



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2019; 7(1): 206-209

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Received: 25-11-2018

Accepted: 29-12-2018

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## *In vitro* insecticidal activities of essential oil of Lemon grass against house fly: *Musca domestica* L.

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**Abstract**

Essential oil was extracted from the fresh leaves of Lemon grass (*Cymbopogon citratus*) by hydro-distillation using Clevenger apparatus at 80° C for 3 - 4 hrs. One hundred gram of fresh leaves yielded maximum of 1.5 to 2 ml of oil and GC-MS analysis revealed 27 different components. *In vitro* efficacy of essential oil against larvae, pupae and adult flies at different concentrations viz., 50, 100, 150, and 200 µl was evaluated. The mortality of larvae, pupae and adult flies had increased with the concentration of the oil increasing. The LD<sub>50</sub> values of oil against larvae and pupae were 29.58, and 18.26 µl respectively. In the control group, 10.30 ± 0.31 and 12.30 ± 0.22 per cent mortality of larvae and pupae respectively were observed. The fumigant toxicity against adult flies showed that the highest concentration of 200 µl caused 100 per cent mortality in 4.3 ± 0.11 minutes against no mortality of flies up to 2 days in the control group. The dead larvae from treated group appeared shrunken and black in colour, while histopathological study revealed bleb formation and intestinal cell damage.

**Keywords:** *Cymbopogon citratus*, essential oil, house fly, *In vitro* efficacy, *Musca domestica*

**Introduction**

The house fly, *Musca domestica* L is a major pest that causes irritation, spoils food, and acts as a vector for more than 100 pathogens of medical and veterinary significance Kumar *et al.* [6]. To control this challenging pest, various classes of insecticides and insect growth regulators are being used in the poultry farms. But, prolonged usage of chemical insecticides has led to emergence of resistant strain, environmental pollution threats of persistence and bio magnifications through the food chain, and development of resistance in insects and residues in products emphasize the need for eco -friendly alternatives. In search of alternative control agent, essential oils with its reported efficacy against various pests, target specificity, and safety to human beings could be an option. Products obtained from certain medicinal plants have proved as alternatives to synthetic chemicals Nithiyagowry [10].

*Cymbopogon citratus* is a native of India and Sri Lanka Zheng *et al.* [17]. The various parts of lemon grass are used for several herbaceous preparation as well as medical remedies Katsukaawa *et al.* [4]. The insecticidal activity of *Cymbopogon citratus* essential oil has been reported against several agricultural Reitz *et al.* [12], Ishilii *et al.* [2], and non agricultural pests. The insecticidal activity of *Cymbopogon citratus* oil has been attributed to its monoterpenes content and composition. Citral, the major *Cymbopogon citratus* oil component, has been implicated as fumigant agent against *Culex pipiens quinquefasciatus*, Yang *et al.* [16], while citral and geranial have been demonstrated for their antifeedant activity against three species of mosquitoes *C. pipiens pallens*, *C. pipiens quinquefasciatus* and *Aedes albopictus* Leal *et al.* [8]. Although the above studies established that essential oil of *Cymbopogon citratus* was a potent insecticide, there have been limited studies on efficacy against house flies. Thus, the present study on insecticidal properties of essential oil of *Lemon grass* (*Cymbopogon citratus*) was evaluated against house flies.

**Materials and Methods****Extraction and analysis of the essential oil**

Essential oil was extracted from the fresh leaves of Lemon grass by hydro -distillation (Figs.1) as per method described by Radunz *et al.* [12].

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**Fig 1:** Extraction by Clevenger apparatus

The essential oil was then submitted to the Indian Institute of Crop Processing Technology (IICPT), Government of India, Thanjavur, Tamil Nadu, for chemical analysis. The oil was stored in glass vials at 4° C until use.

### ***In vitro* trials**

#### **Efficacy of essential oils against larvae**

*In vitro* trials were conducted to evaluate larvicidal properties essential oil of lemon grass. In these trials, 50, 100, 150 and 200 microlitre of essential oils were dispensed into four glass vials containing 1 ml of acetone each and mixed well. The oil dissolved in acetone was then transferred to specimen containers containing 24 ml of water in order to obtain 0.2, 0.4, 0.6 and 0.8 per cent concentrations. These diluted essential oils (25 ml) were then added to 4 plastic containers containing 25 g of larval medium for treatment group, while acetone mixed water was used to prepare control larval medium. One hundred number of third stage larvae were seeded into oil treated and control group container. Each trial was monitored for 4 days and larval mortality was recorded.

