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Original Research

Chemical Composition and Insecticidal Activities of Essential Oils of Myrtaceae against *Tribolium castaneum* (Coleoptera: Tenebrionidae)

Saima Siddique[1]*, Zahida Parveen[3], Firdaus-e-Bareen[2], Abida Butt[4], Muhammad Nawaz Chaudhary[1], Muhammad Akram[5]

[1]College of Earth and Environmental Sciences, University of the Punjab, 54890-Lahore, Pakistan
[2]Department of Botany, University of Punjab, Lahore-54890, Pakistan
[3]Applied Chemistry Research Centre, PCSIR Laboratories Complex, Lahore-54600, Pakistan
[4]Department of Zoology, University of the Punjab, 54890-Lahore, Pakistan
[5]Medicinal Botanic Centre, PCSIR Laboratories Complex, Peshawar-25000, Pakistan

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Abstract

The present study was designed to determine chemical composition of essential oils extracted from different species of the Myrtaceae family and to evaluate their insecticidal activities against *Tribolium castaneum* (Coleoptera: Tenebrionidae**)**. The essential oils of 10 species were extracted by hydrodistillation and analyzed by a gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The main component of *Eucalyptus crebra, E. microtheca, E. rudis* and *Melaleuca quinquenervia* essential oils was 1,8-cineole (31.6-49.7%). *E. melanophloia* and *E. tereticornis* contained *p*-cymene (41.8-58.1%) as a major component, while *Eucalyptus kitsoniana* and *E. pruinosa* essential oils were dominated by α-pinene (25.8-31.4%). Eugenol methyl ether was identified as a major component in *M. bracteata* essential oil (82.3%). α-Pinene (31.4%) was the main component in the *C. viminalis* essential oil. Essential oils of all selected plant species showed good insecticidal activities against *T. castaneum* when compared with pyrethroid as a positive control. *Eucalyptus rudis* proved most potent against *T. castaneum*, followed by *M. bracteata*, *M. quinquenervia*, and *C. viminalis*. The results of this study indicate that essential oil of Myrtaceae leaves have potential to be used in the control of *T. castaneum.*

Keywords: 1,8-cineole, *E. melanophloia*, hydro-distillation, GC-MS, LC_{50}, *M. quinquenervia*, α-pinene

*e-mail: saimesiddique@gmail.com

Introduction

Wheat (*Triticum aestivum* L.) is the major source of protein in human foods and the second main food crop in terms of production [1]. More than 2,000 species of pestiferous insects annually destroy approximately one third of the world wheat production, valued at more than $100 billion [2]. Synthetic chemical insecticides and fumigants are commonly used to manage pestiferous insects throughout the world. Fumigants are preferred as they are convenient to use, economical, and have broad spectrum action and rapid penetration into the commodity [3]. However, the use of synthetic insecticides and fumigants lead to the problems of residual toxicity in non-targeted organisms, development of resistance and resurgence in pestiferous insect populations, adverse environmental impacts such as ozone depletion, pollution, etc. [4]. These problems have necessitated the search for alternative ecologically safe insect pest control methods. Plant-derived insecticides have gained attention in recent years in the pest management industry because of their low toxicity on mammals, short environmental persistence, minimal residual activity, and thus wide public acceptance [5].

Among plant-derived insecticides, essential oils are worth mentioning. They are secondary plant metabolites, aromatic in nature, and give a distinctive odour or flavour to a plant [6]. Chemically, they are complex mixtures comprised of a large number of constituents in variable ratios [7]. The effectiveness of essential oils and their components in the control of stored product pests has been well-established [8-11].

The Myrtaceae (Myrtales) family, comprising at least 133 genera, is a rich source of essential oils. It is found abundantly in Australia, Southeast Asia, and tropical to southern temperate America, while a few are domesticated in Africa [12]. The essential oils from Myrtaceae have demonstrated antibacterial, anti-inflammatory, fungicidal, antioxidant, and antiviral properties [13-15]. Studies have also reported insecticidal activities of different species of Myrtaceae against *T. castaneum* [16-18]**.** α and β-pinene, terpinen-4-ol, 1,8-cineole, and methyl eugenol have been reported as major components in Myrtaceae essential oils, which have been known for their toxicities against pestiferous insects [19-23].

