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# **Research Article**

# REPELLENCY OF ESSENTIAL OIL EXTRACTED FROM CYMBOPOGON CITRATUS AGAINST THE NUISANCE HOUSEHOLD PEST, LUPROPS TRISTIS FAB. (COLEOPTERA: TENEBRIONIDAE)

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## ARTICLE INFO

## ABSTRACT

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Essential oils extracted from aromatic plants have been considered as an important source of natural pesticides. The anti insect activity of essential oil hydrodistilled from lemongrass (*Cymbopogon citratus*) was tested against the rubber litter beetle, *Luprops tristis* under laboratory conditions. Repellent activity of various doses (2, 4, 8, 16  $\mu$ l) of essential oil from *C. citratus* against *L. tristis* adults during different time periods (0.5 h, 1 h, 2 h, 4 h and 8 h) was performed by area preference method. The assays revealed the pest insects' behavioral responses towards the volatile components of essential oil from test plant. Results indicated that the oil exhibited potent repellent activity against *L. tristis* adults (P<0.001) [df=3; F=104.523]. The difference among different times of exposure (P<0.001) [df=4; F=82.028] was also highly significant. The chemical constituents of the essential oil were analyzed by gas chromatography–mass spectrometry.

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# INTRODUCTION

Luprops tristis, the rubber litter beetle, is a nuisance household pest for farming communities particularly in the rubber plantation areas of Kerala. The foray and aggregation of these beetles causes considerable annoyance to most householders along rubber plantation belts. The sustained occurrence and attraction of these beetles towards light, during night is a serious problem for local people. Clusters of several hundreds to thousands crawl into the living rooms and fall off into beds and food from ceilings, and when disturbed, they release an irritating odoriferous phenolic secretion that causes burn to the skin (Sabu et al., 2008). This beetle poses a serious threat to the people living in the vicinity of rubber plantations as it interferes with his welfare and convenience, particularly in summer seasons. They not only cause problems through direct nuisance effects but are also problematic in terms of the use of pesticides directed at them, which can also have human health impacts. This has led to think of measures for their effective suppression.

The problems due to synthetic pesticides and their residues have increased the need for effective biodegradable insecticides with greater selectivity. The natural plant products extracted from plants effectively meet this criterion and have enormous prospective to influence modern agrochemical research. The use of botanical pesticides is now promising as 'phytochemical insecticides' and 'green-chemical insecticides' (Vendan, 2016). Botanicals degrade more rapidly than most chemical pesticides, and are, therefore, considered relatively environment friendly and less likely to kill beneficial pests than synthetic pesticides with longer environmental retention.

Plant essential oils and their constituents exert insecticidal effects or reduce and interrupt insect growth at several life stages. They are safe and ecofriendly. They are more attuned with the environmental components than synthetic pesticides (Isman and Machial, 2006). Plant essential oils are produced commercially from many plant species. The oils are mainly composed of complex mixtures of monoterpenes, biogenetically related phenols and sesquiterpenes. Interests in the use of essential oils were renewed with emerging demonstration of their fumigant and contact insecticidal

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activities to a broad range of pests in the 1990s (Isman, 2000). The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference of these oils with the neuromodulator octopamine (Kostyukovsky *et al.*, 2002) and with GABA-gated chloride channels (Priestley *et al.*, 2003). The purified terpenoid constituents of essential oils are moderately toxic to mammals, but, with few exceptions, the oils themselves or products based on oils are mostly nontoxic to mammals, birds and fishes (Stroh *et al.*, 1998), thereby justifying their placement under "green pesticides".

In the present study, the insect repellent effect of essential oil hydro-distilled from *C. citratus* against rubber litter beetle, *L. tristis* was investigated under laboratory conditions, so that information thus gathered may be utilized for the management of this pest under field conditions.

## **MATERIALS AND METHODS**

#### Insect collection

Newly invaded pre dormancy *L. tristis* adult beetles, collected from an infested residential building were used to assess the behavioral responses (repellent effects) of essential oil from *C. citratus*. Insects were maintained at optimum conditions of temperature  $(27+0.5^{\circ}C)$  and relative humidity  $(70\pm5^{\circ})$ .

#### Essential oil extraction

Fresh leaves of lemon grass were cut into small pieces and subjected to hydro-distillation using a modified Clevengertype apparatus. Condition of extraction was: 50 g of leaves + 600 ml distilled water and 5 h of distillation. Volatile oils were collected in the reservoir on top of the aqueous layer. After extraction, the essential oil was transferred to a glass vial, and dehydrated by treating with anhydrous sodium sulphate. The quantity of essential oils obtained were averaged over three experiments and calculated according to fresh weight of the plant materials. The distilled essential oil was stored in amber coloured glass vials in a refrigerator at 4 °C until being used for the experiments.

