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Simarouba glauca DC: An effective biopesticide against leaf defoliators of Ailanthus and Teak plants

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ABSTRACT

Simarouba glauca DC seeds were collected and oil was extracted by using n-Hexane solvent in soxhlet apparatus. Phytochemical screening of n-Hexane extract of *S. glauca* seed oil showed the presence of cardiac glycosides, saponin and steroids. Physicochemical analysis showed that density of oil was 0.441 g/cc, free fatty acid value was 1.6 mg KOH/g, iodine value was 27.74 g/100g oil, peroxide value was 1.14 meq/kg oil, acid value was 3.21, saponification value was 208 mg KOH/g and unsaponifiable matter was 3%. Gas Chromatography and Mass Spectrometry analysis revealed that the presence of oleic acid, steric acid, palmitic acid, and linoleic acid as major fatty acids with the area percentage of 44.6%, 24.03%, 15.44% and 0.232% respectively. Further laboratory experiment was setup to evaluate *S. glauca* seed oil against Ailanthus and Teak defoliators *viz.*, *Eligma narcissus* Cramer (Lepidoptera: Nolidae) and *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae). Spraying of *S. glauca* seed oil had resulted in 100% larval mortality in both *E. narcissus* and *H. puera*. The larval mortality was due to the insecticidal property of oil through hydrolytic dissociation and excess loss of water. *Simarouba glauca* seed oil acts as a contact poison and it could be used as a biopesticide to control the *E. narcissus* and *H. puera* in the early developmental stages.

Keywords: *Eligma narcissus*, *Hyblaea purea,* Fatty acids.

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INTRODUCTION

Simarouba glauca DC, (Simaroubaceae) is a medium sized ever green tree with tap root system, cylindrical stem and commonly known as paradise tree (Sharanya *et al.,* 2016). The leaf, bark and seed oil of *S. glauca* are useful in curing amoebiasis, diarrhea, cancer and resistance against malaria (Patil and Gaikwad, 2011). Dry seeds of *S. glauca* contain protein and unsaturated fatty acids (Anil Duhan *et al.,* 2011; Rout *et al.,* 2014). Due to the presence of high fatty acids in *S. glauca* seeds, it can be used as edible oil and also has antagonistic activity against many disease causing microorganisms. In earlier reports jatropha seed oil, crude palm and cotton oils, orange peel oil, eucalyptus oil and *Delonix regia* (Hook.)Raf. (Fabaceae) seed oils have been reported with biopesticidal properties (Adebowale and Adedire, 2006; Rahman and Talukder, 2006 ; Obembe, 2017). The insecticidal properties in these oils are due to the presence of various fatty acids. Fatty acids have insecticidal property through hydrolytic dissociation when they used as contact sprays (Dheeraj *et al.,* 2013). However, the present study was aimed to evaluate *S. glauca* seed oil against two different leaf defoliators v*iz.,* the teak defoliating insect *Hyblaea puera* Cramer and ailanthus defoliator, *Eligma narcissus* (Cramer).

Materials and Methods

Collection and processing of seeds

Extensive surveys were carried out to identify and locate the *S. glauca* seed sources in and around Dharmapuri district lies between

12º03'20.41" N latitude and 78º06'28.10" E longitude at the elevation of 492 m ASL, Tamilnadu, India. The identified trees were at the age of 5 years since the tree started bearing fruits when they are at the age of 4-6 yrs old and seeds were collected from identified trees during March and April, 2019 when the fruit set was observed (flowering started in the months of January and February and fruits set in the months of March and April every year in the said locality) and brought to the laboratory. Collected seeds were air dried, processed, tightly packed until analysis.

Morphometric analysis

Twenty five seeds were selected randomly from the collected seed sample and subjected to Image analysis using Lyca Q win V3 to analysis morphometric characters such as Area (cm^2), Length (cm) and Breath (cm).

