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Chemical constituents and toxicity assessment of the leaf oil of *Lantana camara* Linn from Tamilnadu regions

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ABSTRACT

Plant secondary metabolites derived from weeds, medicinal and aromatic plants have been found to be a protective biopesticidal agent, cost effective and safe to ecosystem & public health. In the present investigation, the essential oil of Lantana camara collected from the different agro-climatic conditions of Tamilnadu regions has proven to be interesting source of terpenoid derivatives for their biopesticidal properties against some of the defoliators of forestry tree species. Bioassay confirmation of those terpenoids identified from the essential oil was tested on the target pests such as Hyblaea puera, Eligma narcissus and Atteva fabriciella at different concentrations both in the laboratory and field conditions. The bioactivity interms of larval mortality was observed when the early instars were exposed to preformulated oil applications.

Keywords: *Lantana camara*, essential oil, chemical constituents, biopesticidal properties and preformulated oil.

INTRODUCTION

Lantana camara is a noxious weed of Verbenaceae comprises 650 species spread out over 60 countries, and has been introduced as an ornamental / hedge plant which is now abundantly occurring as a weed throughout India. The oil is used for curing various biological disorders [1], highly toxic to pests [2], repellent effect against stored grains pests supporting indigenous grain protection [3]. Thousands of secondary plant products that possess anti-insect properties which are either purely insecticidal or act as feeding deterrents, growth inhibitors, growth regulators, repellents or oviposition inhibitors against a variety of insect species have been identified [4]. The bioefficacy of the oil may be due to monoterpenes germacene D, 3-elemene, β-caryophyllene, β-elemene, α-copane, α-cadinene [5]. The essential oil of *L.camara* from different regions of the world has been reported to have differences in essential oil composition according to geographic origin of the plants [6]. Bioactive components present in oils have found to act as a short term and long term biopesticides, and remained a neglected aspect, so as less known to commercial industries too. *Cinnamomum osmophloeum* is one of hardwood species indigenous in Taiwan that possess cinnamaldehyde a major compound having significant antifungal activity while compared with other plant components [7]. In the present investigation, some of terpenoid derivatives have been characterized from the leaf essential oil of lantana is capable of disrupting the feeding behaviour insects, and reported that essential oil derivatives are inhibitory to insect and fungal growth [8]. In this respect, various oils were tested for their inhibitory activity towards the growth of some microorganism and disrupt the gut of insects. The oils of heartwood of *Santalum album* and whole part of *Glorrogyme pinnatiflide* exhibited antibacterial activity against some pathogenic bacteria such as *Bacillus mycoides* and *Escherichia coli* [9]. Oils from *Capillipedium foetidus* displayed high antibacterial activity against Gram – positive bacteria and moderate to excellent activity against Gram-negative bacteria. The most attractive aspect is the essential oils and their constituents are commonly used as herbs in traditional medicine and not toxic to humans. Therefore, the bioactive essential oils could be used to formulate green pesticides, which are less toxic & non-deleterious on biocontrol agents, easily biodegradable and no ecological backlash. It can be dealt by combining the essential oil with other non edible oils like oil from *Azadirachta indica* and *Pongamia pinnata*. Considering the importance of bioefficacy of the essential oil, an attempt has been made in the present study to investigate the quality of oil fractions of *L.camara* and screened the biopesticidal properties against some of the forestry pests.

MATERIALS AND METHODS

Extraction of essential oil

L. camara leaves were collected from different agroclimatic zones of Tamilnadu viz.Valparai**,** Kanyakumari, Kothagiri, Lalgudi, Ranipettai, Coonoor, Salem, Melamathur and Thirumangalam (Fig. 1 & Table 1). Leaves were processed, shade dried and hydro distilled at higher temperature in order to isolate the active fraction from the oil glands present in the leaves. The granulated *L. camara* leaves were packed with a sufficient quantity of water and boiled. The influence of hot water and steam liberated essential oil fractions from the oil glands present in the plant tissue. The vapour mixture of water and oil is condensed by indirect cooling with water. The water content of essential oil is nullified by extraction with n-hexane. At this point, the essential oil separates from water and floats to top. The top layer was collected by using separating funnel and used for GC-MS/MS analysis and bioassay studies.

Fig. 1 Collection of *Lantana camara* having different flower colours

GC-MS/MS analysis

GC-MS/MS analysis was performed on a Varian 4000 MS coupled with a Varian 3800 GC, equipped with a cross linked 5% Phenyl 95% dimethyl polysiloxane VF-5MS capillary column (30 m x 0.25 mm i.d, film thickness, 250nm) and operating under the conditions as mentioned below: The oven temperature was programmed as 60°C (10 min), 60°C - 220°C (4°C/min), 220°C (10 min) and 220°C -240°C (1°C/min). Injector and detector temperatures were maintained at 60°C and 240°C respectively. The amount of the sample injected was 1.0 µl in the splitless mode. Helium was used as carrier gas with a flow rate of 1ml/min.