Pakistan is the 9th largest wheat-producing country, accounting for 3.0% of the world's wheat production from an area of 3.57% of the world [24]. The warm and moist climate of Pakistan is conducive to infestation of stored grains by different pests. *Tribolium castaneum* (Herbst) (Coleoptera: Tenibrionidae) is one of the most abundant and detrimental insects that affects stored grains [25]. Estimated weight loss during storage is approximately 4% in Pakistan and accounts for about 1,000 million rupees in losses contributing more than 24% to gross domestic product (GDP) each year [26]. Sun-drying, aluminium phosphine tablets, insecticides, and elemental mercury have been used to control pestiferous insects. Although some degree of control seems to have been achieved, most chemical treatments are unsatisfactory and can be dangerous to health. Pakistan is rich in medicinally and economically rich flora. Myrtaceae is represented by seven genera and 26 species in Pakistan [27]. Keeping in view the insecticidal properties of Myrtaceae and the need for natural fumigants, the present study was designed to explore chemical composition of oil extracted from 10 species (*C. viminalis, E. crebra*, *E. kitsoniana*, *E. melanophloia*, *E. microtheca*, *E. pruinosa*, *E. rudis, E. tereticornis, M. bracteata,* and *M. quinquenervia*) from Pakistan and to assess their insecticidal effects for their potential use as alternatives to chemical fumigants. The selection of species was made on the basis of their abundance and availability.

Material and Methods

Chemicals

Homologous series of C_8-C_{25} *n*-alkanes used in this study were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol was purchased from Merck (Darmstadt, Germany).

Plant Material

Mature leaves of 10 selected species of Myrtaceae were collected from different localities around Pakistan (Table 1). Taxonomic authentication was performed by Prof. Dr. A. N. Khalid at the Herbarium, Department of Botany, University of Punjab, Lahore, Pakistan. Plant specimens were deposited in the same herbarium.

Isolation of Oils

From each plant, fresh leaves weighing 2 kg were hydrodistilled for 3 hours using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [28]. Oils were dried over anhydrous sodium sulfate, filtered, and stored at -4ºC in a freezer until analyzed. The essential oil contents (%) were expressed as volume of essential oil vs.weight of fresh leaves (v/w).

Chemical Analysis of Essential Oils

GC-FID

GC analysis of the essential oils was carried out on a Shimadzu GC 2010 equipped with a flame ionization detector (FID) and AOC-20i autosampler using a DB-5 MS (30 m × 0.25 mm id, 0.25 µm film thickness) capillary column. The column oven temperature was programmed initially at 40-90ºC at the rate of 2ºC/min and then raised to 90-240ºC at the rate of 3ºC/min. The final temperature was held constant for 5 min. Injector and detector temperatures were maintained at 240 and 280ºC, respectively. Essential oil (0.5 µl) was injected in

Table 1. Detail of collected plants from different sites in Pakistan.

Plant Name	Harvesting Month	Locality of Collection	GPS coordinates	Voucher Number
Callistemon viminalis	October 2013	Qarshi Botanical garden, Hattar, Abottabad	Longitude 72.85 °E, Latitude 33.85°N	BDSS #4055
Eucalyptus crebra	March 2013	Pakistan Forest Research Institute, Faisalabad	Longitude 73.11°E, Latitude 31.28°N	BDSS #4023
Eucalyptus kitsoniana	March 2013	Pakistan Forest Research Institute, Faisalabad	Longitude 73.11°E, Latitude 31.28°N	BDSS #4024
Eucalyptus melanophloia	March 2013	Pakistan Forest Research Institute, Faisalabad	Longitude 73.11°E, Latitude 31.28°N	BDSS #4025
Eucalyptus microtheca	March 2013	Pakistan Forest Research Institute, Faisalabad	Longitude 73.11°E, Latitude 31.28°N	BDSS #4026
Eucalyptus pruinosa	March 2013	Pakistan Forest Research Institute, Faisalabad	Longitude 73.11°E, Latitude 31.28°N	BDSS #4027
Eucalyptus rudis	March 2013	Pakistan Forest Research Institute, Faisalabad	Longitude 73.11°E, Latitude 31.28°N	BDSS #4028
Eucalyptus tereticornis	March 2013	Pakistan Forest Research Institute, Faisalabad	Longitude 73.11°E, Latitude 31.28°N	BDSS #4029
Melaleuca bracteata	April 2013	Government College University botanical garden, Lahore	Longitude 74.31°E, Latitude 31.57°N	BDSS #4040
Melaleuca quinquenervia	October 2013	Pakistan Horticulture Authority botanical garden, Lahore	Longitude 74.30°E, Latitude 31.50°N	BDSS #4042

a split-mode ratio of 1:5. Helium was used as a carrier gas at a flow rate of 1 ml/min. Quantification of constituents was done by integration of peak areas without using correction factors.

