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Full Length Research Article

Larvicidal and ovicidal activities of Chloroxylon swietenia (Rutaceae) essential oils against Spodoptera litura (Lepidoptera: Noctuidae) and their chemical compositions

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Objective: To determine the chemical composition, larvicidal and ovicidal activity of the essential oil from *Chloroxylon swietenia (C. swietenia)* against lepidopteran pest *Spodoptera litura (S. litura)*.
Methods: The plants dry leaves were subjected to hydrodistillation using a modified Clevenger-type apparatus. The composition of the essential oil was analyzed by gas chromatography (GC) and GC mass spectrophotometry. *C. swietenia* essential oil and major chemical compositions was tested, against 4 th instar larvae of *S.litura* for 24 h and mortality were recorded at various concentrations(10-250ppm); The 24 h LC_{50} and LC_{90} value of *C. swietenia* oil were determined following probit analysis. *C. swietenia* essential oil and major chemical compositions was tested, against *S.litura* eggs for 120 h post treatment and Percentage of egg hatch ability recorded at various concentrations (25-250 ppm).
Results: Chemical constituents of Twenty compounds were identified in the oil of *C. swietenia* compounds representing to 98.95%. The major Chemical compositions in leaves were α-Pinene, limonene, geijerene, pregeijerene and germacrene D. The essential oil exhibited significant larvicidal activity, with 24h LC_{50} 131.20 ppm and LC_{90} 224.68 ppm. The major Chemical compositions larvicidal activity were also tested. The LC_{50} values of geijerene, limonene, germacrene D, pregeijerene and α-Pinene were LC_{50} 18.31, 20.03, 24.83, 25.23 and 26.35 ppm and LC_{90} 34.77, 38.31, 46.67, 47.27 and 49.78 ppm respectively. The essential oil produced (100% mortality) eggs no hatchability recorded in 200 ppm, however, highest ovicidal activity found in geijerene 125 ppm, This was closely followed by limonene and germacrene D 150 ppm, pregeijerene and α-Pinene had 175 ppm, respectively.
Conclusions: Results of this study show that the leaf essential oil of *C. swietenia* and its five major compositions may be a potent source of natural larvicidal and ovicidal activities against lepidopteran agricultural pest *S. litura*.

Key words: *Spodoptera litura, Chloroxylon swietenia,* Essential oil, GC-MS analysis, Chemical composition, Larvicidal activity , Ovicidad activity

INTRODUCTION

S. litura commonly known as armyworm, is an economically important polyphagous pest in many countries. Causing considerable economic loss to many vegetable and field crop loss due to Insect pests varies between 10% and 30% for major crops [1]. The cutworm *S. litura* is one of the major pests of many important crop plants, whose larvae can defoliate many economically important crops Possessing a high dispersal capability, this pest has often generated high levels of agricultural losses [2-4]. One of the major causes of crop losses is herbivory by larvae and adults of some insect species. The common methods for controlling these types of insects are chemical, biological, and physical control or a combination of these methods [5]. *C. swietenia* is commonly known as East Indian Satin Wood, it is a hardwood tree, native to south India and Sri Lanka, *C. swietenia* belonging to the family Rutaceae. is a medium-sized deciduous tree, growing to 15-20 m tall, with thick, fissured, slightly corky bark. Alternately arranged leaves are 15-22 cm long, pinnately divided into 10-20 pairs of oblong, blunt leaflets. The flowers are small, creamy-white, produced in panicles 10-20 cm long. Buds are round. The fruit is an oblong three-segmented capsule 2.5-4.5 cm long, containing 1-4 seeds in each segment. Ceylon Satinwood is used in folk medicine in Chhattisgarh. In case of a problematic wound, the dried leaves of *C. swietenia* are applied on wound in order to increase the healing process [6]. is a general practice in tribal areas to resort to only herbal medicinal formulations for most clinical disorders and thus traditionally, the crude plant extract of *C. swietenia* is extensively used as topical application for infectious wounds and other bacterial and fungal infections. It would be essential to evaluate the phytochemical composition of this plant because of its widespread utilization as a folk medicine, to ensure that the phytoconstituents are novel and may offer a possible role to play as effective antimicrobial agent. Therefore, leaves and stems oil of C. *swietenia*, to assess the potential use of these extracts for their antimicrobial activity and we present here the results of our investigative study people, and hence, there are no published reports so far available [7]. Management of agricultural pests over the past half century has been largely depending on the use of synthetic pesticides for field and post – harvest protection of crops; potential problems associated with continued long-term

