Repellent and adulticidal efficacy of essential oils of two indigenous plants from Myrtaceae family against *Lasioderma serricorne* F.

K. Anju Viswan*, V.K. Rahana and E. Pushpalatha**

ABSTRACT

Several plant extracts and essential oils are being used as insecticides to reduce environmental pollution and risk of resistance. The aim of this study is to evaluate the repellent and adulticidal properties of essential oils of two indigenous plants from Myrtaceae family, *Melaleuca leucadendron* L. and *Callistemon citrinus* Curtis against the Coleopteran beetle *Lasioderma serricorne* F. included in the family Anobiidae. In the present study repellent activity, direct contact toxicity and indirect toxicity of the two essential oils were evaluated. Significant pest repellency was demonstrated. Undiluted concentrations of essential oils showed 97.5% repellency after 1 hour of exposure. Both the repellent and adulticidal activity was highly dependent upon the oil concentration and exposure time. In direct contact toxicity test both *M. leucadendron and C.citrinus* essential oil exhibited 100% mortality within 1hour. In indirect method, both the essential oils exhibited 100% mortality in a time period of 6 hours. From these observations it is clear that these oils have potential as active insecticides against cigarette beetle.

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Key words: *Lasioderma serricorne, Melaleuca leucadendron, Callistemon citrinus* adulticidal activity, repellent activity.

INTRODUCTION

Indiscriminate uses of synthetic insecticides lead to many negative consequences like environmental pollution and the cause of resistance. To overcome the problem of development of resistance in insects, attention is being given to natural products because of their biodegradable nature. Aromatic plants are most efficient insecticides and essential oils constitute the bioactive fraction of plant extracts. Several experiments on essential oils like eugenol and olive oils proved their biological activity against stored seeds insect pests. Repellency of 48 essential oils used commonly for the flavoring of food was reported by Hori in 2003. The *Laurus nobilis* essential oil shows repellent activity against cigerette beetle (Jauda *et al.*, 2011). Essential oils and extracts from plants were considered as valuable alternative for insect control agents (Govindarajan *et al.*, 2008; Cetin *et al.*, 2011). Forty essential oils from Australian plants were evaluated against mosquitoes, march flies and sandflies. The most effective of these were *Dacrydium franklini*, *Backhousia myrtifolia*, *Melaleuca bracteata* and *Zierria smithii* (Penfold and Morrison, 1952). Repellency properties of nepetalactone isolated from *Nepeta cataria*, against 17 species of insects were reported by Eisner (1964). Alpha pinene, limenone terpinolene, citronellol, camphor, dolichodial, teucrein and isoborneol (Perttunen, 1957; Moore, 1974; Takikawa *et al.*, 1998; Baske *et al.*, 2003). The essentials oil from *M. leucadendron* exhibit significant irritant and repellent properties against *Ae.aegypti* (Noosidum *et al.*, 2008).The volatile oil of *M. leucadendron* possess antimicrobial and antifungal properties (Farag *etal*, 2004). Amer and Mehlhorn evaluated 41 plants against the yellow fever mosquito, malarial vector, filariasis vector and proved the ability of *M. leucadendron* as one of the five best repellent activity plants. *Callistemon citrinus* shows antibacterial activity (Oyedeji *et al.*, 2009). The essential oil from *C. citrinus* leaves were found to be toxic against *Callosobruchus maculates* (F) when applied by fumigation (Sohani *et al.*, 2013). Chloroform extracts of *C. citrinus* reduced the oviposition and larval survival rate when tested against sugarcane stalkborer (Mumtaz *et al.*, 2012).

Target organism, the cigarette beetle *Lasioderma serricorne* is a serious pest of tobacco leaves, cigarettes, cocoa beans, cereals, oil seeds, pulses, spices, dried fruits etc. It occurs throughout the tropical and subtropical areas and breeds on a wide variety of commodities including plant and animal materials and infests during storage and manufacturing (Howe, 1957; Ashworth, 1993). This is the most serious insect pest of stored tobacco (Ryan, 1995). The present study envisages to test the repellency, direct contact toxicity and fumigant toxicity of essential oils of *Callistemon citrinus* and *Melaleuca leucadendron* against *Lasioderma serricorne*.

MATERIALS AND METHODS

Rearing of insect

Cigarette beetle, *L. serricorne* was reared in 1 litre glass container containing turmeric powder and that was covered by a fine mesh of cloth for ventilation. The rearing conditions were darkness in 25±1°C and 65± 5% relative humidity. Adult insects 7-10 day old which are more sensitive to oil treatment were used for the tests. All experiments were carried out under the same environmental conditions as in the culture.

Preparation of essential oils

Leaves of *M. leucadendron* and *C. citrinus* collected from Calicut University campus, washed and shade dried at room temperature for a week. Essential oils were extracted by hydro distillation of dried leaves (100g of each sample in 500mL of distilled water) using a modified Clevenger apparatus for 5 hrs. The oils were dried over anhydrous sodium sulfate and stored in sealed glass vials in refrigerator.