Oil extraction

100 gm of seeds were taken and powdered using electronic blender. Oil was extracted from powdered samples using n-Hexane solvent at 40°C – 55°C in soxhlet apparatus. Oils were collected after the completion of 8^{th} cycle, and then excess solvents were removed using rotary evaporator. Yield of oil was calculated by weighing the concentrated oil and stored in deep freezer (-4°C) for further analysis (Krishnakumar *et al*., 2011).

Phytochemical screening

Extracted oil was subjected to phytochemical and physicochemical analysis as per standard methods of Harborne (1973) and AOAC (1984) respectively.

Collection and laboratory rearing of insects

Both teak defoliator *H. puera* and Ailanthus defoliator *E. narcissus* were collected from Institute of Forest Genetics and Tree Breeding (IFGTB) nursery and brought to Chemistry and Bioprospecting laboratory for mass rearing. Both male and female moths were placed inside cage covered with muslin cloth and fed with 15% of sugar solution. Females laid eggs on muslin cloth. The half of first generation larvae were subjected to laboratory bioassay study and remaining half were allowed to grow mass and cultured in the laboratory for further studies.

Bioefficacy of *S. glauca* seed oil against *H. puera* and *E. narcissus*

Potter's tower method was adapted to find out bioefficacy of *S. glauca* seed oil against Ailanthus leaf defoliator *Eligma narcissus* and Teak defoliator *Hyblaea puera* with different concentrations (0.5%, 1.0%, 1.5% and 2.0%). A 1% of neem oil and distilled water applications were considered as positive control and negative control respectively. Formulated seed oil of *S. glauca* (oil and emulsifier in the ratio of 9:1) was directly sprayed on *E. narcissus* and *H. puera*. Pre-starved (18h) seven numbers of larvae of *E. narcissus* and *H. puera* were used in each treatment with five replicates. Larval mortalities were observed every 24 hrs until 96 hrs and post treatment changes were also noted until adult emergence. Percentage of larval mortality was calculated using the following formula:

$$\text{Percent of larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of treated larvae}} \times 100$$

GC-MS/MS analysis of *S. glauca* seed oil

GC-MS/MS analyses

Oil was subjected to GC-MS to separate mixture and identify the active compounds.

Statistical analyses

Oil yield was correlated with seed morphometry to check any correlation between the oil yield and their morphometric characters. Duncan Multiple Range Test (DMRT) was used to check whether a statistically significant difference among treatments at the respective time intervals. All the statistical analysis was done by SPSS v16.0 software. Results with $p<0.05$ were considered as statistically significant.

RESULTS

Morphometric analysis of *S. glauca* seeds showed area of 2.02 ± 0.03 cm^2, 2.17 ± 0.01 cm length and of 1.19 ± 0.01 cm breadth. During the extraction process 100g of seed yielded 33.2 ± 0.63 (n=10) percent. Correlation of oil yield with morphometric analysis indicated that oil yield is not dependent on any morphometric characters (Table 1).

Table 1. *S.glauca* oil yield with respect to seed morphometry

Parameters	Oil yield	Area	Length	Breadth
Oil yield	1			
Area	0.4756	1		
Length	0.0262	0.7410	1	
Breadth	0.5252	0.9169	0.4441	1

Phytochemical screening of n-Hexane extract of *S. glauca* seed oil showed the presence of cardiac glycosides, saponin and steroids. However, other phytochemicals such as alkaloids, flavonoids, protein, phenols, tannins, resins, triterpenoids and starch were absent in extracted oil. Results are tabulated in Table-2. Physicochemical analysis revealed the following parameters: density of oil - 0.441 g/cc, free fatty acid value - 1.6 mg KOH/g, iodine value - 27.74 g/100g oil, peroxide value - 1.14 meq/kg oil, acid value - 3.21, saponification value - 208 mg KOH/g and unsaponifiable matter - 3%.