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use of toxic insecticides include pest resistance and negative impact on natural enemies. In addition, increasing documentation of negative environmental and health impact of synthetic toxic insecticides and increasingly stringent environmental regulation of pesticides [8]. Plant-derived materials play an important role in traditional methods of protection against insect infestations. Although these types of methods are little used in developed countries, they still play significant roles in pest control programs of developing countries. Essential oils isolated from plants consisting of mainly bioactive monoterpenes may have attractive or repellent effects. In some cases, they show an insecticidal action such as inhibition of molting and respiration, reduction in growth and fecundity, cuticle disruption, and effects on the invertebrate octopamine pathway [9]. Essential oils are also effective repellents against some insect species [10] and their vapors and pure constituents also show toxic effects against larvae and adults of some insects [11-14]. Essential oils from plants may have minimal direct and/or indirect effects on natural enemies for ecological equilibrium [15]. For these reasons, essential oils are currently under investigation for their broad-spectrum pest control properties. Although numerous reports exist on insecticidal activity of plant essential oils [16-20]. In this present investigation was undertaken to study the Larvicidal and ovicidal activities of *C. swietenia* (Rutaceae) essential oils against *S. litura* (Lepidoptera: Noctuidae) and their chemical compositions.

MATERIALS AND METHODS

Plant material and essential oil extraction

Leaves of *C. swietenia*, collected from the forest region of Ooty, Uthagamandalam District, Tamilnadu, India. the plant material were collected in the month of February 2010, were air dried and hydrodistilled in a clavenger apparatus for 4 h. the distilled oil was dried over anhydrous sodium sulphate and stored under nitrogen atmosphere until further use. The plant material was identified and the voucher specimens (AU ZOO 362) were deposited at the department of Zoology, Annamalai University, Annamalai Nagar, Tamilnadu, India.

Gas Chromatography Analysis

Analysis was carried on a varian-gas chromatograph equipped with a flame ionization detector and a BPI (100% dimethyl polysiloxane) capillary column. Helium at a flow rate of 1.0 ml min-1 and 8 psi inlet pressure was employed as a carrier gas. Temperature was programmed from 60 to 2200C at 5oc min-1with a final hold time of 6 min. The injector and detector temperatures were maintained at 250 and 3000C, respectively. The sample (0.2 µl) was injected with 1:20 split ratio.

Gas Chromatography – Mass Spectrometry Analysis

Gas chromatography – mass spectrometry (GC-MS) analysis was performed on an Agilent 6890 GC equipped with 5973 N mass selective detector and an HP – 5 (5% phynylmethyl polysiloxane) capillary column. The oven temperature was programmed from 50 to 2800C at the rate of 40C min-1 and held at this temperature for 5 min. The inlet and interface temperatures were 250 and 2800C, respectively. The carrier gas was helium at a flow rate of 1.0 ml min-1 (constant flow). The sample (0.2µl) was injected with a split of 20:1. Electron impact mass spectrometry was carried at 70 eV. Ion source and quadrupole temperatures were maintained at 230 and 1500c respectively.

Insect rearing

S. litura (Lepidoptera:Noctuidae) cotton leaf worm were cultured and maintained in the laboratory on castor leaves *(Ricinus Communis)*. Rearing conditions were a 12 h photo regime at 28±2°C and 75±5% relative humidity. Insect cultures were continuously refreshed with wild moths captures by a light trap in the vicinity of the agricultural farm of Department of Agricultural, Annamalai University, Annamalai nagar. Generally fourth instar larvae were used in the experiment.

Larvicidal activity

The larvae of *S.litura* were collected from the insect rearing cage. Larvicidal activity of the essential oil and five major components of α-Pinene, limonene, geijerene, pregeijerene, and germacrene D isolated from the essential oil of *C. swietenia.* Based on the wide range and narrow range tests, essential oil was tested at 50,100, 150,200 and 250 ppm and each compound was tested at 10, 20, 30, 40, and 50 ppm. Essential oil or individual compounds were dissolved in Polysorbate 80 (Qualigens) was used as an emulsifier, Experiments were conducted for 24 h at room temperature (28±2°C and 75±5% relative humidity and a photo period of 16:8 (L/D). A leaf – dipping method was used to evaluate the activity of the test samples [21]. Leaf disk (6.5cm) of castor were used for evaluating larvicidal activity of the sample against *S.litura* three leaf disks per dose were separately dipped in each test solution for 30second. Solvents were evaporated under a fume hood for 2h. Castor leaves were washed with 70% double distilled alcohol and air-dried for 15 min before dipping in to the required amount of plant products. The larval were transferred individually on treated and control (disk treated with solvent, polysorbate 80 and distilled water only) leaf disks places in petri disk. The percentage of leaf damage was gravimetrically estimated every 12 hours. With an additional initial check after 6 hours mortality was determined 24 hours after larvae were placed on disks. All moribund pest larvae were considered as dead.

