Chemical Composition, Insecticidal and Oviposition Deterrent Activities of the Essential Oil of Eucalyptus globulus against Bemisia tabaci (whitefly)

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ABSTRACT

Plant secondary metabolites are considered to be rich source of bioactive compounds for the discovery and development of molecules for human health and crop protection. These metabolites were found effective, non-persistent, environmentally safe, target specific and an alternative to toxic synthetic drugs and pesticides. To identify the bioactive products and molecules from plant resources, the essential oil from the leaves of *Eucalyptus globulus* was isolated by hydro-distillation method and analyzed by GC and GC-MS and evaluated for pest control activity against *Bemisia tabaci* (whitefly), an economically important insect. Chemical analysis showed the presence of thirteen compounds in the oil and 1,8-cineole (60.0%), limonene (14.0%, p-cymene (8.41%), α-terpineol (3.29%), 4-terpineol (3.34%) and β-fenchol (2.31%) were identified as main compounds. The oil and 1, 8-Cineole were evaluated for their insecticidal and ovipositional deterrent activities against *Bemisia tabaci* (whitefly) under lab conditions. Results showed that *Eucalyptus* oil caused considerable mortality of whitefly, ranged from 21.560 ±1.218 to 84.907 ±1.218% and 23.423 ±0.902 % to 86.210 ± 0.902 % at 0.07% to 0.9% concentrations at 24 and 48hr of treatment while that of 1,8-cineole showed 31.357± 0.787 to 76.807 ± 0.787 % and 34.863 ±0.745 to 78.783 ±0.745% mortality. Mortality of whitefly ranged from 15.260 ±1.218 to 16.420 ±0.902 % control. Similarly, the number of eggs laid by *B. tabaci* were found to be less in oil (18.667±1.072 to 5.667±1.072) than 1,8-Cineole (17.333±1.063 to 9.0±1.063) at 0.01 to 0.05% concentration compared to control (29.0±1.072 to 29.667±1.063). The oil (-21.67 to -67.29) and 1,8-Cineole (-26.34 to -53.45) also possessed promising ooviposition deterrence index. 1,8-cineole was identified as a bioactive molecule responsible for the insecticidal activity and ovipositional deterrent activity of the oil.

Keywords: *Eucalyptus globulus*; 1, 8-cineole; Essential Oil; *Bemisia tabaci*; Toxicity

Introduction

Eucalyptus (Family: Myrtaceae) is a large genus of evergreen aromatic trees, indigenous to Australia, Tasmania and New Guiana. About 100 species of *Eucalyptus* are known and used for medicinal purpose and in paper industry. *Eucalyptus globulus* Labill, is native to the forests of New South Wales, Victoria and Tasmania [1]. It was introduced in India and grows well from 5000 to 8,300 ft in northern, southern and eastern parts of India [1,2]. Dried leaves are used in chronic bronchitis and asthma while leaf oil is used as antiseptic and in upper respiratory tract and skin problems and as mosquito repellant [2-4]. Literature survey revealed that the essential oils from *Eucalyptus* species growing in different parts of the world have been extensively studied for their chemical compositions and biological activity. The leaves of *Eucalyptus* species are reported to contain 0.9 to 1.2% oil and many of them are rich source of 1,8-Cineole [1-4]. In brief, the oils from *E. spathulata, E. microtheca,* and *E. torquata* were found to contain 1,8-cineole (6.6-69.6 %) and α-pinene (2.3-14.5%) and aromadenderene (0.3-10.5%) as main compounds [5]. The oils from *E. melliodora* and *E. anceps* grown in Rwanda contained 1,8-cineole (33.49-47.7%), aromadendrene (1.0-18.01 %) and α-pinene (7.8-13.69 %), respectively [6].

Earlier reports showed that the oils of *E. intertexta*, *E. platypus* and *E. leucoxylon* from Iran had 1,8-cineole (26.1-85.5%) and α-pinene (7.3-31.7%) as main compounds [7] while *E. globulus*, *E. camaldulensis*, *E. gomphocephala*, *E. sideroxylon*, *E. robusta E*. *tereticomis* and *E. viminalis* from Algerian contained 1,8-cineole (3.5-81%) and α-pinene (2.8-24%) [8]. The leaves oil of *E. globulus, E. radiata* and *E. smithii* growing in Zambia were found to have highest concentration of 1,8-cineole (70.1-86.4%) [9]. Michelle et al. (2000) reported [10] that *Eucalyptus* of Finland origin also contained 1,8-cineole (3.2-88.6%,) and α-pinene (11.4-57.8%). The leaf of *E. campanulata* was rich in E-methyl cinnamate (94-99%) from a New England [11].