Table 1. Collection of *Lantana camara* in different zones of Tamilnadu

S. No.	Agroclimatic zones	Area/Districts
1	Cauvery Delta zone	The zone covers Thanjavur district, Musiri, Tiruchirapalli, Lalgudi, Thuraiyur and Kulithalai taluks of Tiruchirapalli district, Aranthangi taluk of Pudukottai district and Chidambaram and Kattumannarkoil taluks of Cuddalore and Villupuram district.
2	North Eastern zone	The zone includes Chengleput district, North Arcot district, Cuddalore and Villupuram district excluding, Chidambaram and Kattumannarkoil taluks and Ariyalur.
3	Western zone	Includes Periyar district, Coimbatore district, Tiruchengode taluk of Salem district, Karur taluk of Trichirapalli district and Northern part of Madurai district.
4	North Western zone	This zone covers Dharmapuri district excluding hilly areas, Salem district excluding Thiruchengodu taluk and Peramabalur taluk of Tiruchirapalli district.
5	High Altitude zone	Covers Nilgiris, Kodaikanal, Elagiri, Javadhi, Kollimalai, Pachamalai, Yercaud, Valparai, Anamalai, Palani and Podhigaimalai.
6	Southern zone	The zone comprises of old Ramanathapuram, Nellai Kattaboman, V.O. Chidambaranar, Kamarajar and Dindigul taluk of anna district, Natham, Melur, Thirumangalam, Madurai South and Madurai North taluks of Madurai districts and Puddukottai district excluding Aranthangi taluk.
7	High Rainfall zone	This zone consists of only Kanyakumari district.

Identification of phytocompounds

Interpretation on mass-spectrum of GC-MS/MS was conducted using the database of National Institute of Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, molecular formula, retention time and retention indices of the chemical constitutents of the test materials were ascertained.

Bioassay of essential oil against forest pests

The efficacy of essential oil at different concentration ranging from 250 ppm to 10,000 ppm was tested on the major defoliator's viz., *Hyblaea puera, Eligma narcissus and Atteva fabriciella* in terms of insecticidal properties. Bioassay was performed on the 3rd day of 1st instar larvae of *H. puera, A. fabriciella* and *E. narcissus* in the laboratory condition with multiple replications to minimize error, and to confirm the toxicity of essential oil in comparison with nimbicidine and monocrotophos. The preformulated essential oil was scaled up with suitable adjuvant, and tested for its efficacy against the defoliators. Leaf disc bioassay on the larvae of all three defoliators was carried out and recorded the mortality rate upto 72 hrs of incubation period in different doses of samples ranging from 250ppm - 10000ppm. The sample was processed for bioactive fractions of insecticidal action against the 3rd day of the 1st instar's teak & ailanthus defoliators, *H. puera, E.narcissus* and *A. fabriciella.*

RESULTS

GC-MS/MS analysis of *Lantana camara* essential oil

L. camara leaves extracts analysis revealed the presence of triterpenoids, steroids, alkaloids, flavonoids, tannins etc. and the oil percentage was calculated and founded the yield of essential oil is high in Valparai and Coonoor – High altitude zone as shown in Table 2. This account describes the deveopment of a new class of biopesticide formulation derived from the secondary metabolite group of *L. camara.* The essential oils are being classified as potential biocontrol agents for pest and disease management. Extracts from the leaves tested to possess antimicrobial, fungicidal, insecticidal and nematicidal activity according to geographic origin of the plants. Those properties of natural chemicals could act as an alternative pesticide, and to be utilized on a large scale production & formulation of promising botanical insecticides. The essential oil, such as eugenol, linalool and geraniol gives promising larvicidal activity. The monoterpenes E-anethol and E-nerolidol were found to be the active principles of the most toxic essential oils. Among monoterpenes they found that (1R) - (+) - a-pinene and (1S)-(-) - α-pinene were most toxic while menthone, 1, 8-cineole, linalool and terpineol were less toxic [10]. Kanat, *et al.* (2004) [11] reported that essential oils of wood turpentine, thyme herb, cypress berry and styrax are most effective in terms of mean mortality time against larvae of pine processionary moth.