GC-MS analysis revealed the presence of oleic acid, steric acid, palmitic acid, and linoleic acid in *S. glauca* seed oil as major compounds with the area percentage of 44.6%, 24.03%, 15.44% and 0.232% respectively (Fig.1; Table 2).

Studies on bioefficacy revealed that *S. glauca* oil when treated at 1.5% and 2% furnished 100% larval mortality in *E. narcissus* at 96 hrs after application (Table 3). The larval mortality was highly significant with hours *viz.*, 24h to 96h (df=8, f=7.34, $p<0.001$) and not significant with the treatments *viz.*, T_1 to T_6. Regression analysis showed that time interval (hours) significantly increased larval mortality in all treatments *viz.*, T_1 ($r^2 = 0.99$, n=5, p=0.001), T_2 ($r^2 = 0.97$, n=5, p>0.001), T_3 ($r^2 = 0.97$, n=5, p>0.001), T_4 ($r^2 = 0.95$, n=5, p=0.01), and T_5 ($r^2 = 0.98$, n=5, p>0.001) (Figure 2)

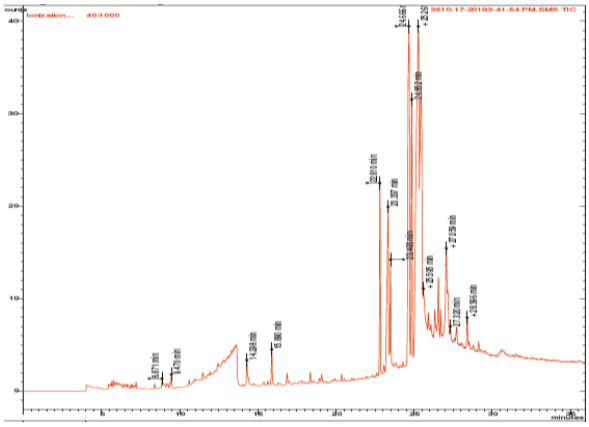

Figure 1. GC-MS/MS Chromatogram of *S. glauca* seed oil

Table 2. Gas Chromatography and Mass Spectrometry analysis of *Simarouba glauca* seed oil

Scientific Name	Common Name	Retention Time	Area %	Molecular Formula	molecular weight
9-Octadecenoic acid (Z)-	Oleic acid	24.96	44.568	$C_{18}H_{34}O_2$	282.5 g/mol
Octadecanoic acid	Stearic acid	25.1	24.03	$C_{18}H_{36}O_2$	284.5 g/mol
Hexadecanoic acid	Palmitic acid	23.112	15.448	$C_{16}H_{32}O_2$	256.42 g/mol
Diethylmethyl-borane	-	27.059	4.249	$C_5H_{13}B$	83.97 g/mol
2,3- 9,12,15-Octadecatrienoic acid	-	25.959	2.758	$C_{18}H_{30}O_2$	278.4 g/mol
Eicosanoic acid	Arachidic acid	26.922	2.291	$C_{20}H_{40}O_2$	312.5 g/mol
2-Pentadecyn-1-ol	-	15.09	1.554	$C_{15}H_{28}O$	224.38 g/mol
9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid	25.985	0.642	$C_{20}H_{36}O_2$	280.4 g/mol

Table 3. Larval mortality of *Eligma narcissus,* data indicates mean larval mortality percentage ± SD.

Treatments	Time interval			
	24 h	48 h	72 h	96 h
T_1 (0.5% SO)	0 ± 0^a	28 ± 4.90^b	60 ± 6.32^c	80 ± 6.32^d
T_2 (1% SO)	8 ± 4.90^a	40 ± 6.32^b	80 ± 6.32^c	95 ± 4.47^c
T_3 (1.5% SO)	20 ± 0^a	52 ± 8^b	84 ± 7.48^c	100 ± 0^c
T_4 (2% SO)	28 ± 4.90^a	64 ± 7.48^b	88 ± 4.90^c	100 ± 0^c
T_5 (1% NO)	20 ± 0^a	48 ± 4.90^b	84 ± 4^c	100 ± 0^d
T_6 (DW)	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Mean followed by the same letter in row indicates that not significant at 5% level by DMRT