The oil compositions were analyzed by gas chromatography mass spectrometry (GC-MS). The essential oils were analyzed using Agilent Technologies 6850 Network GC system equipped with HP5MS column (30m x 0.25mm and film thickness 0.25um) and MS 5975 CVLMSD with triple axis detector Agilent Technologies. Initial temperature of the oven was 60°C and maximum temperature 325°C and equilibration time was 0.50 min. Ramps were done in the rate 3 for final temperature 150°C and rate 5 for final temperature 250°C. Total running time was 51 minute. A sample of 1µl was injected using split mode (split ratio 50:1). All quantifications were carried out using a built in data handling programme provided by the manufacturer of the gas chromatograph. The composition was reported as a relative percentage of the total peak area. The identification of the essential oil constituents was based on a comparison of their retention times to published data and spectra of authentic compounds. Compounds were further identified and authenticated using their mass spectra compared to C:\ Database\ NIST08.L search libraries.

Repellency bioassay

Repellency assays were done according to the experimental method described by Jilani and Saxena (1990). Whatman filter paper (radius- 5cm) was cut in half. Test solutions were prepared by dissolving the essential oils in alcohol. Each solution was applied to half a filter paper disc as uniformly as possible with micropipette. The other half treated with only alcohol acted as control. The treated and control halves were attached to their opposites using adhesive tapes and placed in petridishes. 20 adult beetles were released at the centre of each filter paper disc. The dishes were then covered and sealed with Para films. Three replications were used for each concentration. Observation on the number of insects present on both the treated and untreated halves was recorded after 1hr, 6hr and 12hr respectively. Three trials were done for each concentration. Numbers of cigarette beetle present on treated and untreated portions of the experimental paper halves were recorded after various hours of exposure. Percentage repellency (PR) was calculated (Nerio *et al.*, 2009).

Contact toxicity

The contact toxicity of both oils against cigarette beetle was evaluated on Whatman filter paper disc, which were treated with essential oils. The treated filter papers were placed in glass petridishes. 20 adults were introduced in to the petridishes and that were keep in darkness. In the control groups only water was applied in the filter papers. Each treatment was replicated for three times. Insect mortality was recorded each hour.

Indirect contact toxicity or Fumigant toxicity

Essential oils were applied on Whatman filter paper stripe. Treated filter papers were placed at the bottom of 250ml glass jars. 20 adults were placed in small plastic tubes (3.5cm diameter and 5cm height) with open ends covered with clothes mesh. The tubes were hung at the geometrical centre of the glass jar and then sealed with air tight lids. In the control groups filter paper treated only with water was used. Mortality was observed in each hour. Percentage mortality was calculated.

RESULTS AND DISCUSSION

Table 1 shows data on percent repellency observed using the essential oils of the selected plants tested against cigarette beetle. Our results showed that the essential oils of both *M. leucadendron* and *C. citrinus* have significant adulticidal and repellent activity against cigarette beetle. Comparing the results observed on repellency bio assays using the selected essential oils a slight increase in the activity is found for *C. citrinus* than *M. leucadendron*. The activity of repellency was dependent upon the concentration and exposure time.

Table 1. Data on percent repellency observed using the essential oils of the selected plants tested against cigarette beetle.

Conc. (%)	% repellency in different duration		
	1hr	6 hr	12hr
Callistemon citrinus			
10	68.0±1.0	52.0±1.7	45±1
50	85.0±2.6	70.0±1.0	57±2
100	97.5±1.8	82.0±2.0	70±2
Melaleuca leucadendron			
10	66.0±1.0	50.8±0.34	44.5±2.3
50	84.0±2.0	68.5±1.8	56.2±1.9
100	97.5±1.32	81.6±1.9	69.8±0.92

Contact toxicity and fumigant toxicity of the essential oils of the selected plants were conducted for two different concentrations. In direct contact toxicity test both *M. leucadendron and C.citrinus* essential oils of 100% concentration exhibited 100% mortality within 1hour. Data on contact toxicity and fumigant toxicity of *C. citrinus* and *M. leucadendron* essential oils against *L. serricorne* is provided in fig.1 and 2. Maximum 4 hours is needed for the 100% mortality of insects while using *C. citrinus* and 5 hrs for *M. leucadendron* in direct contact method (Fig.1). In direct contact assay the essential oils kills the insects faster than the fumigant assay.

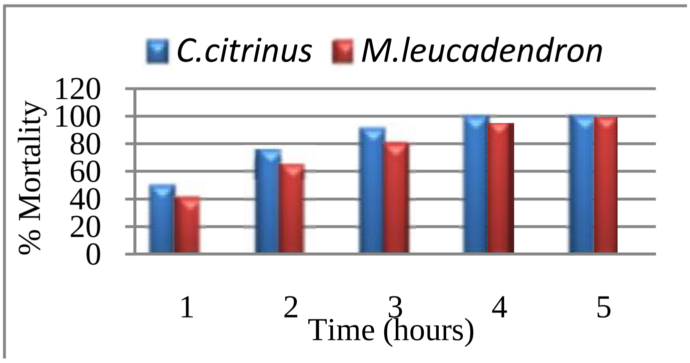

Fig1. Contact toxicity of *C. citrinus* and *M. leucadendron* (10%) essential oils against *L. serricorne.*

In indirect method, both the pure essential oils exhibited 100% mortality in a time period of 6 hours. Whereas 10% concentration of the essential oils of the selected plants shows 100% mortality within 24 hrs of exposure period (Fig. 2).