### Repellency bioassay with 'choice chamber'

Experiments were conducted in a 'choice chamber' by area preference test as described by Mc Donald et al. (1970). Test arena consisted of Whatman no.1 filter papers, cut into two halves. Different test solutions were prepared by dissolving 2, 4, 8, 16 µl of essential oil in 0.5 ml acetone. Each solution was applied to a half filter paper disc as uniformly as possible with a micropipette. The other filter paper halves were treated with acetone alone. The essential oil treated and untreated half discs were air dried to evaporate the solvents completely. Full discs were then remade by attaching treated halves to untreated halves of the same dimensions with cello tap. Each filter paper was placed in a petri dish. The circular edge was pasted on to the Petri dishes to prevent insect moving underside of the filter paper, and 10 adults of mixed sex of Luprops beetles were released separately at the center of each filter paper disc. Each 'choice chamber' was then covered with the lid of the Petri dish inside of which was pasted a correctly fitting black paper. Each treatment was replicated 6 times. The number of adult

beetles present on control (Nc) ant treated (Nt) stripes were recorded after 0.5 h, 1 h, 2 h, 4 h, and 8 h intervals.

Percentage repellency was calculated for each replicate at the above mentioned intervals and the means of different treatment periods were taken. All repellency assays were carried out in laboratory at  $27\pm2^{\circ}$ C; and  $75\pm5\%$  relative humidity.

# Analysis of essential oils by Gas-Chromatography and Mass spectrometry (GC-MS)

1  $\mu$ l of the analyte was injected in splitless mode into a Agilent model GC6890 N coupled with a HP 5975 B mass selective detector. HP 5 column was used with helium as a carrier gas. During the run the temperature programming was as follows 40 °c for- 3 min and rise at 5 °C / min to 280 ° C, iso therm for 10 min; Post run 10 min at 300 ° C. Retention time and mass spectra were compared with the libraries (MP and NIST) and co injecting with standards.

# **RESULT AND DISCUSSION**

Percentage of repellency due to the treatment of various doses (2, 4, 8 and 16  $\mu$ l) of essential oil from *C. citratus* in choicechamber bioassay is presented in table I. Statistical analysis of the experimental data was performed using ANOVA for testing the significance of repellent effects of the essential oil on the test insect (Table II). All doses of essential oil showed significant repellent activity against *L. tristis* adults (P<0.001) [df=3; F=104.523]. The difference among different times of exposure (P<0.001) [df=4; F=82.028] was also highly significant (Table.II). Figure I presents the time-course repellent activities of various doses of *C. citratus* essential oil against *L.tristis* adults. The significant difference in mean repellency among different time periods and different doses were tested using Duncan's Multiple Comparison Test (DMCT).

 Table – 1 Repellency of various doses of essential oil from Cymbopogon citratus against L.tristis adults

	Corrected percentage repellency rates at different durations of							
Dose(µl)	exposures (hours)							
	.5	1	2	4	8			
2	$31.74 \pm 7.44$	$35.71{\pm}8.58$	$45.24{\pm}5.32$	$37.3 \pm 3.97$	$16.67 \pm 7.45$			
4	$59.13 \pm 6.27$	$59.13{\pm}6.27$	$63.09{\pm}3.77$	$59.13{\pm}6.27$	$35.71 \pm 8.58$			
8	$84.98 \pm 6.72$	$84.98{\pm}6.72$	$87.96{\pm}\ 4.57$	$86.11{\pm}3.93$	$45.24 \pm 5.32$			
16	$93.98{\pm}4.21$	$96.29{\pm}2.34$	$96.29{\pm}2.34$	$92.13{\pm}4.08$	$69.05{\pm}3.77$			
			ē.					

Values are expressed as means SEMs±(n=6) Sample: 10 insects × 4 doses ×6 replications =total 240 insects

**Table II** ANOVA of data showing the repellent effects

 *Cymbopogon citratus* on *L.tristis* adults

Source of variables	df	MS	df Error	MS Error	F	P Level
Dose	3	23721.96	231.00	226.95	104.52	<0.001**
Time	4	18616.64	231.00	226.95	82.03	<0.001**

\*\*Shows very high significance

From the results, it was found that the time periods 0.5 h, 1 h, and 2 h forms homogenous group while 4 h and 8 h are different from each other and also from other time periods. DMCT to find out difference in mean repellency due to

different doses shows that all doses are different from each other in mean repellency.

Fig. I Time-course repellent activities of various doses of *Cymbopogon citratus* essential oil against *L.tristis* adults



The repellency observed in our bioassay was found to be significantly dose-dependent (p<0.001). It was found that the rate of repellency of the insects increased with increase in dose of oil during different periods of treatment. The difference among different times of exposure was also highly significant (P<0.001). From the data of repellency (Figures I) it seems apparent that the essential oil of various doses maintained more or less higher activity up to 2 h of exposure increased. However, these effects were found to be statistically significant (Tables II). From the results, it is thus inferred that the test oils contain several volatile compounds having various degrees of volatility. The initial high repellency during smaller duration of exposure may be due to presence of highly volatile low molecular weight compounds in the oil.