E. globulus leaf has been reported to contain high percentage of oil (0.9-1.2%) and 1,8-Cineole (upto 62.0%) in its leaves [3]. *E. globulus* growing in Jammu and Kashmir, India showed higher percentage of p-cymene (31.9%) compared to 1,8-Cineole (17.5%) in the leaves [12]. 1,8-cineole (74.2%) was also identified as a main compound in oil from leaves oil of *E. radiata* subsp. *robertsonii* together with α-terpineol (11.6%) from India [13]. The *Eucalyptus* oil is reported to possess antimicrobial [14-17], antifungal [18,19], antiviral [20], anthelmintic [21], allelopathic [22,23], insecticidal [24-26], acaricidal [27] and antibacterial [28] activities. Studies showed that the leaves of many *Eucalyptus* species are rich source of 1,8-cineole, however its percentage vary with age of the leaves and the geographical distribution [4,29]. Literature survey revealed that *E. globulus* leaf oil possesses pest control property but has not been evaluated against *Bemisia tabaci* and the bioactive has not been identified.

Bemisia tabaci (Hemiptera:Aleyrodidae), commonly known as whitefly, is a phloem feeder polyphagous agriculturally important pest in crops worldwidely. Its persistent sap sucking nymphs and adults causes tremendous loss to the vigor and photosynthetic parts of the plant due to formation of sooty made up of a secretion of honeydew. It is an important vector of about 100 plant viruses, responsible for leaf curl diseases in e.g. cotton, tomato, brinjal, papaya, and mosaic disease of several agricultural and horticultural crops, resulting in severe yield and economic losses [30]. An adult female whitefly lays about 150-200 eggs during its life period. Currently it is controlled by synthetic pesticides but these pesticides are harmful to the environment and non-targeted organisms including humans [31]. Plant based products with quick knock-down effect are the most preferred pest control measure for whiteflies but oviposition deterrent products are also considered as an alternative way of its control.

Search for target specific and biodegradable natural product with less or no toxicity to end users and environment is continuing worldwidely. The essential oil of *E. globulus* leaves and 1,8-Cineole have not been evaluated for their insecticidal activity against *B. tabaci* and thus chosen for present work. The study describes the isolation and characterization of essential oil and its bioactive compound from *E. globulus* oil and determine their effect on mortality and ovipositional deterrent activity against *B.tabaci*.

Materials and Methods

Plant material

Isolation of essential oil

The cleaned and fresh leaves (400g) of *Eucalytpus globulus* were collected from the campus of IARI, New Delhi and subjected to hydrodistillation for 4 hours using a Clevenger-type apparatus. The distillate was extracted with diethyl ether, the ethereal layer was dried over anhydrous sodium sulphate and ether distilled of on gently heated water bath. The yield of the oil obtained was found to be 0.86% on dry weight basis (w/w) and was stored at 4-8°C.

Separation of 1,8-cineole from essential oil

Eucalyptus oil (5.0g) was adsorbed on silica-gel (25g, 60-100mesh) and then column chromatographed over silica-gel (200g, 60-120mesh). The column with eluted with hexane and polarity of the eluant was increased using ethyl acetate. The fractions eluted with hexane:ethyl acetate (97:3) gave 1.5g of the compound (EO-1, 1.5g) and was identified as 1,8-Cineole (97.5%) by comparing with its authentic sample and GC-MS.

Gas chromatography

Analysis of the oils from *E. globulus* was performed using a Gas Chromatography-Flame Ionisation Detector (GC-FID, Shimadzu model 2010 Plus) equipped with a capillary column DB-5 (30 m × 0.25 mm, film thickness 0.25 µm). Helium was used as the carrier gas at a flow rate of 1ml/min (split mode). The oven temperature was held at 60°C, then programmed at 3°C/min to 230°C with split mode (1:20) and injection volume was 0.2µl. The injector and the detector temperatures were kept at 250°C and 260°C, respectively. The relative percentages of the individual components were calculated based on GC peak area (FID response) without using correction factors.