GC-MS

The identification of components was carried out on a GCMS-QP 2010 Plus (Shimadzu, Japan) operating in electron ionization mode at 70 eV. Mass units were monitored from 35 to 500 AMU. A DB-5 MS (30 m × 0.25 mm id, 0.25 µm film thickness) capillary column was used. Column conditions and injector and detector temperatures were the same as in GC analysis.

Linear retention indices were calculated using a homologous series of *n*-alkanes (C_8-C_{25}) under the same temperature-programmed conditions. The components were identified by comparison with linear retention indices (RI) from literature, mass spectra with those of NIST mass spectral library, or co-injection with standards [29-30].

Collecting Insects

Tribolium castaneum adults were obtained from the laboratory cultures (Department of Zoology, University of Punjab) maintained in the dark in an incubator at 30±1ºC and 60±5% relative humidity. Insects were reared on wheat flour mixed with yeast (10:1) containing 12-13% moisture content. Adult unsexed insects, 7-14 days old, were used in the bioassay.

Toxicity as Fumigant

The activity of essential oils against *T. castaneum* was evaluated using the method of Pires et al., (2006) [31].

Different concentrations of essential oils were selected on the basis of preliminary tests. Tested concentrations of oils ranged from 2-25 µl for *Eucalyptus* species and 0.4-0.9 µl for *Melaleuca* and *Callistemon* species. The filter paper discs (2 cm diameter, Whatman No. 10, Sigma Aldrich) were impregnated with different oils using micropipette (Gilson, Inc.). Then treated discs were attached to the under surface of the screw cap of a glass container (22 ml) and assumed 100% volatilization of the oils in the container. Ten 7-14-day-old *T. castaneum* adults were placed in each container with 0.5 g of wheat flour as food before capping. The control groups consisted of a similar setup without the disc of essential oil. The experiment was conducted at 30±1ºC and 65±5% relative humidity. Mortality of *T. castaneum* adults was recorded after 4, 8, 12, and 24 h from commencement of exposure. Insects were considered dead if they showed no leg or antennal movements on touch with fine brush. For bioassay, 6-8 concentrations of essential oil from each species of Myrtaceae were used. Each concentration and control was replicated 10 times.

Statistical Analysis

Hierarchical cluster analysis was done on the basis of the percentage composition of essential oils (with compounds of >0.1%). A dendrogram was obtained

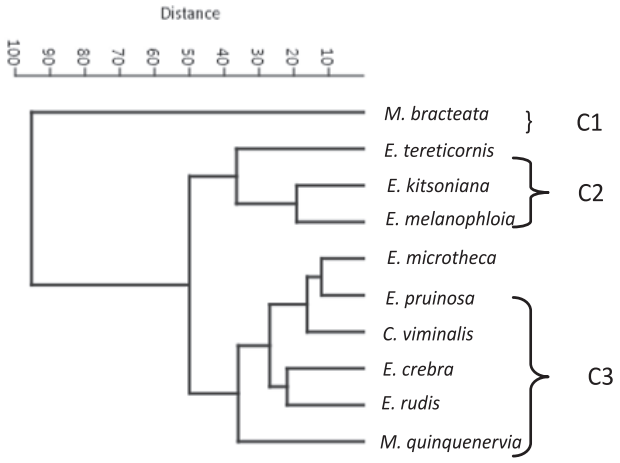

Fig. 1. Dendrogram showing hierarchical cluster analysis of the ten essential oils from different species of Myrtaceae species based on Euclidean linkage distance.

by plotting the pair groups using the Euclidean linkage distance method showing dissimilarity of the analyzed oils within the range 0-100 (Fig. 1). For statistical analysis PAST (version 3.11) was applied.

The data were corrected using Abbott's formula for the mortality in the controls [32]. The mortalities were subjected to probit analyses using Minitab 17.0 to estimate LC_{50} and LC_{95} values of essential oils against *T. castaneum*. LC_{50} values were subjected to one-way ANOVA to determine variation in the activity of essential oils. Means were separated at the 5% significance level using Tukey's Test.

