250 and 280°C respectively. Helium, with a flow rate of 1.0 ml/min, was the carrier gas. EIMS: electron energy, 70 eV. Ion source and quadrupole temperatures were maintained at 230 and 150°C respectively.

The preparative GC analysis was carried out on a Hewlett Packard GC equipped with a Thermal Conductivity Detector (TCD) and a stainless steel column packed with 5% OV 101 on Chromosorb W-HP 100/120 mesh. Injector and detector temperatures were 250 and 260°C respectively. The column temperature was 150°C for isothermal analysis and 200–240°C at 20°C/min for the programmed analysis. Nitrogen was employed as a carrier gas with a flow rate of 100 ml/min.

GLC facilitated identification of individual components by comparing the retention indices (RI) of the peaks on the BP-1 column determined with reference to a saturated mixture of C_8–C_{22} *n*-alkanes and linear interpolation with those reported in the literature[9–11]. Further identification of the components was accomplished by GC–MS, by comparing their mass spectra with those in Wiley and NIST mass spectral databases resident in the system.

Toxicity of essential oils and compounds isolated from the oils was evaluated by topical application to fourth instar larva of *H. armigera*. The oils and compounds were tested at varying concentrations to establish LD_{50} and LD_{90} values, using five replicates for each dose. The compounds were prepared in acetone as the carrier. Acetone alone served as control and was applied to the dorsum of the insects using a topical dispenser. Monocrotophos was used as a positive control sample. All treated larvae were transferred onto a 2 cubic cm block of diet placed in petri plates, which were kept in sealed boxes and held in a growth chamber under a photoperiod regimen, light : dark (16 : 8 h). Subsequently, mortality was recorded and further observations were made on the behaviour of the larvae.

Antifeedant activity was investigated using the leaf disc choice method. Fresh leaf discs (3 cm diameter) of cotton were made using a cork borer. The pure oil and the compounds at varying concentrations in acetone were painted onto one side of the leaf discs. Azadirachtin was used as a positive control sample. Discs treated with acetone alone served as control. Freshly moulted third instar larvae were taken and starved for 4 h prior to testing. One larva was placed in each petri plate containing leaf discs with a small piece of wet cotton to prevent desiccation. Experiments were carried out in quintuplicate. Progressive consumption of leaf area by the larva after 24 h was recorded in control and treated discs using a ΔT leaf area meter, and subsequently a feeding deterrence index was calculated[12].

Probit analysis[13] was conducted to determine LD_{50}, LD_{90}, and DC_{50} representing the concentrations that caused 50 and 90% mortality and 50% feeding deterrence along with 95% confidence limits.

The essential oils from leaves and stems of *C. swietenia* were pale yellow in colour with an yield of 0.28 and 0.07% respectively. They were subsequently analysed by GC and GC–MS. A total of 22 compounds amounting to 92.4% in leaves and 77% in stems have been identified (Table 1). The major components in leaf oil are limonene (16.23%), pregeijerene (10.86%), geijerene (24.45%) and germacrene D (13.69%), while stem oil is rich in methyl eugenol (12.15%), limonene (12.86%), pregeijerene (9.42%), geijerene (12.08%) and germacrene D (6.54%). Both the oils are composed mainly of monoterpenes and sesquiterpene hydrocarbons, accompanied by appreciable amounts of oxygenated monoterpenes. Nevertheless, oxygenated sesquiterpenes have also been noticed as minor constituents.

The essential oils and isolated compounds were tested for their toxic and antifeedant activities against *H. armigera*. Among the whole range of compounds tested, the extracted essential oils, geijerene (Grn) and pregeijerene (Pg) displayed potent insecticidal activity with $LD_{50} <$ 50 μg/larva, whereas LD_{50} of monochrotophos was 3.6 μg/larva (Table 2). Germacrene D (Gm D) and limonene (Lm) showed intermediate toxicity. Mortality percentage at different concentrations of oils was recorded (Figures 1–2), where leaf oil could induce 100% mortality at a concentration of 52.7 μg/larva. The stem oil offered 100% mortality only at 61.6 μg/larva, whereas 8.1 μg/larva concentration of monochrotophos caused 100% mortality.

It is well known that various essential oils and their constituents exhibit acute toxic effects against insects. The present study was undertaken because unusually geijerene and pregeijerene were present in considerably large amounts in the essential oil of *C. swietenia* and no activity related to these compounds has so far been reported. Further, the oil as such has shown remarkable insecticidal activity which is presumably due to relatively

Table 1. Percentage composition of oils from leaves and stems of *Chloroxylon swietenia* DC