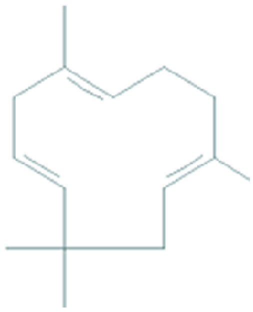
The essential oil from leaves of *L. camara* was processed, extracted and purified for analyzing by the standard GC-MS/MS method of estimation. The spectral character was interpreted on mass-spectrum of GC-MS/MS by using the database of National Institute of Standard and Technology (NIST) having the reference of 62,000 profiles. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, molecular formula, retention time and retention indices of the components of the test materials were ascertained. Oil fraction analysed by GC-MS/MS has revealed major compounds of the said nature. About thirty six compounds were characterized from essential oil of *L.camara*, some of them like α-Copaene, Germacrene D & B, α – Cubebene, β – Elemene, α – Guaiene, α – humulene, Aromadendrene, β – Selinene, α – Selinene, Caryophyllene oxide, Nerolidol, Spathulenol and Delta – Cadinene, have expressed tritrophic interactions as reported by earlier findings as well as insecticidal activity in terms of larval mortality against teak defoliator (Table 3-6, Figs. 2-3). Some of the compounds such as Isotridecanol, phytol, Lycopersen, Isochiapin B and Serverogenin acetate are known for their application in pharmacology [12]. Standardised method of operating procedure for essential oil estimation: Varian 4000 MS coupled with a Varian 3800 GC, equipped with a cross linked 5% Phenyl 95% dimethyl polysiloxane VF-5MS capillary column (30 m x 0.25 mm i.d, film thickness, 250nm) and operating under the conditions as mentioned below: The oven temperature was programmed as 60°C (10 min), 60°C - 220°C (4°C/min), 220°C (10 min) and 220°C -240°C (1°C/min). Injector and detector temperatures were maintained at 60°C and 240°C respectively. The amount of the sample injected was 1.0 µl in the split less mode. Helium was used as carrier gas with a flow rate of 1ml/min.

Table 2. Comparison of *L. camara* essential oil and phytoconstituents

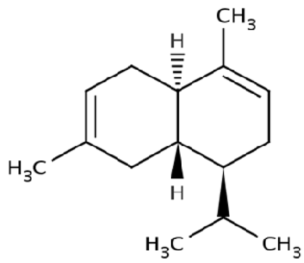
Place of collection	Oil Percentage (%)	Phytochemicals	*L. camara* essential oil +/-
Valparai	0.44	Cardiacglycosides	-
Kanyakumari	Nil	Alkaloids	+
Kothagiri	0.01	Flavonoids	+
Lalgudi	Nil	Glycosides	-
Ranipettai	Nil	Saponins	-
Coonoor	0.21	Tannins	+
Salem	Nil	Steroids	+
Melamathur	0.01	Triterpenoids	+

Table 3. GC-MS/MS profiles of *Lantana camara* essential oil

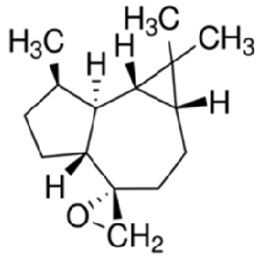
S. No	Retention Time	Retention indices	Name of the compound	Peak Area	Molecular weight	Molecular formula
1	26.486	802	Bicycloelemene	0.970	204	$C_{15}H_{24}$
2	27.005	818	α – Cubebene	4.226	204	$C_{15}H_{24}$
3	28.017	830	α – Copaene	1.187	204	$C_{15}H_{24}$
4	28.487	879	β – Elemene	2.404	204	$C_{15}H_{24}$
5	29.653	900	Bicyclo [5.2.0] Nonan, 2-Methy	4.509	204	$C_{15}H_{24}$
6	29.826	850	Germacrene B	3.179	204	$C_{15}H_{24}$
7	29.992	877	α – Guaiene	4.268	204	$C_{15}H_{24}$
8	30.704	855	α – Humulene	3.463	204	$C_{15}H_{24}$
9	30.820	906	Aromadendrene	6.257	204	$C_{15}H_{24}$
10	31.290	877	Napthalene	1.472	204	$C_{15}H_{24}$
11	31.499	905	Germacrene D	4.109	204	$C_{15}H_{24}$
12	31.754	912	β – Selinene	1.635	204	$C_{15}H_{24}$
13	31.839	884	Epi – Bicyclosesquiphellandren	0.977	204	$C_{15}H_{24}$
14	31.976	873	α – Selinene	6.747	204	$C_{15}H_{24}$
15	32.575	770	1-Hydroxy-1, 7-dimethyl-4-iso	2.119	222	$C_{15}H_{26}O$
16	32.636	911	β – Cadinene	3.600	204	$C_{15}H_{24}$
17	33.662	845	- Caryophyllene oxide	1.127	220	$C_{15}H_{24}O$
18	33.988	887	Nerolidol	4.841	222	$C_{15}H_{26}O$
19	34.924	833	Salvial – 4 (14) – en – 1 – one	2.862	220	$C_{15}H_{24}O$
20	34.980	877	Veridifloral	0.764	222	$C_{15}H_{26}O$
21	35.444	761	12-Oxabicyclo [9.1.0] dodeca – 3	2.296	220	$C_{15}H_{24}O$
22	35.574	777	1 – Napthalenamine, 4 – bromo	1.321	220	$C_{15}H_{24}O$
23	35.932	796	(-) – Spathulenol	4.087	220	$C_{15}H_{24}O$
24	36.064	839	Isospathulenol	1.632	220	$C_{15}H_{24}O$
25	36.208	830	Tetracyclo [6.3.2.0 (2,5) .0 (1,	1.188	220	$C_{15}H_{24}O$
26	36.303	856	Delta – Cadinene	2.399	204	$C_{15}H_{24}$
27	36.450	860	1-Napthalenol, 1, 2, 3, 4, 4a, 7	2.596	222	$C_{15}H_{26}O$
28	36.707	831	1R, 4S, 7S, 11R-2, 2, 4, 8 – Tetrame	3.211	204	$C_{15}H_{24}$
29	36.816	776	Alloaromadendrene Oxide – (2)	1.220	220	$C_{15}H_{24}O$
30	37.158	808	Aromadendrene Oxide– (2)	3.592	220	$C_{15}H_{24}O$
31	37.675	768	6-isopropenyl-4,8a-dimethyl-	1.407	220	$C_{15}H_{24}O$
32	38.122	750	4,4 – Dimethyl – 3 – (3 – methyl but	5.599	202	$C_{15}H_{22}$
33	38.229	770	1H – Cycloprop [e] azulen – 7 – ol,	4.970	220	$C_{15}H_{24}O$