Results and Discussion

Essential Oils Yield

Hydodistillation of fresh leaves yielded 1.7±0.1% of essential oil. The oil content of *Callistemon viminalis* was higher than previous reports [33-34]. The essential oils yields ranged between 0.1-1.9% and 0.1-1.3% for selected *Eucalyptus* and *Melaleuca* species, respectively (Table 2). The oil contents of *E. rudis* and *E. crebra* were in accordance with previous reports [16, 25, 38-40]. However, in *E. microtheca*, *E. melanophloia, E. kitsoniana*, *E. tereticornis*, *M. quinquenervia*, and *M. bracteata,* oil yields were found to be in lower amounts than the previous reports [38, 41-44]. No data was available to compare oil yield of *E. pruinosa* in literature. The variability in essential oil yield can be attributed to the season of harvest and age of the leaves (immature, mature, or senescent).

GC-MS Analysis

The essential oil from *C. viminalis* leaves was found to be rich in monoterpene hydrocarbons (59.8%), followed by oxygenated monoterpenes (37.6 %). Monoterpene hydrocarbons were represented by α-pinene (31.4%), *p*-cymene (15.9 %), limonene (5.6 %), and α-phellandrene (4.3 %) as major constituents. Among oxygenated monoterpenes, 1,8-cineole (24.9%) was the principal component, followed by α-terpineol (11.2%) (Table 3). The richness of *C. viminalis* essential oil in α-pinene conflicted with previous studies, where 1,8-cineole (47.9-82.0%) was reported as a major constituent of the oil [33, 42-44]. Carvone, *p*-cymene-3-ol, 3-allyl-2-methoxyphenol, nerol acetate, and eugenol methyl ether among monoterpenes, and *ar*. tumerone among sesquieterpenoids (although in small concentrations) have been reported for the first time in essential oil from *C. viminalis*.

Eucalyptus rudis, E. microtheca, and *E. crebra* essential oils contained 1,8-cineole (31.6-49.7%) and α-pinene (16.9-32.5%) as major components. *Eucalyptus kitsoniana* and *E. pruinosa* essential oils were rich in α-pinene (25.8-29.5%) and *p*-cymene (8.2-24.8%). *Eucalyptus melanophloia* and *E. tereticornis* contained *p*-cymene (41.8-58.1%) as a major component. The richness of *E. crebra*, *E. rudis*, *E. microtheca,* and *E. pruinosa* essential oils in 1,8-cineole and α-pinene was in conformity with previous reports [22, 45-46]. *Eucalyptus rudis* oil contained higher 1,8-cineole (48.5%) and α-pinene (32.5%) contents when compared to the Tunisian variety of *E. rudis* (19.9% and 3.9-14.5%, respectively). Limonene, which has been identified in *E. rudis* oil, was absent in the Tunisian variety of *E. rudis* [23, 47-48]. 1,8-Cineole and α-pinene were reported previously as prominent components in *E. tereticornis* essential oil contrary to *p*-cymene in evaluated *E. tereticornis* species [49-50]. 1,8-Cineole was reported as a principal constituent in Tunisian variety of *E. kitsoniana,* whereas α-pinene has been identified as a major component in *E. kitsoniana* oil from Pakistan [48].

Eugenol methyl ether (82.3%) was identified as a major compound along with a significant amount of methyl cinnamate (11.4%) in *M. bracteata* essential oil. This is in agreement with a previous report [19, 41, 51]. *Melaleuca quinquenervia* essential oil mainly contained monoterpenes (96.0%). The major components identified were 1,8-cineole (31.0%), followed by *p*-cymen-8-ol (19.7%) and α-terpineol (9.9%) in oxygenated monoterpene fraction, while the monoterpene hydrocarbon fraction contained *p*-cymene (16.5 %) as a major component, followed by limonene (6.8%), α-pinene (4.2 %), and terpinolene (4.2 %). *Melaleuca quinquenervia* essential oil showed similarity to the previous results as 1,8-cineole being the major component [52-53]. However, *p*-cymen-8-ol (19.7%), linalool (1.5%), eugenol methyl ether (1.1%), and α-phellandrene (2.0%) among the monoterpenes and caryophyllene (0.1%), caryophyllene oxide (0.4%), and epiglobulol (0.9%) as sesquiterpenes that were present in essential oil of *M. quinquenervia* have not been stated previously.

These differences in the essential oils compositions might arise from differences in environmental (climatic, seasonal, geographical) factors [54].

Table 2. Chemical composition of essential oils from different Myrtaceae species.