The major chemical constituents of essential oil were identified using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Nineteen constituents were identified from essential oil from lemongrass with Geaniol (64.37%) and Carveol (46.25%) accounting for major portion of the constituents identified. The other constituents identified include 6 methyl 5 heptene 2 one (0.31%), Eucalyptol (0.40%),  $\beta$  pinene (0.81%), Cis Verbenol(0.59%), L. Borneol(1.89%), Decanal(0.41%), 3 carene (0.33%), L.P. pinene(3.13%), Cyclosalivene (0.20%), B. pinene (1.82%),  $\beta$  Elemene(0.52%),  $\beta$  caryophyllene (6.11%),  $\alpha$  caryophyllene (0.85%),  $\alpha$  curcumene (0.22%),  $\beta$ cubenene (0.92%), Zingiberene (0.20%), and  $\gamma$  cadenene (2.96%). Additional minor compounds were detected but not identified as their concentrations were too small to be analyzed with mass spectroscopy.

The results of the present investigation are in agreement with the earlier findings on the repellent effect of *C. citratus* on various insect pests. Essential oils isolated from *C. citratus* were tested for repellent activity and contact toxicity against *Tribolium castaneum* by Verbel *et al.*, (2010) and suggested this plant as a potential candidate for insect repellents. The essential oil of *Cymbopogon* species (*C. citratus*, *C. nardus* and

*C. martini*) were very effective against mosquito species such as, *Anopheles culicifacies, Anopheles quinquefasciatus* etc. (Ansari and Razdan, 1995; Senthilkumar *et al.*, 2009; Amer and Mehlhorn, 2006; Trongtokit *et al.*, 2005). Repellent effect of *C. citratus* against the maize weevil, *S. zeamais* was reported by Parugrug and Roxas (2008). Ethanol extracts of *C. citratus* were tested against rice weevil, *Sitophilus oryzae* to determine their insecticidal properties (Saljoqi *et al.*, 2006). The results revealed that the tested materials had repellent and deadly effects against the pest as compared with the control. Geranial and neral were described as the major mosquito-repellent metabolites of an essential oil isolated from *C. citratus* leaves (Soares and Uchida, 1998). Probably, these compounds could be responsible for higher bioactivity of *C. citratus* essential oil against *L.tristis*.

Our results get support from previous studies that tested repellency of lemongrass oil against household and other insects. Lemongrass oil presented repellent activity against Periplaneta americana, the domestic cockroach (Ahmad et al., 1995). This oil showed excellent results both in direct and indirect application, against the dipteran species that cause cutaneous myiasis (Subramanian and Mohanan, 1980). It has both repellant and toxic effects against arthropods. A methanol extract of lemongrass shows various degrees of repellency and larvicidal effect against a malaria vector, Anopheles arabiensis. Karunamoorthi et al. (2010) also reported the use of essential oil of lemongrass as a repellent. It can provide protection against bites of Anopheles darlingi and Mansonia sp. The screening for bioactive principles from several Chinese medicinal herbs showed that the essential oil of Cymbopogon distans aerial parts possessed strong repellency against the booklouse, Liposcelis bostrychophila, and the rust red flour beetle, T. castaneum (Zhang et al., 2011). Result showed that geraniol and citronellol were strongly repellent against the booklouse, L. bostrychophila, whereas citronellal, geraniol and citronellol exhibited stronger repellency against the red flour beetle. There are many reports, where citronellol and transgeraniol were demonstrated to repel mosquitoes, sand flies, human body lice, aphids, and stored product insects (Mumcuoglu et al., 1996; Muller et al., 2008; Hieu et al., 2010). These findings suggest that the essential oil show potential for development as a natural repellent for household applications against L. tristis.

The results obtained in the present study are encouraging. The GC-MS analysis of the essential oil revealed that the oils are comprised mainly of monoterpenoid and sesquiterpenoid hydrocarbons many of which have been extracted from other plants and reported to possess anti insect properties against various stored product pests (Tapondjou et al., 2005; Stamopoulos et al., 2007; Sahaf et al., 2007). In a repellency bioassay against stored product pests, two volatile compounds  $\alpha$ -pinene and  $\beta$ -caryophyllene repelled *T. castaneum* adults significantly even at 0.025% concentrations (Chaubey, 2012). The toxicity and repellency of the corn mint, Mentha arvensis oil to American cockroaches and German cockroaches were determined by Appel et al. (2001). Corn mint oil, containing menthol and menthone as the main components, was repellent and toxic to both species. Essential oil components, such as citronellic acid, carvacrol, and geranial, can potentially be used as repellents for cockroach prevention (Steltenkamp et al.,

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1992). Both thyme and rosemary oils have been found to be repellent to *Myzus persicae*, and the essential oil constituents linalool, camphor and  $\alpha$ -terpineol demonstrated repellency at higher concentrations (Hori, 1998).

In repellency assay with *C. citratus*, adult *L. tristis* that were given the choice between untreated controls and essential oil treated filter paper discs significantly chosen the control areas. This suggests that *L. tristis* is able to detect the repellent sources through olfaction and avoid them when given the choice. As a result of their highly volatile nature, essential oils are not persistent in the environment and are less likely to leave residues on food products. These favorable properties of *Cymbopogon* oils suggest that products based on them may be viable options as a part of IPM in residential building with *L. tristis* infestation.

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