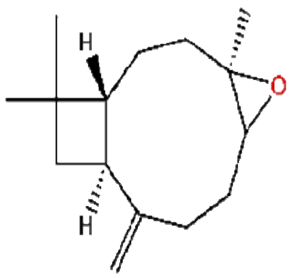
α- Humilene



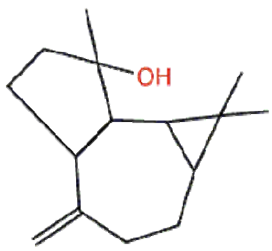
β-Cadinene



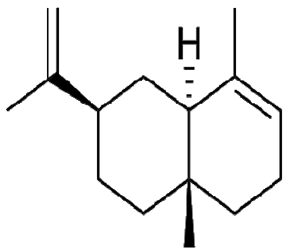
Aromadendrene II Oxide



Caryophyllene II oxide



Spathalenol



α – Selinene

34	39.806	734	6-isopropenyl-4,8a-dimethyl-	0.952	220	$C_{15}H_{24}O$
35	41.920	948	Phthalic acid, butyl hexyl e	0.958	306	$C_{18}H_{26}O_4$
36	47.777	853	2-Hexadecen – 1 – ol, 3, 7, 11, 15-	1.859	296	$C_{20}H_{40}O$

Fig.2. Sructure of individual compounds of *Lantana camara* essential oil.

Table 4. Biological properties of individual compounds of *L. camara* essential oil

S.no	Compound name	Biological properties
1	α - Copaene	Pest attractant
2	β – Elemene	Anti-carcinogenic
3	α – humulene	Anti-carcinogenic
4	Aromadendrene II oxide	Antimicrobial
5	β – Selinene	Antibacterial
6	α – Selinene	Has pharmacological uses
7	Caryophyllene II oxide	Antifungal, anti inflammatory, analgesic and attractants to predators.
8	Spathulenol	Immunoinhibitory molecule.
9	Delta – Cadinene	Insecticide

Efficacy of essential oil over forest pests

The efficacy of essential oil at different concentration ranging from 250 ppm to 10,000 ppm was tested on the major defoliator's viz., *H. puera*, *E.narcissus* and *A. fabriciella* in terms of insecticidal properties. In leaf disc bioassay on the larvae of all three defoliators, low mortality was reported in 72 hrs of incubation period in different doses samples tested ranging from 250ppm - 1000ppm. Though there was an insignificant mortality rate, it could express antifeedant activity within 24 hrs in all concentrations. Further, the concentration increased to 10 fold (2500ppm to 10000 ppm) expressed insecticidal and antifeedant properties, and sustained biopesticidal effect was observed from 24 to 72 hrs of experiment on *H.puera* brought 25 to 62% larval mortality. Similar effect was noted on ailanthus defoliators, *A. fabriciella* and *E. narcissus* when compared to nimbicidine (0.5%) and monocrotophos (0.05%). It was observed that the feeding behaviour and larval mortality rate depends on the dose of toxic metabolites consumed by the insects when they were allowed to feed on feeds, and its availability to the larvae for its determinable action. It is confirmed that the *L.camara* essential oil produces a lower impact on larval growth rate but has expressed significant antifeedant activity which may be the concentration of secondary metabolites in the essential oil required more to contain the above said insect pest (Fig. 4). The group of metabolites belongs to sesquiterpenes are distributed predominantly in oil but their individual concentrations are not enough to compete with the insect pests. In case of positive control (not treated) there was no larval mortality, but in the negative control growth rate of larvae was completely arrested by nimbicidine (0.5%), exhibits 100% larval mortality in 72 hrs and in monocrotophos (0.05%) 100% larval mortality was seen in 24hrs of incubation period.