Compounds	RI	Content (%)									
		C. vim	*E. cre*	*E. kit*	*E. mel*	*E. mic*	*E. pru*	*E. rud*	*E. ter*	*M. bra*	*M. qui*
α-pinene	932	31.4	16.9	25.8	24.1	31.0	29.5	32.5	0.7	0.2	4.2
Camphene	944	-	0.2	0.2	0.3	0.6	0.4	0.1	-	-	0.1
β-pinene	974	-	-	-	-	-	0.3	-	-	-	-
α-phellandrene	1,002	4.3	0.2	2.0	1.0	Tr	0.1	tr	0.3	0.1	2.0
δ-3-carene	1,004	0.2	0.1	0.2	0.3	-	-	tr	0.2	tr	Tr
p-cymene	1,026	15.9	16.1	24.8	41.8	2.4	8.2	1.6	58.1	1.7	16.5
Limonene	1,024	5.6	4.1	4.0	6.2	5.7	4.6	8.4	-	0.2	6.8
1,8-cineole	1,032	24.9	49.7	3.3	3.3	31.6	24.2	48.5	6.5	0.3	31.0
cis-β-ocimene	1,043	0.5	Tr	Tr	0.0	-	-	0.1	-	tr	0.0
γ-terpinene	1,055	1.3	0.2	1.2	0.8	0.1	-	0.2	0.5	tr	0.1
Terpinolene	1,083	0.6	0.1	0.6	0.6	0.1	tr	0.1	0.2	0.3	4.2
α-terpineol	1,186	11.2	4.1	7.2	2.9	4.8	3.9	2.4	2.1	0.9	9.9
2-caren-10-al		-	-	-	-	-	-	-	0.4	-	-
Linalool	1,095	0.8	-	Tr	0.8	-	0.1	-	0.5	1.0	1.5
α-campholenal	1,127	-	0.1	-	0.1	0.1	0.1	-	-	-	-
Trans-pinocarveol	1,135	Tr	2.0	0.2	-	2.2	1.3	0.9	0.1	-	-
Camphene Hydrate	1,145	-	0.1	0.1	0.1	0.2	0.2	tr	-	-	-
Citronellal	1,148	-	-	-	-	-	-	-	-	tr	-
2(10)-pinen-3-one	-	-	1.0	0.1	0.1	0.8	0.5	0.4	-	-	-
Borneol	1,165	0.1	1.0	0.7	1.9	3.2	1.9	0.4	0.1	-	-
p-menthan-3-ol	1,167	-	-	-	-	-	-	-	-	0.1	-
p-cymen-8-ol	1,183	0.2	0.5	0.3	0.8	0.3	0.4	0.2	3.6	0.4	19.7
2-Isopropenyl-5-methylhex-4-enal	1,198	-	-	Tr	-	-	-	-	-	0.1	0.1
Citronellal	1,223		-	-	-	-	-	-	-	0.4	-
Carvone	1,245	0.1	-	-	-	-	-	-	-	-	-
p-cymen-3-ol	1,287	0.2	0.1	0.1	0.1	Tr	0.1	0.4	0.4	-	-
α-cubebene	1,345		-	-	-	-	-	-	-	tr	-
3-allyl,2-methoxy phenol	-	-	-	-	-	-	-	-	-	0.3	-
Nerol acetate	1,365	0.1	-	-	-	-	-	0.1	0.2	tr	-
α-Copaene	1,374	-	-	-	-	Tr	-	-	0.1	Tr	-
Methyl cinnamte	1,379	-	-	-	-	-	-	-	-	11.4	-
Fenchol	-	-	-	-	-	-	-	tr	-	-	-
Eugenol methyl ether	1,402	0.2	0.1	0.2	0.5	-	0.1	0.8	0.7	82.3	1.1
β-Eudesmene	-	-	-	-	-	Tr	-	-	-	-	-
Caryophyllene	1,408	Tr	0.4	Tr	Tr	Tr	-	-	0.1		-
β-Caryophyllene	1,417	Tr	Tr	Tr	0.3	0.2	0.2	0.1	-	tr	0.1
Humulen- (IV)	-	-	-	-	-	0.1	-	-	-	-	-
Germacrene D	1,484	-	tr	-	0.1	Tr	tr	0.5	0.2	0.2	0.1

Table 2. Continued.