Gas chromatography-mass spectrometry

Analysis of the essential oils of *E. globulus* was performed on a Gas Chromatography-Mass Spectrometry (GC-MS, Thermo DSQ) equipped with a capillary column DB-5 (30 m × 0.25 mm, film thickness 0.25 µm). Chromatographic conditions were as follows: helium as carrier gas at a flow-rate of 1 ml/min (split mode, 1:20); injection volume was 0.2µl; injector temperature was 250°C, respectively. The column temperature was held at 60°C, and then programmed at 3°C/min to 230°C. The column was coupled directly to the quadrupole mass spectrometer at EI mode at 70eV with the mass range of 28-400 a.m.u range at 1 scan/s. Individual compounds were identified by comparing their mass spectrum with library, authentic sample, Kovat's index as well as literature [32].

Pest control activity against Bemisia tabaci

Preparation of test solutions

E. globulus oil and 1,8-Cineole were used for evaluation for their contact toxicity and ovipositional deterrent activities against *Bemisia tabaci*. The stock solution (1.0%) of the oil and 1,8-Cineole were prepared separately by mixing with distilled water and surfactant (Tween-80, 1%) followed by stirring using magnetic stirrer for 30 minutes. Different concentration of test solutions for contact toxicity (0.07 to 0.9 %.) and ovipositional deterrent activity (0.01 to 0.05%) were prepared from the stock solutions.

Rearing of insect

B. tabaci (whitefly) used for the experiment belong to New Delhi population and were reared on cotton plants in an isolated chamber in the Insect Proof Climate Control facility at Division of Entomology, ICAR-IARI New Delhi.

Contact toxicity bioassay

Contact toxicity was carried out by Leaf-dip method as per IRAC with some modifications [33]. The fresh, cleaned and matured cotton leaves were treated with the test solutions (0.07, 0.1, 0.3, 0.7, & 0.9%) and control (1% Tween-80) by dipping for a minute and left for drying for half an hour. The petiole of treated leaves was placed inside agar cubes kept in perforated petriplates. Whiteflies were aspirated from the culture chamber and exposed to CO_2 and then released in the petriplates. The sides of the plates were sealed with paraffin. Tween-80 (1%) solution was used as negative and Imidacloprid as positive control. Each experiment was conducted in triplicates and the mortality of the adults was recorded after 24 and 48 hours.

Ovipositional deterrence bioassay

The clip cage bioassay method was used for ovipositional deterrent activity. Different concentrations (0.01 to 0.05%) of test solution and cotton plants (30-45 days) with three mature leaves were used in this study. The leaves were treated by dipping in different concentration of test solution and control (1% Tween-80) for a minute and dried for an hour. The plants were tied with stick and twine for providing the necessary support.10 adult female Whiteflies were released in each cage containing treated leaves. Each experiment was conducted in triplicates and the numbers of eggs laid were recorded after 48 hours of treatment.

Statistical analysis

Statistical analysis of the data was carried out using SAS Package (Statistical Analysis System package) software while EC_{50} values were calculated by probit analysis in POLO software. Oviposition Deterrence Index (ODI) was calculated according to the formula [34] given by Huang et al., (1995) where

ODI= [(T-C)/(T+C) * 100] where T= no of eggs laid on the treated leaves
C= no of eggs laid on the control leaves

ODI values vary from -100 to 100 where -100 represents absolute deterrence and +100 represent absolute stimulant. An ODI value in the negative range represents potential oviposition deterrence activity.