#### **Efficacy of essential oils against pupae**

A total of six *in vitro* trials were conducted to evaluate efficacy against pupae of house flies. In these trials 100 numbers of 3 - 4 days old pupae were seeded into plastic containers containing treated and control larval medium and thoroughly mixed. Each trial was monitored for 4 days and adult fly emergence was recorded.

#### **Efficacy of essential oils against adult fly – Fumigation**

Six trials were carried out to determine the adulticidal properties of essential oil against house flies by method as described by Wang *et al.* (2001) [15] with some modifications. In these trials, the cotton balls soaked in 50, 100, 150 and 200 microlitre of essential oil in 1 ml of acetone solution, were placed into a glass jar containing 100 flies and monitored for fly mortality for half an hour

#### **Statistical analysis**

The data collected in the *in vitro* trials were analyzed by probit analysis to determine the LD<sub>50</sub> values of oils against different stages of house flies Fenny [1].

#### **Results and Discussion**

In the present study, the fresh leaves of lemon grass yielded maximum of 1.5 - 2.0 ml of oil. Whereas in the earlier study a maximum of 0.5 ml of essential oil was extracted from Lemon grass using steam distillation method Sathish Kumar [14]. The difference in the oil yield may be due to method of distillation employed.

In this study, the gas chromatography analysis revealed the presence of 27 different compounds, of which Limonene (7.09%), Eucalyptol (13.90%), β-Citral (21.03%), Citral (32.49%) and Geraniol acetate were found in high level, while 3- Cyclohexen-1-ol, 4-methyl -1- (1- methyl ethyl)-(R) (3.99%), Camphor, [(1R,4R)-(+)-] (2.37%) were found low quantity (Table.1).

**Table 1:** Components of essential oil of lemon grass

S. No	RT	Name of the compound	Percentage
1.	2.04	Camphene	1.80
2.	2.45	Limonene	7.09
3.	2.52	Eucalyptol	13.90
4.	2.96	Fenchone	1.03
5.	3.13	Camphor, [(1R,4R)-(+)-]	2.37
6.	3.80	Borneol	0.53
7.	4.56	β-Citral	21.03
8.	4.63	3- Cyclohexen-1-ol, 4-methyl -1- (1- methylethyl)-(R)	3.99
9.	4.96	Citral	32.49
10.	5.14	Geraniol formate	1.57
11.	6.10	Geraniol acetate	5.46
12.	6.69	Caryophyllene	1.10
13.	8.20	Propanoic acid, 2-methyl-, 3,7 dimethyl- 2,6- Octadienylester, (E)	1.03
14.	8.69	Caryophyllene oxide	1.66

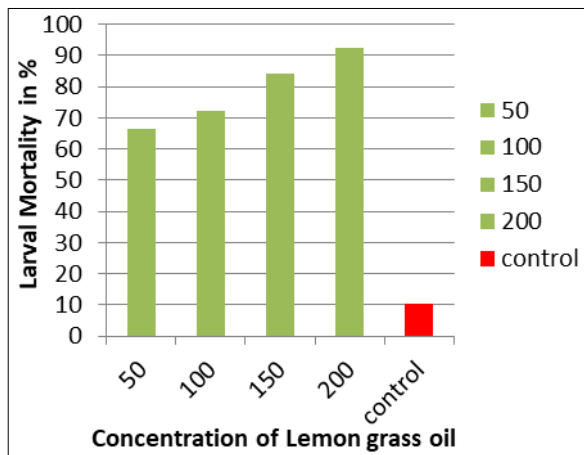
Kumar *et al* [5], also stated that Citral, 1,8 – Cineole were identified as major compounds in the gas chromatography analysis of essential oil of lemon grass. Further, the quantity and composition of essential oils may be influenced by various factors such as isolation method, climate, soil composition, plant organ, age and vegetative cycle Kumar *et al.* [6]. In the present study, essential oil of lemon grass at different concentrations *viz.*, 50,100,150 and 200 µl caused 66.50 ± 0.52, 72.16 ± 0.24, 84.16 ± 0.25, 92.66 ± 0.23 per cent mortality of larvae (Figs.2) respectively with the LD<sub>50</sub>

value of 29.58 against 10.30 ± 0.31 mortality in control group. The percentage mortality of larvae increased with the increasing concentration of oil with LC<sub>50</sub> value of 104 ppm in *in vitro* trials conducted by direct dipping of larvae into the essential oils Morey *et al.* [9].