Ovicidal activity

For ovicidal activity, scales from the egg mass of *S.litura* were carefully removed using fine brush. *S. litura* 500 eggs were separated into five lots each having 100 eggs and dipped in 25,50,75,100,125,150,175 and 200 ppm concentrations of plant essential oil and five major components. Control is maintained above. Numbers of eggs hatched in control and treated were recorded and the percentage of ovicidal activity was calculated by the following formula [22]. For each experiment, five replicates and the hatch rate was assessed 120 h post treatment.

$$\text{ovicidal activity} : \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} x\ 100$$

Statistical Analysis

The average mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics chi-square values were calculated by using the software using statistical package of social science (SPSS) version 13.0 for windows, significance level was set at $P<0.05$.

Table 1. Percentage composition of the oils from leaves of *Chloroxylon swietenia.*

Peak	Compounds	RT(min)	Concentration (%)
			leaves
1	α-Pinene	939	10.23
2	Myrcene	985	1.25
3	Limonene	1025	18.35
4	Linalool	1086	0.12
5	Geijerene	1143	26.27
6	Decanal	1192	0.42
7	Geraniol	1241	1.08
8	Pregeijerene	1286	14.61
9	Methyl cinnamate	1342	1.18
10	Geranyl acetate	1370	1.03
11	β-Elemene	1389	0.21
12	Methyl eugenol	1403	2.25
13	α-Humulene	1446	1.42
14	Germacrene D	1481	14.69
15	Bicyclogermacrene	1493	0.72
16	d-Cadinene	1536	0.89
17	Spathulenol	1562	0.74
18	Caryophyllene oxide	1574	1.27
19	γ-Cadinol	1641	1.69
20	β-Bisabolol	1672	0.53

RT- Retention time (min)

Table 2. Larvicidal activity of leaves *C. swietenia* essential oil and major chemical compositions against fourth instar *S. litura* larvae

Compound	Concentration (ppm)	24h Mortality(%)	LC_{50} (ppm) (LCL- UCL)	LC_{90} (ppm) (LCL- UCL)	Chi-square*
Essential Oil	Control 50 100 150 200 250	1.6±1.2a 19.6±1.6b 32.4±0.8c 58.6±1.2d 74.8±1.4e 100±0.0f	131.20 (105.32-157.89)	224.68 (191.22-286.76)	14.614*
α-Pinene	Control 10 20 30 40 50	1.8±1.4a 28.8±1.2b 37.4±0.8c 53.8±0.6d 73.6±1.2e 92.6±1.4f	26.35 (20.44-20.44)	49.78 (20.44-65.84)	13.734*
Limonene	Control 10 20 30 40 50	1.8±0.8a 31.2±0.6b 52.4±1.2c 79.6±1.4d 86.4±1.6e 98.6±1.4f	20.03 (14.81-24.79)	38.31 (32.40-48.70)	13.247*
Geijerene	Control 10 20 30 40 50	2.2±0.8a 34.6±0.6b 56.2±1.2c 81.4±1.4d 92.6±1.6e 100±0.0f	18.31 (13.94-22.32)	34.77 (29.74-43.09)	10.812*
Pregeijerene	Control 10 20 30 40 50	1.2±1.2a 29.6±0.8b 39.8±1.4c 55.6±1.2d 76.4±1.4e 95.4±0.8f	25.23 (18.88-31.61)	47.27 (39.16-63.93)	16.549*
Germacrene D	Control 10 20 30 40 50	1.6±1.2a 29.8±1.4b 41.6±0.8c 54.6±1.2d 77.8±0.6e 96.2±1.2f	24.83 (18.39-31.23)	46.67 (38.59-63.37)	16.953*

Values in a column with a different superscript are significantly different at $p<0.05$ level (DMRT test).
Each value (mean ± SD) represents mean of four values. *significant at $p<0.05$ level.

RESULTS

Yield and chemical composition was analysed by gas chromatography (GC) and GC mass spectrophotometry. Chemical constituents of twenty compounds were identified in the oil of *C. swietenia* representing to 98.95% (Table-1). The major components in essential oil were α-Pinene (10.23%) limonene (18.35%), geijerene (26.27%), pregeijerene (14.61%) and germacrene D (14.69%). The percentage compositions of remaining fifteen compounds ranged from 0.21% - 2.25%. The essential oil of *C. swietenia* exhibited significant larvicidal activity (Table 2), with 24h LC_{50} 131.20 ppm and LC_{90} 224.68 ppm. Larvicidal activities of the five major compounds of essential oil were also tested. The LC_{50} and LC_{90} values of geijerene, limonene, germacrene D, pregeijerene and α-Pinene were LC_{50} 18.31, 20.03, 24.83, 25.23 and 26.35 ppm and LC_{90} 34.77, 38.31, 46.67, 47.27 and 49.78 ppm respectively. Mean percent hatchability of the ovicidal activity was observes 120 h post treatment (Table-3). *C. swietenia* essential oil produced (100% mortality) eggs no hatchability recorded in 200 ppm, however, highest ovicidad activity found in geijerene 125 ppm, This was closely followed by limonene and germacrene D 150 ppm, pregeijerene and α-Pinene had 175 ppm, respectively. In general, mortality increased with increasing concentration of compounds and exposure time. *C. swietenia* leaves and their compounds may have benefits in pest control programs.