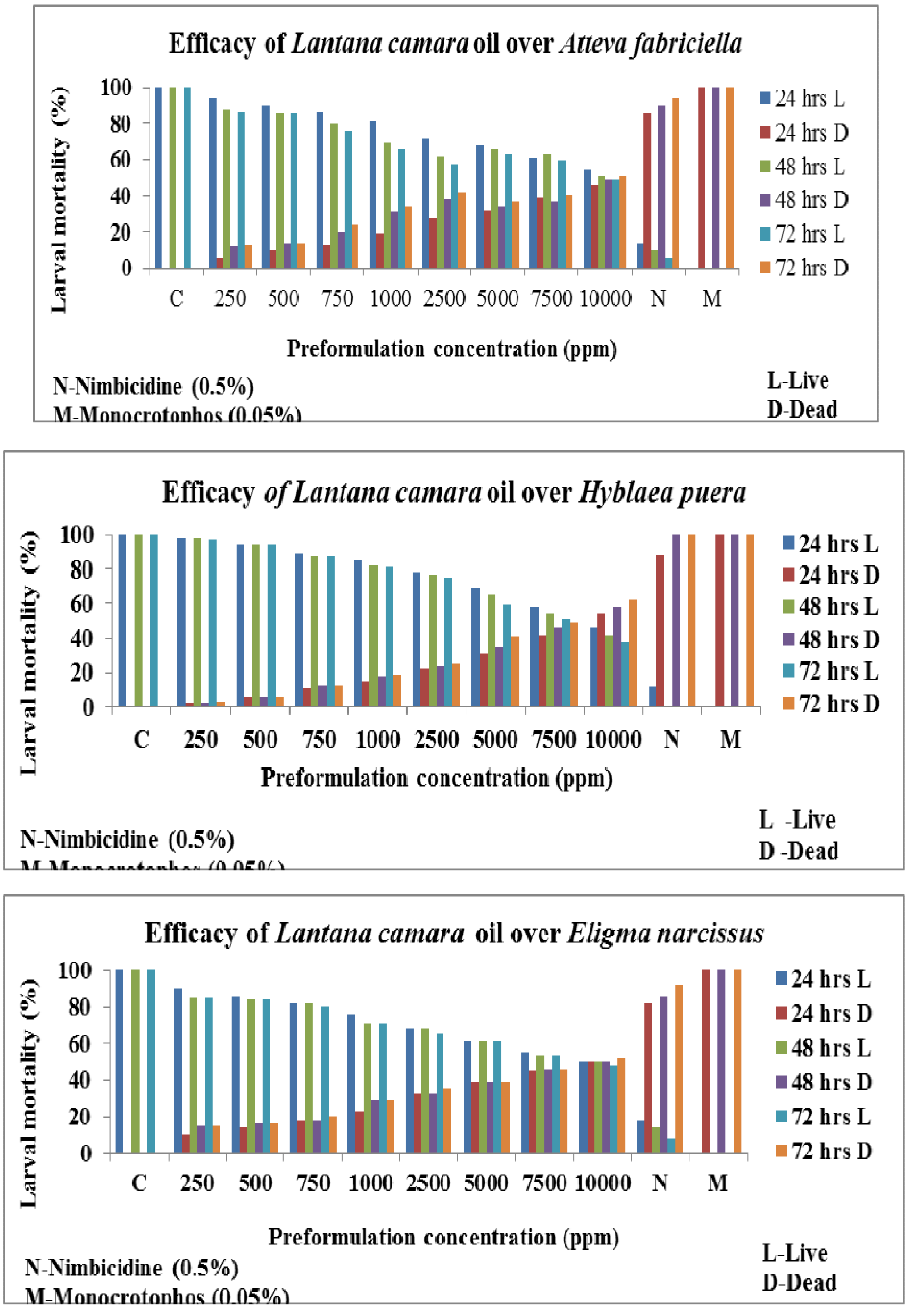


Fig .3. Efficacy of *Lantana camara* essential oil over target defoliaters

Comparative efficacy of bioactive compounds of essential oil

Natural resources like medicinal/aromatic plants/weeds would be the best source to obtain a variety of biological utility; therefore, such plants should be investigated for characterizing new molecules and to understand of their properties, safety and efficacy. The plant world comprises a rich storehouse of toxic constituents represent the secondary metabolites that could be tapped for use as pesticides [13]. For example, pyrethrum and neem are well established plant based botanicals commercially. Pesticides based on plant essential oils have recently entered the marketplace, and the use of rotenone appears to be waning [14]. In the present study, biopesticidal impact of individual or in combination of plant secondary metabolites on target organisms was studied with reference to larval mortality. The most significant effect of known plant products on the defoliators of teak and ailanthus, *H. puera, A. fabriciella* and *E. narcissus* is behavioural (antifeedant effect) and brought reduction in nutritional indices (consumption rate). Such findings and reports have been studied in a sizable number of insect pests of agri and forestry importance with reference to plant chemicals including phenols, phenolics, terpenoids, sequeterpenes, coumarins, flavonoids, furanocoumarins, polyacetylene and even alkaloids that are among the most acutely toxic plant products [15, 16]. It was demonstrated by them that mixtures of plant chemicals are more deterrent than single compounds, and it confirms the findings of the present study on teak and ailanthus defoliators. Essential oils, often complex mixtures of dozens or hundreds of terpenoids and other constituents in relatively low concentration may function generally throughout the plant kingdom as an antifeedant in front line of defense [17]. A number of plant substances have been considered for use as insect antifeedant or repellents but apart from some natural mosquito repellents, little commercial success has ensured for plant substances that alter target organisms behaviour. Numerous defensive chemicals either single or in combination belonging to various categories (terpenoids, alkaloids, glycosides, phenols and tannins) which cause behavioural and physiological effects on pests have already been identified. Therefore, bioactive compounds of plant origin are considered as ecologically safe alternative and the plant extracts with complex mixtures of bioactive compounds have been widely investigated for their insecticidal, repellent, ovicidal and antifeedant properties. Teak defoliator, *H. puera*, Ailanthus defoliator *E.narcissus* & *A. fabriciella* are major insect pests of forestry tree species in India reported to attack and cause severe damage to tree species. It has become difficult to manage them because of widespread development of resistance to conventional insecticides.