Compounds	RI	Content (%)									
		C. vim	*E. cre*	*E. kit*	*E. mel*	*E. mic*	*E. pru*	*E. rud*	*E. ter*	*M. bra*	*M. qui*
Epiglobulol	1,585	0.1	2.5	0.5	6.9	2.5	9.7	1.0	-	tr	0.9
Germacrene B	1,559	-	tr	-	2.8	-	-	tr	0.2	tr	-
(*E*)-nerolidol		-	-	Tr	-	-	-	-	-	-	-
Caryophyllene oxide	1,582	Tr	-	-	-	-	-	-	-	tr	0.4
ar. Tumerone	-	0.2	-	-	-	-	-	-	-	-	-
Di-epi-α-cedrene	-	-	-	-	Tr	-	-	-	-	-	-
Total		98.2	99.5	71.5	95.8	85.9	85.7	97.8	74.5	99.9	98.7
Monoterpene hydrocarbons		43.9	21.8	34	33.3	37.5	34.9	41.3	1.9	0.8	17.4
Oxygenated monoterpenes		37.2	58	11.6	9.2	42.9	32.2	52.7	9.9	2.6	42.4
Sesquiterpene hydrocarbons		0.3	0.4	tr	3.2	0.3	0.2	0.6	0.6	0.2	0.2
Oxygenated sesquiterpenes		0.3	2.5	0.5	6.9	2.5	9.7	1.0	-	tr	1.3
Aromatic compounds		16.5	16.8	25.4	43.2	2.7	8.7	2.2	62.1	96.2	37.3
Others		-	-	Tr	-	-	-	-	-	0.1	0.1

RI = Retention Indices relative to C9-C25 *n*-alkanes on the DB-5 column; tr = trace < 0.05 %
*Plant abbreviations: *Eucalyptus kitsoniana*: *E. kit*; *Eucalyptus crebra*: *E. cre*; *Eucalyptus melanophloia* : *E. mel*; *Eucalyptus microtheca*: *E. mic*; *Eucalyptus pruinosa*: *E.pru*; *Eucalyptus rudis*: *E. rud*; *Eucalyptus tereticornis*: *E.ter*; *M. bracteata*; *M. brac*; *M. quinqueneveria*: *M. qui; Callistemon viminalis : C. vim;* RI : Retention Index; -: not detected; tr; traces.

Cluster analysis was carried out to examine the differences and similarities among the studied species on the basis of chemical compositions. The dendrogram sorted the essential oil samples into three clusters: C1, C2, and C3 (Fig. 1). C1 consisted of *M. bracteata* containing phenylpropanoids (eugenol methyl ether); C2 consisted of *E. tereticornis, E. melanophloia,* and *E. kitsoniana* that predominantly contained *p*-cymene; and Class C3 connects three genera viz. *Callistemon*, *Eucalyptus,* and *Melaleuca*. It consisted of *E. rudis, E. microtheca, E. crebra, E. pruinosa, C. viminalis,* and *M. quinqueneveria,* and is characterized by high 1,8-cineole content.

Fumigant Activity

The essential oils from *Callistemon, Eucalyptus,* and *Melaleuca* species exhibited significant fumigant toxicity against *T. castaneum* (Table 3). Conspecific comparison of essential oils of *Melaleuca* species showed variable activities against *T. castaneum* ($F_{1, 4}$ = 22.14, p = 0.009). *Melaleuca bracteata* (LC_{50} = 200.3 µl/L) was more toxic than *M. quinquenervia* (LC_{50} = 236.3 µl/L). The essential oils from *Eucalyptus* species also caused different mortalities in *T. castaneum* ($F_{6, 14}$ = 16.21, p = 0.000). The LC_{50} values of *Eucalyptus* species ranged from 146.3-1046.1 µl/L. *Eucalyptus rudis* was the most lethal fumigant against *T. castaneum* (LC_{50} = 146.3 µl/L), while *E. pruinosa* was the least toxic (LC_{50} = 1046.1 µl/L) among the evaluated *Eucalyptus* species. The fumigant potential of *C. viminalis* could not be compared, being the single selected species from *Callistemon* genus. However, the overall comparison of essential oils from *Callistemon*, *Eucalyptus,* and *Melaleuca* genera showed high susceptibility of *T. castaneum* toward all of them.