Results

The yield of the essential oil in the leaves of *Eucalyptus globulus* obtained by hydro-distillation method was found to be 0.86% on fresh weight basis. In GC-MS analysis, thirteen compounds constituting 97.33% of the oil, were identified (Tables 1 and 2 and Figure 1). The oil was found to be a mixture of monoterpenes and oxygenated monoterpenes with trace percentage of alkane hydrocarbon. Oxygenated monoterpenes constituted the major portion (69.51%) of oil and 1,8-Cineole (60.0%) was found to be major compound followed by 4-Terpineol (3.34%), trans-Pinocarveol (3.29%) and α-Terpineol (2.31%) but endo-Fenchol (0.57) was found in less percentage. Its oil also found to contain monoterpenes (27.49%) and Limonene (14.0%) has been found as a main compound followed by p-Cymene (8.41%), α-Pinene (3.83%), γ-Terpinene (0.51%), α-Phellandrene (0.31%), Myrcene (0.23%) and β-Pinene (0.2%). Nonane (0.33%) was identified as alkane hydrocarbon in the oil. None of the sesquiterpenes and their derivatives were identified in this oil. Further 1,8-cineole (98.0% purity), a major compound was isolate from essential oil by column chromatography and its identity was confirmed by GC-MS.

Both the essential oil and 1,8-Cineole were evaluated for the insecticidal and ovipositional deterrent activities against *Bemisia tabaci* using leaf dip method at seven different concentrations (0.07, 0.09, 0.1, 0.3, 0.5, 0.7 and 0.9%). The oil caused dose dependent mortality of *B. tabaci* with highest (84.907±1.218 and 86.210±0.902%) mortality at 0.9% concentration, while 1,8-Cineole caused 76.807±0.787 and 78.783±0.745 % mortality at 0.9% at 24 and 48 hr of treatment, respectively. It was observed that longer exposure time increased mortality of adult female whiteflies. The LC_{50} values of *Eucalyptus* oil were found to be 0.323 and 0.304% at 24 and 48 hours and that of 1,8-Cineole were 0.415 and 0.351% concentrations.

Compound	KI**	KI *	Conc. (%)
Nonane	898	900	0.33
α-Pinene	931	932	3.83
β-Pinene	972	974	0.20
Myrcene	986	988	0.23
α-Phellandrene	1001	1002	0.31
p-Cymene	1021	1020	8.41
Limonene	1025	1024	14.0
1,8-Cineole	1026	1026	60.0
γ-Terpinene	1052	1054	0.51
endo-Fenchol	1116	1114	0.57
trans-Pinocarveol	1133	1135	3.29
4-Terpineol	1175	1174	3.34
α-Terpineol	1185	1186	2.31
Compound identified			97.33
Monoterpenes			27.49
Oxygenated monoterpenes			69.51
Alkane hydrocarbon			0.33

KI= Kovat's Index, *Adams, 2007, **Calculated using C_8-C_{20} hydrocarbons (Sigma) in DB-5 capillary column

Table 1: Essential oil composition of *Eucalyptus globulus* leaves

Concentration of test solutions (in %)	**Mortality** (Mean ± SD)			
	***E. globulus* oil**		**1,8-Cineole**	
	24hr	**48hr**	**24hr**	**48hr**
0.07	21.560 ±1.218^b	23.423 ±0.902^b	31.357± 0.787^b	34.863 ±0.745^b
0.09	38.433 ±1.218^c	38.747 ±0.902^c	39.88± 0.745^c	39.880 ±0.745^c
0.1	39.490 ±1.218^c	41.807 ±0.902^c	40.660± 0.787^c	44.547 ±0.745^d
0.3	50.187± 1.218^d	54.467 ±0.902^d	51.977± 0.787^d	55.997 ±0.745^e
0.5	62.500 ±1.218^e	68.480 ±0.902^e	56.407 ±0.787^e	60.600 ±0.745^f
0.7	70.047 ±1.218^f	71.577 ±0.902^e	58.730 ±0.787^e	61.467 ±0.745^f
0.9	84.907 ±1.218^g	86.210 ± 0.902^f	76.807 ± 0.787^f	78.783 ±0.745^g
Control (1% Tween-80)	15.260 ±1.218^a	16.420 ±0.902^a	15.260± 0.787^a	16.420 ±0.745^a

Values given in the table are mean±SD of three replicates, and the same letter showed an insignificant difference at α=0.05 (Tukey's HSD test) in a column

Table 2: Effect of *E. globulus* oil and 1,8-Cineole on the mortality of *Bemisia tabaci*

Similarly, ovipositional deterrent activity of the essential oil and 1,8-cineole was also carried out at lower concentrations (0.01-0.05%) using clip-cage method. It also showed that both oil and 1,8-cineole possessed egg laying inhibitory capacity of adult females of *B. tabaci*. The number of eggs laid by female was drastically reduced by both oil (5.667±1.072 eggs) and 1,8-cineole (9.00±1.063eggs) at 0.05% concentration compared to the control (29.00±1.072 to 29.667±1.063 eggs) at 48hr of treatment. Oviposition Deterrence Index of the oil and 1,8-cineole indicated that the number of eggs laid by females decreased with increase in the dose of the treatments. Oviposition Deterrence Index also exhibited negative values of all the tested concentrations thus indicating their potential oviposition deterrent action (Table 3).