Compound	RI	Percentage composition: Leaf	Stem	Method of identification
α-pinene	937	2.41	0.89	a, b, c
Myrcene	984	1.84	0.56	a, b, c
Limonene	1024	16.23	12.86	a, b, c
cis-β-Ocimene	1035	1.18	1.88	a, b, c
trans-β-Ocimene	1040	4.94	2.95	a, b, c
Linalool	1085	0.82	0.32	a, b, c
Geijerene	1143	24.45	12.08	a, b, c
Terpinene-4-ol	1166	0	0.35	a, b, c
Geraniol	1240	0	0.65	a, b, c
Pregeijerene	1285	10.86	9.42	a, b, c
Delta elemene	1337	0.49	0.61	a, b, c
Geranyl acetate	1370	0.88	2.20	a, b, c
β-Bourbonene	1386	1.21	0	a, b, c
β-Elemene	1389	0.67	0	a, b, c
Methyl eugenol	1403	3.15	12.15	a, b, c
β-Caryophyllene	1421	2.27	0	a, b, c
α-Humulene	1446	1.48	1.56	a, b, c
Germacrene D	1480	13.69	6.54	a, b, c
Bicyclogermacrene	1493	1.78	2.81	a, b, c
d-Cadinene	1536	0.74	0.55	a, b, c
Nerolidol (stereochemistry is unknown)	1544	0	1.92	a, b, c
Spathulenol	1564	0	2.21	a, b, c
Caryophyllene oxide	1574	0.42	0	a, b, c
τ-Cadinol	1642	0.75	1.83	a, b, c
β-Bisabolol	1672	0.93	0.82	a, b, c
Farnesol (stereochemistry is unknown)	1699	1.26	1.85	a, b, c
Monoterpene hydrocarbons		26.60	19.14	
Oxygenated monoterpenes		4.85	15.67	
Sesquiterpene hydrocarbons		57.64	33.57	
Oxygenated sesquiterpenes		3.36	8.63	

RI, Retention indices determined on BP-1 column.
a, Retention time; b, Retention indices; c, Mass spectra.

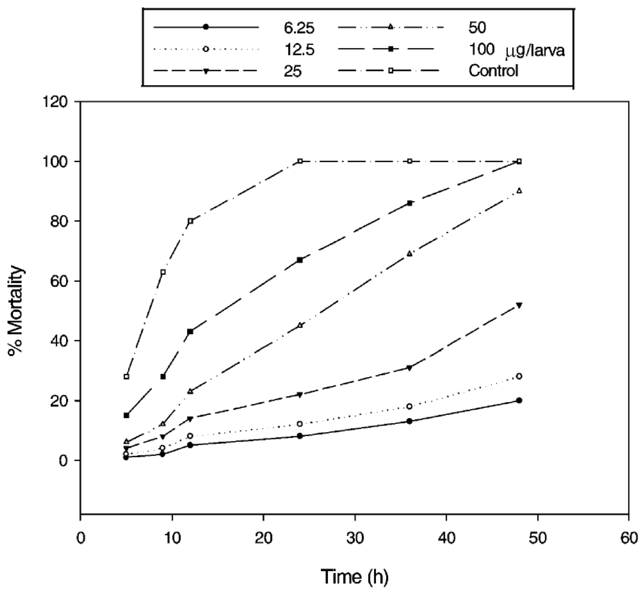

Figure 1. Per cent mortality of larva of *Helicoverpa armigera* at different concentrations of leaf oil and at different time intervals.

Table 2. Effect of essential oils and isolated compounds of *C. swietenia* on *Helicoverpa armigera*

Compound	Acute toxicity LD$_{50}$ (μg/larva)	Acute toxicity LD$_{90}$ (μg/larva)	Feeding deterrence DC$_{50}$ (μg/sq. cm)
Leaf oil	22.3 (17.462–27.138)	43.7 (36.440–51.040)	78.4 (66.839–89.921)
Stem oil	26.9 (17.035–36.725)	49.8 (33.395–66.205)	80.5 (67.609–93.431)
Geijerene	39.4 (28.062–50.818)	77.5 (65.266–89.734)	89.8 (78.996–100.600)
Pregeijerene	45.8 (33.820–57.780)	91.6 (76.400–106.920)	99.6 (86.112–113.090)
Germacrene D	++	++	145.9 (133.240–158.560)
Limonene	++	++	182.3 (166.280–198.320)
Monochrotophos	3.6 (2.017–5.223)	6.2 (3.879–8.561)	NT
Azadirachtin	NT	NT	28.8 (17.660–39.940)

Numbers in parentheses are 95% confidence limits. NT, Not tested.

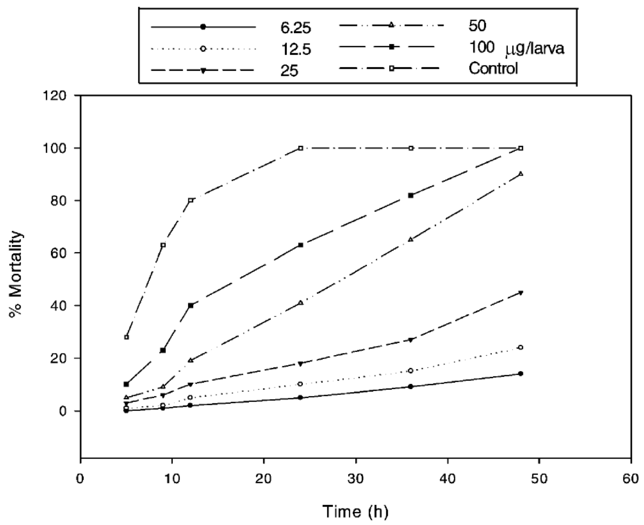

Figure 2. Per cent mortality of larva of *H. armigera* at different concentrations of stem oil and at different time intervals.