A review of literature showed few reports on insecticidal activity of selected Myrtaceae species against different insects. Ndomo et al. 2010 studied fumigant and contact toxicities of *C. viminalis* essential oil against *Acanthoscelides obtectus* and *Callosobruchus maculates* adults [55]. Various researchers have evaluated insecticidal activities of *E. tereticornis* essential oil from Argentina, Cuba, and Korea against *Anopheles stephensi, Aedes aegypti,* and *Drosophila melanogaster* [55-58]. Fumigant toxicity of *E. rudis* essential oils from Tunisia has been investigated against *Ectomyelois ceratoniae* [47].

The fumigant activities of evaluated essential oils could be attributed to their major volatile components (eugenol methyl ether, *p*-cymene, 1,8-cineole, α-pinene). Eugenol methyl ether has been reported to possess insecticidal/acaricidal activity against several insects/mites [59-60]. *p*-Cymene has also been implicated as an insecticide in some studies [61]. α-Pinene has been known to show fumigant toxicity against *Sitophilus oryzae*, *T. castaneum*, and *Triboleum confusum* [62-63]. The effectiveness of 1,8-cineole has been documented against many stored-product insects [22]. This implies that the higher the 1,8-cineole content, the lower the LC_{50} values and vice versa. However, LC_{50} values of *E. rudis, E. microtheca, E. crebra, C. viminalis,* and *M. quinquenervia* did not follow an inversely proportionate relationship with 1,8-cineole content. This suggests that other components present even in minor amounts contribute toward the insecticidal activity. The minor components in the evaluated oils (linalool, limonene, δ-terpinene, and α-terpineol) have

Table 3. Fumigant toxicity of essential oils from different species of Myrtaceae after eight hours.

Essential oils	LC50 (µl/L air)	LC_{95} (µl/L air)	95% Fiducial limits of LC_{50}	Fit of Probit line			
			LCL – UCL (µl/L air)	Slope	X^2	Df	P
C. viminalis	257.76	405.29	368.96-463.89	5.14	7.84	4	0.097
E. crebra	289.28	392.56	364.99-443.18	8.25	1.79	4	0.618
E. kitsoniana	314.23	440.89	407.23-502.58	7.30	3.07	3	0.381
E. melanophloia	615.09	800.45	761.68-867.54	9.77	1.67	5	0.892
E. microtheca	321.15	528.77	466.49-664.01	4.55	4.92	3	0.178
E. pruinosa	1,046.13	1,532.67	1,369.92-2,139.50	6.33	0.01	2	0.997
E. rudis	146.35	243.83	216.42-298.03	4.420	0.56	2	0.754
E. tereticornis	601.42	973.09	862.35-1,199.72	4.76	5.96	3	0.114
M. bracteata	200.32	281.84	260.64-321.37	7.24	11.53	3	0.009
M. quinquenervia	236.33	449.70	376.30-603.75	3.26	1.32	3	0.724
Pyrethroid (Fury)	186,750	437,520	157,980-217,670	2.19	7.04	4	0.134

been known for their toxicities against stored pests [64]. Thus a chemical composition and insecticidal-activity relationship deduced from our results demonstrates that the insecticidal activity of essential oils is attributed to the presence of major and minor components in the essential oils.

The low activity of *E. pruinosa* essential oil, although rich in 1,8-cineole and α-pinene, could be explained on the basis of the low volatility of its active compounds as these toxic compounds exhibit their volatile properties during bioassays. Similarly, the good activity of *M. bracteata* essential oil with eugenol methyl ether, a phenylpropanoid derivative, and low volatile compound in comparison to terpenoids could be due to partial inhalation or ingestion through the insect body [65]. This indicates that the bioactivities of essential oils are possibly due to mixtures of constituents that have multiple effects on multiple targets. The LC_{50} values of studied essential oils were lower than positive control (pyrethroid), implicating their efficacy as fumigants.

Conclusions

The above findings suggest that the fumigant toxicities of essential oils of *Eucalyptus*, *Melaleuca,* and *Callistemon* genera were quite promising. They showed potential for use as natural fumigants for the control of the stored grain pest (*T. castaneum*) as an alternative to currently used synthetic fumigants. An important aspect in the commercial application of plant-essential oil-based pesticides is the availability of sufficient quantities of plant material. These selected species are fast growing and widely cultivated in Pakistan. Moreover, the essential oil yield of the most toxic species in the present study (*E. rudis, M. bracteata, M. quinquenervia,* and *C. viminalis*) is fairly good, supporting feasibility of their exploitation as natural fumigants. However, further investigations are needed in the future to determine the safety of essential oils to humans.

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