In *Hyblaea puera*, 100% larval mortality was observed in 2% of oil concentration at 96 hours of after application (Table-4). The larval mortalities were significant with hours v*iz.*, 24h to 96h (df=12, f=2.32, p=0.01) and not significant with the treatments *viz.*, T_1 to T_6. Regression analysis indicates that time interval significantly increase *H. puera* larval mortality in all treatments *Viz.*, T_1 (r^2 = 0.98, n=5, p>0.01), T_2 (r^2 = 0.99, n=5, p>0.001), T_3 (r^2 = 0.98, n=5, p>0.01), T_4 (r^2 = 0.98, n=5, p>0.01), and T_5 (r^2 = 0.99, n=5, p=0.001) (Figure-2).

In other concentrations where the mortality was less, the remaining larvae which were alive after 96 hours were continuously observed. The observation revealed that although larvae were pupated, the pupal stage has deformities such as shrunken and incomplete form of pupae which didn't developed as adults.

Table 4. Larval mortality of *H. puera.* Table indicates mean larval mortality percentage ± SD.

Treatments	Time interval			
	24 h	48 h	72 h	96 h
T_1 (0.5% SO)	7.14 ± 8.2^a	21.42 ± 8.2^{ab}	35.71 ± 8.2^b	57.14 ± 11.7^c
T_2 (1% SO)	10.71 ± 7.1^a	28.57 ± 11.7^b	57.14 ± 11.7^c	78.57 ± 8.2^d
T_3 (1.5% SO)	14.28 ± 11.7^a	42.85 ± 20.2^b	67.8 ± 18.0^c	85.71 ± 11.7^c
T_4 (2% SO)	25 ± 13.7^a	53.57 ± 13.7^b	82.14 ± 13.7^c	100 ± 0^c
T_5 (1% NO)	17.85 ± 7.1^a	35.71 ± 8.2^b	57.14 ± 11.7^c	75 ± 7.1^d
T_6 (DW)	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Mean followed by the same letter in row indicates that not significant at 5% level by DMRT)

Probit analysis was done to determine LC_{50} and LC_{90} values for all tested time intervals v*iz.*, 24, 48, 72 and 96 hrs against *E. narcissus* and *H. puera*. LC_{50} value of 0.399 ml and 0.65 mL were obtained for the *E. narcissus* and *H. puera* respectively, 96 h after application. LC_{90} value of 0.72 ml and 1.35 mL were obtained against *E. narcissus* and *H. puera* respectively, 96 h after application (Table 5).

Table 5. LD_{50} and LD_{90} value of *E. narcissus* and *H. puera* at different time intervals

Tested plants	Time interval (Hrs)	LD 50	95 % Confidential limit		LD 90	95 % Confidential limit		Chi Square value
			LCL	UCL		LCL	UCL	
Eligma narcissus	24	2.41	-	-	3.56	-	-	0.966
	48	1.44	0.79	4.66	2.82	1.92	17.84	0.819
	72	0.68	-0.249	1.17	1.68	1.18	3.78	0.382
	96	0.399	-0.103	0.736	0.72	0.482	2.08	0.599
Hyblaea puera	24	2.96	-	-	4.83	-	-	0.961
	48	1.75	1.21	5.12	3.24	2.22	14.64	0.821
	72	1.05	0.56	1.57	2.13	1.61	4	0.649
	96	0.65	0.23	0.97	1.35	1.02	2.25	0.409