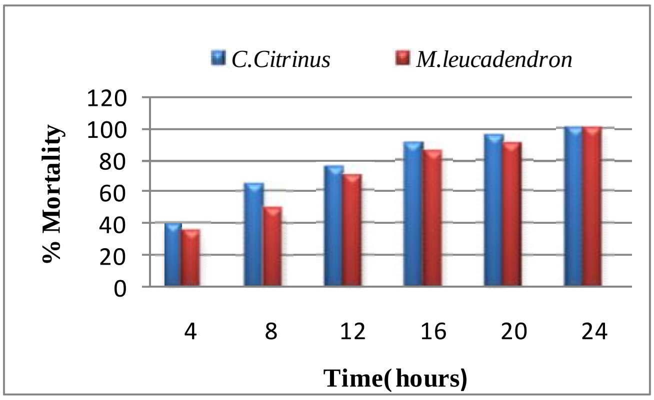

Fig 2. Fumigant toxicity of *C. citrinus* and *M. leucadendron* (10%) essential oil against *L. serricorne.*

The results showed that both the essential oils have active biocontrol agents against *L. serricorne*. Table 2 and 3 provide data on chemical constituents of the essential oil *C. citrinus* and *M. leucadendron* with percentage of abundance and retention time. The GCMS analysis showed that *C. citrinus* contains terpineol, duroquinol, eucalyptol, selinine and pinene as major components. Terpineol constitutes 32.68% of the *C. citrinus.* The main compounds present in M. *leucadendron* are selenene, pinene, carophyllene, terpineol and limenone. The major compound in *M. leucadendron* was selinene. The common compounds present in *C. citrinus* and *M. leucadendron* were a pinene, carophyllene, terpineol, limenone, terpene4ol, cymene, spathulenol, selinene.

Genus Eucalyptus and Family Myrtaceae was identified as botanical insecticides (Papachristo and Stamopoulos, 2002; Negahban and Moharraonipour, 2002). Forty one plant extracts and 11oil mixtures were evaluated against mosquitoes. It was reported that five most effective oils were those of *Listea cubeba*, *Melaleuca leucadendron*, *Melaleuca quinquenervia*, *Viola odorata* and *Nepeta cataria* shows maximum repellency against mosquitoes (Amer and Mehlhorn, 2006). The fumigant and repellent effects of *Ocimum gratissimum* oil and its constituents were potential alternatives to synthetic fumigants in the treatment of *Sitophilus oryzae*, *Tribolium cactaneum*, *Oryzaephilus surinamensis*, *Rhyzopertha dominica* and *Callosobruchus chinensis* (Ogendo *et al.*, 2008). The insecticidal and repellent properties of *C.citrinus* against callasobruchus maculatus were described by Sohani *et al.* (2013).

Table 2. Data on chemical constituents of the essential oil *C.citrinus* with percentage of abundance and retention time:

Compound	%	RT
Terpineol	32.68	14.74
Duroquinol	16.86	28.19
Eucalyptol	7.37	8.41
β- Selinene	4.74	30.79
α- Pinene	4.39	5.56
Spathulenol	3.74	32.32
Globulol	1.57	30.46
Linalool	1.46	10.84
Geraneol	1.3	17.17
Terpene-4-ol	1.3	13.93
Carophyllene	1.01	23.96
P- Cymene	0.84	8.15
Eugenol	0.74	21.43
D-Limenone	0.65	8.29
Farnesol	0.53	34.80
Aromadendrone	0.50	25.62
Phytol	0.49	43.47
Alloaromadendrone	0.43	31.13

In conclusion the study clearly demonstrated that the essential oils from both *C. citrinus* an**d** *M. leucadendron* are showing both very high repellent and adulticidal properties against cigarette beetle.

Table 3. Data on Chemical constituents of the *M. leucadendron* essential oil with percentage of abundance and retention time:

Compound	%	RT
γ –Selinene	39.66	31.17
α- Pinene	9.59	5.59
Carophylline	9.07	24.04
Terpineol	5.97	14.62
Limonene	3.2	8.41
α- Gurjunene	3.11	27.09
Ledol	2.63	2.63
Terpene-4-ol	1.86	13.97
P- Cymene	1.73	8.18
β- Pinene	1.35	6.71
β- Eudesmol	1.21	32.86
Linalool	1.15	10.86
Spathulenol	1.08	25.66
γ- Terpene	0.93	9.33
Borenol	0.60	13.46
β- Selinene	0.52	26.68
Camphene	0.47	5.94
Carene	0.47	10.39

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