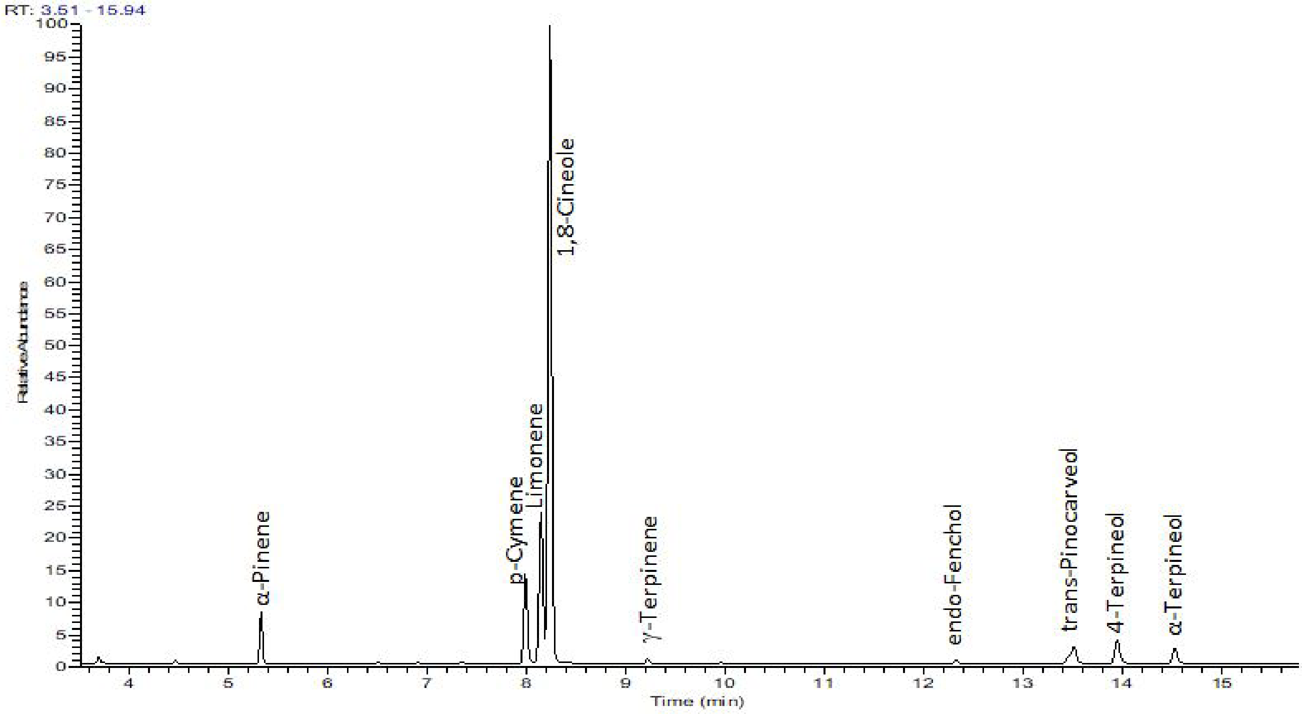

Figure 1: Total ion chromatogram of *Eucalyptus globulus* leaf oil

Concentration of test solutions (in %)	Number of eggs laid after 48hr		Oviposition deterrence index	
	E. globulus oil	1,8-Cineole	*E. globulus* oil	1,8-Cineole
0.01	18.667±1.072^{b}	17.333±1.063^{b}	-21.67	-26.34
0.02	14.667±1.072^{bc}	15.667±1.063^{bc}	-32.81	-30.88
0.03	9.667±1.072^{cd}	14.000±1.063^{bcd}	-49.99	-35.88
0.04	8.667±1.072^{d}	11.667±1.063^{cd}	-53.97	-43.54
0.05	5.667±1.072^{d}	9.000±1.063^{d}	-67.29	-53.45
Control	29.000±1.072^{a}	29.667±1.063^{a}	-	-