Effect of caryophyllene II oxide and aromadenrene II oxide characataerized from the essential oil of *L. camara* on target defoliators

Based on the preliminary laboratory bioassays, the essential oil fractions of *L. camara* was identified as caryophyllene II oxide and aromadendrene II oxide, and were selected for further bioactive confirmation feeding bioassay studies. Individual identified compounds were tested against *H. puera, A. fabriciella* and *E. narcissus* in a different concentration ranging from 250 ppm to 1000 ppm till 72 hrs and the results were compared with nimbicidine (biocontrol) and monocrotophos (synthetic control) which exhibited 100% and 88% larval mortality respectively. The results show that caryophyllene II oxide and aromadenrene II oxide exhibited significant repellent activity, and the efficacy of the components was higher when used individually. The synergistic effect is still higher when the components were used in combination in terms of larval mortality (60%-75%) at 48 hrs, and the assay was monitored till 72 hrs. The individual compound exhibit 54% - 58% larval mortality of target pest *H. puera* in 24 hrs at 10000ppm and 70 – 75% in 72 hrs of experimental period (Table 5). In case of *A.fabriciella* and *E. narcissus* similar bioactivity was recorded and the result confirms the activity is sustained and wider to most of the forest defoliators. The essential oils and their individual compounds from medicinal and aromatic plants have been known to exhibit antifeedant properties against a number of insects [18]. Synergism between bioactive components of a plant may result in unexpected metabolic outcomes within the plant and within the organism that consumes it which shows that the individual components of essential oil is not met with sufficient toxicity and larvae can resist the antibiotic effect of the invidual components of essential oil even at higher concentration of 1000 ppm.

Table. 5 Effect of caryophyllene II oxide and aromadenrene II oxide of essential oil of *Lantana camara* on *H.puera, E. narcissus* and *A. fabriciella* larval mortality

Bioactive compounds	*Hyblaea puera* Larval mortality (%)											
	24 Hrs				48 Hrs				72 Hrs			
	1000 ppm	2500 ppm	5000 ppm	10000 ppm	1000 ppm	2500 ppm	5000 ppm	10000 ppm	1000 ppm	2500 ppm	5000 ppm	10000 ppm
Caryophyllene II oxide	25	40	48	54	32	49	52	65	35	53	58	71
Aromadendrene II oxide	22	39	50	55	30	50	55	68	37	54	62	70
Caryo+aroma	24	39	49	58	32	50	56	68	36	55	61	75
***Eligma narcissus* larval mortality**												
Caryophyllene II oxide	20	31	41	45	29	37	49	54	35	44	51	62
Aromadendrene II oxide	21	32	42	47	28	38	49	55	33	45	53	65
Caryo+aroma	22	33	44	49	30	40	55	57	34	47	55	67
***A.fabriciella* larval mortality**												
Caryophyllene II oxide	24	38	45	53	30	45	56	66	31	51	60	70
Aromadendrene II oxide	21	36	47	55	31	48	57	64	32	52	59	69
Caryo+aroma	24	39	49	55	32	50	56	68	36	55	61	73

No larval mortality was observed in control, whereas nimbicidine and montocrotophos brought 80- 100% mortality at 0.5 and 0.05% respectively.

Efficacy of the preformulation against target defoliators

In view of the aforementioned bioassay studies and the promising results obtained for the *L. camara* essential oil and individual components, preformulation has been developed and evaluated in the laboratory. On testing the formulation at different concentrations (1000ppm, 2500ppm and 5000ppm) the preformulation showed high efficacy at 5000ppm concentration in all the pests (Table 6).