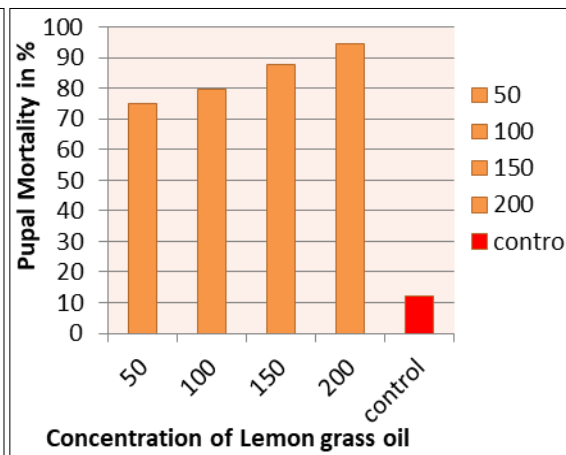
In this study, essential oil of lemon grass produced 75.00 ± 0.31, 79.83 ± 0.31, 87.60 ± 0.27 and 94.5 ± 0.31 per cent mortality of house fly pupae (Figs.4.) at the concentrations of 50,100,150 and 200 µl respectively with the LD<sub>50</sub> value of 18.26 µl against 10.16 ± 0.28 per cent mortality in control

group. The LD<sub>50</sub> values obtained in the present study, albeit the difference in the plant oil, were much lower than the LD<sub>50</sub>

values of lemon grass oil (486 µl/ L) against pupae in fumigation assay Kumar *et al.* [7].



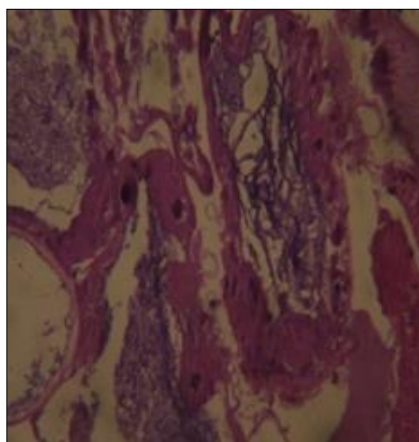
**Fig 2:** Larval mortality in response to Lemon grass oil at different concentration



**Fig 3:** Pupal mortality in response to Lemon grass oil at different concentration

Histopathological changes that include bleb formation (Figs. 3) and damages in the intestinal wall of larvae were observed in the present study. The intestinal damages in the larvae might have occurred as a result of detrimental effect of essential oils against digestive enzymes and subsequent destruction of midgut epithelium Kumar *et al.* [6]. In this study, the different concentration of oil *viz.*, 50, 100, 150 and 200 µl took 13.5 ± 0.21, 09.10± 0.24, 6.5 ± 0.18 and 4.3 ± 0.11 minutes to cause 100 per cent mortality in adult flies as opposed to no mortality in control group up to 2 days

(Figs.5.). Further, the adult flies showed behavioural changes such as convulsion, hyperextension of legs and abdomen and lying on their back and died. Similar symptoms in house flies were recorded when treated with pure monoterpenes Picollo *et al.* [11]. The occurrence of neurological symptoms is attributed to activation of receptor of octopamine, which is a neuromodulator and induce hyperextension of the legs and abdomen by increasing frequency of the legs. Further, the fumigant toxicity of the oil is also dependant on the volatility Isman *et al.* [3].



**Fig 4:** Larva from treated group showing bleb formation (H&E stain 400x)



**Fig 5:** Dead fly lying at the of bottom of the bottle

**Conclusion**

*In vitro* study on insecticidal efficacy of essential oil of Lemon grass (*Cymbopogon citratus*) against house fly revealed that the efficacy was found to be proportional to the concentration of the oil. The histopathological changes observed in the dead larvae indicate that mortality of larvae might to intestinal damage.

**Acknowledgement**

The authors are thankful to the Dean, Veterinary College and Research Institute, Namakkal for facilities provided.

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