Values given in the table are mean±SD of three replicates, and the same letter showed an insignificant difference at α=0.05 (Tukey's HSD test) in a column

Table 3: Effect of *E. globulus* oil and 1,8-Cineole on ovipositional deterrency against *Bemisia tabaci* and their oviposition deterrence index (ODI)

Discussion

The essential oil from *E. globulus* leaves is reported to possess insecticidal and antimicrobial activities [2,4]) but its oil and 1,8-cineole has not been evaluated earlier against whiteflies, an agriculturally important insect and vector of many plant virus diseases [40]. In this study, *E. globulus* oil was found to have better insecticidal and ovipositional deterrent activities against whiteflies compared to the 1,8-cineole, which could be due to the synergistic activity of other compounds present in the oil (4,41). However, 1,8-cineole, the main compound of the oil, also showed insecticidal as well as ovipositional deterrent activity against whiteflies and were concentration dependent. Earlier studies showed the leaves of *Eucalyptus* species growing in Portugal (1.3-2.7%), Morocco (1.9-2.7%), Argentina (2.68%), Ethiopia (0.95-1.32%), Algeria (1.87%) and Bangladesh (0.08-3.5%) contained a different percentage of oil and were rich in 1,8-cineole (up to 88.63%), however, the percentage of 1,8-cineole and composition of the oil vary with location, age, season, and even within different varieties of the same species [35-39]. Similar studies were also carried out in India, which showed the percentage of the oil in the leaves varied from 0.8-1.2% [4,14,38,40,41]. *E. globulus* leaves oil has been reported to contain the highest percentage of 1,8-cineole (up to 62.0%) from India, but its percentage varied with the origin, age maturity of leaves and season [2,4]. 1,8-Cineole percentage in Eucalyptus oil has been the main criterion of quality of the oil [4]. The oil obtained in this study was found to have a high percentage of 1,8-Cineole (60.0%), similar to some of the high yielding species (28.40 to 66.1%) reported from various locations of India [4,39]. Similar study carried out using the essential oils of ten Egyptian plant species against eggs and 3rd instar nymphs of *B. tabaci* the results showed that *Artemisia absinthium*, *Cyperus articulates*, and *Thyme vulgaris* oils exhibited potent ovicidal activity while *A. absinthium* oil showed reduction in its populations (87.6%), followed by *C. articulates* (85.0%), *Thymes vulgaris* (81.9%), *Mentha longifolia* (78.6%) and *Syzygium aromaticum* (51.7%) [42]. The essential oil from *Gardenia jasminoides* was found to have promising effect in controlling the adult and nymph of whiteflies and mites [43,44]. Likewise, the essential oils from *Schinus terebinthifolius* fruits and *Eucalyptus citriodora* leaves showed concentration dependent activity against *B. tabaci* and the castor bean whitefly (*Trialeurodes ricini* Misra) but the oil from *S. terebinthifolius* fruit were most potent, compared to *C. citriodora* oil [45]. These results suggest that both, oil and 1,8-cineole showed better ovipositional deterrent activity than insecticidal activity even at low concentration (0.05%), compared to the control against whiteflies but concentration dependent. and can be used for controlling whiteflies after their evaluation in field conditions.

Conclusions

The leaves of *E. globulus* possessed therapeutic, antimicrobial and pest control properties. Its leaves are used as a natural source of 1,8-cineole rich oil in India. The essential oil and 1,8-cineole showed dose dependent potent ovipositional deterrent than insecticidal activity against *Bemisia tabaci*. Its leaves were found rich source of 1,8-cineole and ursolic acid and ursolic acid lactone. This is the first report, wherein oil and 1,8-cineole were found to possess insecticidal and ovipositional deterrent activity against *B. tabaci*. Thus, both of them could be useful for development of oil-based product for controlling the population of whiteflies in agriculture and integrated pest management for production of safe edible agricultural produce after evaluation in field conditions.

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