Table. 6 Efficacy of the preformulation against target defoliators

Target defoliators	Larval mortality (%)									Nimbicidine 0.5%	Control
	24 Hrs			48 Hrs			72 Hrs				
	1000 ppm	2500 ppm	5000 ppm	1000 ppm	2500 ppm	5000 ppm	1000 ppm	2500 ppm	5000 ppm	0.5% at 24 hrs	
H. puera	30	48	55	36	45	62	44	51	70	82	0
A. fabriciella	29	45	53	38	48	58	43	55	67	89	0
E. narcissus	24	39	47	32	50	56	39	52	62	89	0

Developing suitable formulations for application at nursery level

Bioassay on defoliators of teak and ailanthus with the oil fractions of *L. camara* leaves in respect of lab and field screening has given lead to make effective pre-formulation. Bio-pesticide formulations include an active ingredient, a suitable carrier, and added adjuvant. Each of this contributes to the efficacy of bio-pesticides on the target insect pest. The formulation was tested for its compatible, protection, and usable with common handling equipment. Active formulation was developed with mixing of neem oil, pungam oil, adjuvant and emulsifier. Bioassay directed, isolation, and purification of some secondary metabolites identified from the leaves of *L. camara* viz. caryophyllene II oxide and aromadendrene II oxide (0.1mg) were added with the above mixtures and bioefficacy was confirmed on the experimental organisms. The use of biological pesticides, based on plant extracts to the target pests and diseases has been proposed for many years. If produced, formulated and applied in appropriate ways, such biopesticides can provide ecological and effective solutions to pest problems. The essential oil has been proven to be effective in controlling insect pests that have developed resistance to chemical pesticides. However, despite the enormous potential of biopesticides as substitutes for chemical pesticides and for use in IPM programs, their development, commercialization and use has not yet lived up to expectations.

The essential oil extracted from *L. camara* leaves was found to be efficient against the target defoliators, based on which the individual components were selected and tested for their efficacy. From the promising results obtained from the crude extracts and the individual components, formulation in the nursery level was evaluated. On testing the formulation at different concentrations (1000ppm, 5000ppm and 10000ppm) the individual components showed high efficacy than the fraction of *L. camara* essential oil at 5000 and 10000ppm concentration in all the pests.

Tree PAL^{H} : Leaf essential oil based biopesticide for the management of teak and ailanthus defoliators

The bioactivity was evaluated on the key insect pests of Ailanthus and Teak based on the preliminary study conducted both in the laboratory and field conditions which reveals that the oil possesses antifeedant and pesticidal properties. The oil formulation showed effectiveness in managing the insect pests in terms of larval mortality; *Hyblaea puera* (72-97%), *Eligma narcissus* (56-76%) and *Atteva fabriciella* (58%-73%). The formulation was also found to act as feeding deterrents and repellents against the target species. Therefore, oil seed fractions in combination with essential oil fractions were considered as promising biopesticide against these pests and the formulation named as **Tree PAL$^{\mathbf{H}}$** had been prepared and released.

Application of Tree PAL$^{\mathbf{H}}$

100 ml of Tree PAL^{H} stock may be added to 10 liters of water and sprayed to infested plants at a week intervals. The requirement of Tree PAL^{H} formulation per hectare of plantation is worked out to be 100 ml.

DISCUSSION

The bioactivity of essential oil fractions of lantana in different doses has been evaluated both in lab and field conditions on teak and ailanthus defoliators. Although a number of articles have appeared in the past on various aspects of essential oils, bioactive emphasis on sustainability, safety and economics of essential oils in insect-pest management, but still the doses of essential oil of lantana is needed to manage the defoliators of teak and ailanthus viz. *H.puera, E. narcissus* and *A. fabriciella.* Several essential oils constitutes a wide range of desirable biological properties worth exploring the possibility for effective utilization in pest management [19] due to their naturally occurring anti-microbial properties conferred by monoterpenes, diterpenes and hydrocarbons with various functional groups. In the present investigation, it is reported that *L.camara* essential oil was higher in Valparai and Coonoor of high altitude region. *L. camara* is a rich source of botanicals which have resulted in the identification and isolation of terpenoids which expressed potent insecticidal behaviour when the defoliators of teak and ailanthus were subjected for nutritional behavioural studies; the other related biochemical profiles extracted from the leaves, such as flavonoids, phenylethanoid glycosides, furanonaphthoquinones, iridoid glycosides, steroids [20] triterpenes and flavonoids [21] have been used for the treatment of various biological disorders. The aforementioned phytoconstituents were screened and confirmed their presence in the essential oil of *L.camara* extracted from high altitude regions. The essential oil showed a moderate positive effect towards the growth of larvae which would lead to limited decrease in larval growth of *E.narcissus & A. fabriciella.* During September-December, when there was a pest build-up in the study area, a few larvae of *E. narcissus* were found dead and hanging on the leaves showing symptoms of bacterial infection.