DISCUSSION

Isman *et al* [23] have been reported that Three of the essential oils were highly toxic to the cutworm *S. litura* : oils of *Satureia hortensis*, *Thymus serpyllum* and *Origanum creticum*. Oil of *Mentha arvensis* was the only other oil producing at least 50% mortality. Pavela [24] reported that twenty essential oils applied by fumigation were highly toxic to the third instar of *S. littoralis* larvae. Two essential oils *Nepeta cataria* and *Thuja occidentalis* were highly toxic with LC_{50}≤10.0 mL/m3 (5.5 and 6.5 mL/m3, respectively). Five essential oils *Salvia sclarea*, *Thymus mastichina, Origanum majorana, Pogostemon cablin* and *Mentha pulegium* were toxic with LC_{50} between 10.1 and 20.0 mL/m3 (11.9, 19.3, 19.6, 14.8 and 11.5 mL/m3, respectively). Jayasankar *et al* [25] reported that mentha oil showed minimum ovicidal activity at 0.25% concentration 18.33 ± 3.15 and maximum ovicidal activity at highest concentration tested (2.0% - 28.99 ± 7.11). Ovicidal activity recorded from 0.50 and 1.0% were less significant (23.25 ± 4.66 and 24.74 ± 5.47 respectively). Neem oil showed maximum ovicidal activity at 2.0% concentration. Elumalai *et al* [26] they have been reported that maximum ovicidal activity was found in *Ocimum basilicum* and *Zingiber officinale. S.litura* eggs were 100% of mortality (No hatchability) recorded in 300 ppm, respectively. Oil of *Origanum creticum* was significantly less toxic. The insecticidal action of *Thymus* and *Satureia* oils are likely due to their major constituents (thymol and carvacrol), which were found to be toxic in our previous experiments (LD_{50} 25.5 and 42.7, respectively) [27]. Krishnappa *et al* [28] they have been reported that *Tagetes patula* volatile oil contained 10 compounds and they were tested against the fourth instar larvae of *S. litura* for their antifeedant activity by leaf disc bioassay. Among the compounds tested Terpinolene was the most effective feeding deterrent agent against *Spodoptera litura* in the laboratory condition. Anandan *et al* [29] they have been reported that crude extracts of *H. suaveolens* and *M. corchorifolia* against *S. litura*, four fractions obtained from *H. suaveolens*, fraction III was found to inhibit the feeding ratio of the *S. litura* and it is apparent from the table. While in *M. corchorifolia* only three fractions have been obtained, among them fraction II was found to induced more feeding deterrent activity at 2000 ppm concentration. Krishnappa *et al* [30] reported that The *Clausena dentate* leaves essential oil against Armyworm, *S. litura* it produce significant larvicidal activity, with 24 hrs LC_{50} 111.54 ppm and LC_{90} 205.38 ppm, respectively. The major chemical compositions larvicidal activitieswere also tested. The LC_{50} and LC_{90} values of sabinene LC_{50} 21.42 ppm and LC_{90} 40.39 ppm, respectively. This was closely followed by biofloratriene LC_{50} 23.31 ppm and LC_{90} 43.62 ppm. Earlier Anandan *et al* [31] reported that Efficacy of ethyl acetate, methanol and aqueous extracts of *Acrous Calamus, Corchorus aestauaus, Cammelina bengalinsis, Emblica ficus religiosa, Lantena Camera* were tested at 1000 ppm for their antifeedant activity against fourth instar larvae of *Helicoverpa armigera (H. armigera)*using leaf disc (no-choice) method. The aqueous extract of *C. collinus* was found to have maximum antifeedant activity followed by *E. fisheri F. religiosa*, and *C. aestauaus.* Baskar *et al* [32,33] reported that the ethyl acetate extract of *Couroupita guianensis* exhibited 69.7% against *H. armigera* at 5% concentration. The antifeedant activity was due to the presence of steroids, phenols, flavonoids and alkaloids in the ethyl acetate extract of leaf.

Conflict of interest statement

We declare that we have no Conflict of interest.

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