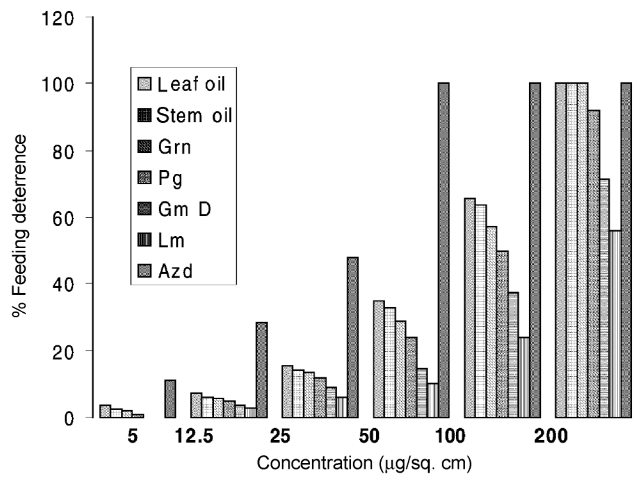

Figure 3. Effect of essential oil and isolated compounds on feeding deterrence of *H. armigera*.

large amounts of sesquiterpenes coexisting with geijerene and pregeijerene. The typical neurotoxic symptoms observed in insects, including tremors, lack of coordination, ending finally in paralysis and death are perhaps due to the blockage of neurotransmitters by the essential oil and its components. On the basis of the data of previous studies involving toxicity of conventional insecticides[14], geijerene is seven times less toxic than the standard organophosphate, monocrotophos; 35 times less toxic than the organochlorine, endosulfan and 81 times less toxic compared to the carbamate methomyl. Topical exposure to the oils and the isolated compounds delayed larval development through decreased rate of growth.

Further the essential oils and compounds were also tested for their efficacy as insect antifeedants against *H. armigera*. Both the oils exhibited significant feeding inhibition. The effective concentrations of leaf and stem oils that caused 50% reduction in larval feeding were 78.4 and 80.5 μg/sq. cm respectively. Among the compounds tested, geijerene and pregeijerene demonstrated the most effective feeding deterrence with DC$_{50}$ of 89.8 and 99.6 μg/sq. cm (Table 2). A minor deterrence was observed at 45 μg/sq. cm, which approached 100% deterrence at 197 and 234 μg/sq. cm respectively, for geijerene and pregeijerene. The percentage feeding deterrence at various concentrations of oils and the isolated compounds is presented in Figure 3. The leaf and stem oils could induce 100% deterrence significantly at 134 and 145 μg/sq. cm respectively, while azadirachtin exhibited 100% deterrence at 50 μg/sq. cm. Historically, the essential oils and individual compounds from medicinal and aromatic plants have been known to exhibit antifeedant properties against a number of insects[15,16]. Several essential oil compounds demonstrated feeding deterrence in a dose-dependent manner, but were less active (3–4 fold) than azadirachtin[17]. However, no reported data are available for geijerene and pregeijerene. These results suggest that the present oils and the isolates served as effective toxicants and antifeedant deterrents. Nevertheless, a mixture comprising these compounds may be more effective towards the total insecticidal and deterrent activity of the oil of *C. swietenia* with major phytochemical performance by sesquiterpenes, which agrees with published reports[18,19], however, the synergistic action of other phytoconstituents of the oil cannot be disregarded.

The present study indicates that the essential oils and sesquiterpenes of *C. swietenia* exhibit insecticidal properties and compare favourably with the commercial insecticide, monochrotophos. Moreover, these results may lead to the development of newer and more selective natural products, particularly geijerene and pregeijerene as effective insecticides, especially with respect to synergy.

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Pollination biology of large cardamom (*Amomum subulatum*)

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Amomum subulatum* Roxb. (family Zingiberaceae) is the large cardamom of commerce cultivated in tropical wet evergreen forests of the Eastern Himalayas of India, Nepal and Bhutan. This study seeks to identify floral visitors and pollinators, examine floral adaptations for pollination and evaluate pollination efficiency. Studies were carried out in two flowering seasons (2005, 2006) in a 6-ha plantation located adjacent to a degraded reserve forest in the Sikkim part of the Himalayas. Only two flower visitor species, a bumble-bee (*Bombus haemorrhoidalis* Smith) and a honey bee species (*Apis cerana* F.) were recorded. The bumble-bee was the effective and only pollinator, but *A. cerana

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