In the present study, essential oil chemical profiles were observed and exhibited plant defensive chemicals against the defoliators of teak and ailanthus. The primary metabolites, such as proteins, carbohydrates, fatty acids, phytosterols, nucleotides, amino acids, and organic acids are found in all plants and perform metabolic roles that are essential and usually evident [22]. The essential oils are being tried as potential candidates for weed, pest and disease management. The oil is reported to possess insecticidal and repellent activities towards bees [23]. Murugesan *et al.*, (2012) [24] reported that the components present in the *L. camara* essential oil was α-Selinene, Caryophyllene oxide, Spathulenol, α- Copaene, α-Cubebene, α-Guaiene, α-Humulene, α-Selinene, β-Elemene, β-Cadinene, β-Selinene, δ-Cadinene, 12-Oxabicyclo(9,10)dodeca-3,7, 1H-Cycloprop(E)azulen-7-ol, 1-Hydroxy-1,7-dimethyl-4-isopro,1-Naphthalenamine,4-bromo, 1R,4S,7S,11R-2,2,4,8-Tetramethy, 2-Hexadecen-1-ol,3,7,11,15-ter, 4,4- Dimethyl-3-13-methyl but-3-e, 6-Isopropenyl-4, 8a-dimthyl-1,2 and aromadendrene oxide-(2) which were found to be responsible for the non-preference mechanism of defoliators in the nursery and young plantations of teak and ailanthus as well as through non-preference for oviposition. Senthilkumar *et al*., 2012 [25] also reported various ethnobotanicals bioactive compounds in *Trichilia connaroides* leaf extract through GC- MS/ MS analysis. Besides this, the presence of extraneous chemicals in the essential oil of *L. camara* prevent higher defoliation to the crop which is basically different from the chronic effects of feeding, growth and survival offered by a number of chemical substances. Several investigators studied the effects of chronic ingestion of certain allelochemicals on growth, survival, pupation and the possible role of allelochemicals on the feeding activity of herbivorous insects. Some other workers demonstrated the role of several plant allelochemicals on the feeding activity of herbivorous insects [15]. The phytotoxic constituents present in the plant represent the secondary metabolites been documented to arthropod pests, but it should be continued to the larger extend, yet only a handful of botanicals are currently used, and there are few prospects for commercial development of new botanical products. Pyrethrum and neem are well established commercially. Pesticides based on plant essential oils have recently entered the marketplace, and the use of rotenone appears to be waning [14]. In this study, biopesticidal impact of secondary metabolites either individually or in combination on defoliators of teak and ailanthus viz. *H. puera*, *E. narcissus* and *A. fabriciella* was studied with reference to feeding behaviour and larval mortality. It was observed that the toxic effect of those botanicals identified from lantana essential oil disrupted the feeding behaviour through antifeedancy nature and brought about a rapid nutritional reduction in consumption rate. Essential oils, often complex mixtures of dozens or hundreds of terpenoids and other constituents in relatively low concentration may function generally throughout the plant kingdom as an antifeedant in front line of defence [17]. Therefore, bioactive compounds of plant origin are considered as ecologically safe alternative and the plant extracts with complex mixtures of bioactive compounds have been widely investigated for their insecticidal, repellent, ovicidal and antifeedant properties. We are reporting first time in the present investigation that the compounds isolated from the leaf essential oil of lantana, viz., Aromadendrene II oxide and Caryophyllene II oxide were found to be active and exhibiting antifeedent and repellent properties on the defoliators of teak and ailanthus.

CONCLUSION

Bioassay confirmation of essential oil and individual compounds identified from *L.camara* was tested on the target pests of teak and ailanthus viz., *H. puera, E.narcissus* and *A. fabriciella* at different concentrations ranging from 250ppm to 1000ppm and from 2500ppm to 10,000 ppm in the laboratory. The results of the bioassay on the target insect pests indicated that the efficacy of essential oil is significant in terms of insecticidal property. Individual compounds such as caryophyllene II oxide and aromadendrene II oxide was tested on target pests at the concentrations ranging from 250ppm to 1000 ppm to further confirm the bioefficacy. The identified compounds belong to sesquiterpenes which are being reported first time for the biopesticidal properties against the defoliators of teak and ailanthus. The inhibitory effects of metabolites were found that they were not largely affected by the survival growth and on morphological malformations. Field level biopesticidal applications & experiments were carried out in *Ailanthus excelsa* field station, Salem and State forest nurseries located at Thirumoorthi, Aaliyar and Amaravathi, IFGTB research nursery and Kerala Forest Research Institute (KFRI) field station, Nilambur, Kerala to confirm the efficacy of the crude extracts, formulated extracts and individual compounds in both laboratory and nursery conditions. Based on the above mentioned findings, suitable preformulation were developed and tested at the doses of 10,000 ppm in comparison with synthetic pesticides such as nimbicidine and monocrotophos. Bioassay directed isolation and identification of bioactive compounds of *L.camara* essential oil was found to be effective against the target defoliators. This holds the potential of attempting the formulation of a number of promising biopesticides towards the objective of chemical pesticide free crops. With the promising results obtained both in laboratory and field trials, an eco-friendly new botanical called "Tree-PAL^{H}" *Lantana camara* leaves essential oil based biopesticide was developed and released for the benefit of farmers. The product has received good feedback from farmers and has scope for scaling up through Direct to Consumer Initiatives of ICFRE.

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