DISCUSSION

During the survey *S. glauca* seed sources were identified in Palayamudur area, Dharmapuri District, Tamilnadu. *Simarouba glauca* grow well in semiarid dry and saline land areas and cultivated in various states such as Tamilnadu, Gujarat, Maharashtra, Karnataka and Andhra Pradesh. It is capable of growing in marginal wetlands or dry land with degraded soil (Patil and Gaikwad, 2011). The percentage of oil

extracted was 33.2 ± 0.63 from 100g of seeds. Anil *et al.* (2011) reported that *S. glauca* seeds contain about 57.22% of oil. However, in our study the yield was comparatively low. This may be attributed with the results of Ketkar (2000). He reported that the yield of neem oil depended on soil, age, distance and rainfall. Hence, oil content in *S. glauca* seed may be influenced by similar abiotic and biotic factors. A number of questions regarding oil yield in *S. glauca* remain to be addressed. In this percent study, free fatty acid value was higher in *S. glauca* seed oil than with previous study done by Anil *et al.* (2011). Free fatty acid value indicated the presence and freshness of fat. Adebowale and Adedire (2006) stated that the presence of high unsaponifiable matter (3.8%) in *Jatropha curcas* L. is an advantage to use as natural insecticide. In the present study the percentage of unsaponifiable matter was estimated as 3%. This may correlated with its insecticidal property as reported by Adebowale and Adedire (2006).

GC-MS/MS profile of *S. glauca* seed oil showed that it contains more fatty acids such as oleic acid, steric acid, palmitic acid, and linoleic acid. Abhishek Raj (2014) reported that neem seed oil contains major fatty acids *viz.*, oleic acid, palmitic acid, stearic acid and linolic acid. Similarly, GC-MS/MS studies revealed the presence of fatty acids such as oleic acid, palmitic acid, stearic acid and linolic acid. In an another study, Ekere *et al.* (2015) reported that Fatty Acid Methyl Ester and Fatty Acid Butyl Ester converted seed oil of *Monodora tenuifolia* contains palmitic and linoleic acid as major compounds and have been used as insecticides in East Indies, Malaysia and Srilanka.

Both 1.5% and 2% concentrations of *S. glauca* seed oil resulted in 100% mortality of both *E. narcissus* and *H. puera* at 96 hours after application. Many investigations showed that various seed oil sources effectively acted as bio insecticide on wide range of insects. Siegler and Popenoe (1925) reported that fatty acids have an insecticidal property which act through hydrolytic dissociation when they used as contact sprays. Similar observation was made by Dheeraj *et al.* (2013). He reported that potassium salts of fatty acid mixed with synthetic pyrethroids, acts as an insecticides and they may cause death of insect by excess loss of water. Neem oil treated teak leaf furnished 48% of larval mortality in *H. peraea* and also showed antifeedant activity (Murugan e*t al*., 1999). Jatropha seed oil showed significantly high mortality of *Callosobruchus maculatus*, *Callosobruchus chinensis* and *Sitophilus zeamais* (Adebowale and Adedire, 2006). Amaugo and Emosairue (2003) reported that aqueous and acetone extracts of seed kernel were high antagonistic against stem borers and extracts were superior to the other plant extracts. Similarly, Hill and Schoonhoven (1981) found that crude palm and cotton oils and orange peel oil were highly effective against adult of *Zabrotes subfasciatus*. Equally, eucalyptus oil from *Eucalyptus camaldulensis* caused significant high mortality against *Caryedon serratus* (El-Atta and Ahmed, 2002) and *Eucalyptus globulus* essential oil extracted by acetone was highly effective against *C. maculatus* (Rahman and Talukder, 2006). Seed oil extracts of *Delonix regia* caused high mortality of adult *S. zeamais* with the concentration of 2.0% (Obembe, 2017). Plant products other than neem were not practically used much for agriculture and forestry pest control due to lack of research on it. Other than neem, around 58 botanical sources were identified as potential source to control various pests. Among those, *S. glauca* seed oil have a potential to control two different leaf defoliators v*iz.*, *E. narcissus* and *H. puera* effectively during early developmental stages without harming the environment.

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