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Comparative Evaluation of Lemon Grass and Orange Essential Oils as a Green Pesticide against House Flies *Musca domestica* in India

N. Rani[1, 2*], G. Ponnudurai[1, 2] and Aditi Kalita[1, 2]

[1]*Department of Veterinary Parasitology, Veterinary College and Research Institute, Namakkal, TANUVAS, Tamil Nadu, INDIA*
[2]*Veterinary College and Research Institute, Namakkal, TANUVAS, Tamil Nadu, INDIA*

**Corresponding author: N Rani; E-mail: ranideepan99@gmail.com*

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ABSTRACT

The house fly *Musca domestica* is a cosmopolitan insect associated with vectoring of etiological agents. Pest management strategies for house flies are needed. Chemical control method commonly used against this pest, though effective, has some disadvantages, such as development of insect resistance and bioaccumulation. Presently bioinsecticides, especially those derived from plants have been increasingly evaluated in controlling insect of medical and veterinary importance. In order to search for effective and ecofriendly control agents, the essential oils of Sweet orange (*Citrus sinensis*) and lemon grass (*Cymbopogon citrates*) were evaluated larvicidal, pupicidal and adulticidal activity against house flies. The two essential oils were Gas chromatography / mass spectroscopy analysis of two oils showed that Citral (32.9 %), β-Citral (21.03 %), Eucalyptol (13.9%) and Limonene (7.09%) as main components of lemon grass. While Limonene (53.31%), Carvol (7.74%), *Cis*-carveol (5.54%) were the major constituents of Orange EO. The $(LD)_{50}$ value for larvicidal activity of lemon grass and orange were 29.58 µl & 33.28 µl respectively. The $(LD)_{50}$ value for pupicidal activity of the lemon grass was 18.2 µl while orange oil $(LD)_{50}$ = 48.27 µl . In the adulticidal activity assay to determine the Eos of lemon grass and orange at the concentration of 200 µl took a minimum time of 4.3 ± 0.11 minute to cause 100 per cent fly mortality followed by orange oil is 10.5 ± 0.22 minutes. The result revealed that the essential oils of Lemon grass and orange oil have possessed larvicidal, Pupicidal and adulticide properties against *Musca domestica*.

HIGHLIGHTS

- *Musca domestica* is the most common pest in poultry farms in India.
- House fly is known to transmit many pathogens.
- Essential oils of *Citrus sinensis* and *Cymbopogon citrates* has larvicidal, pupicidal and adulticidal activity against house flies.

Keywords: *Citrus sinensis, Cymbopogon citrates,* essential oils, larvicidal, pupicidal and adulticidal

The housefly, *Musca domestica* L. (Diptera: Muscidae) is a common pest in India. Adult house flies has been shown to transmit more pathogens (De Jesús *et al*., 2004) such as protozoa cysts, helminth parasites, enteropathogenic becteria and enterovirus (Tarelli *et al*., 2009 and Hanan, 2013). The housefly is categorized as an important contributing factor in the dissemination of various infectious food-borne diseases, such as cholera, typhoid, shigellosis, bacillary dysentery, tuberculosis and infantile diarrhoea in human populations (Olsen *et al*., 2001). In view of this and nuisance caused by the flies and vector potentiality for suitable methods for housefly control are warranted.

Many insecticides such as organochlorines and organophosphates, and more recently pyrethroids and spinosad, have been used for housefly control. However,

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houseflies have developed resistance to these pesticides (Scott *et al*., 2000) and health and environmental risks associated with these compounds, necessitated alternative methods of fly management. In this sense, essential oils (EOs) and natural terpenes (Ts) are potential alternatives and environmental friendly insecticides (Regnault-Roger, 2000; Shono *et al*., 2003).

Eos compounds and their derivatives are considered to be alternative means of controlling many harmful insects and rapid degradation in the environment has increased specificity that favours beneficial insects Essential oils have been shown to be relatively nontoxic to fish, birds and mammals and easily biodegrade in the environment (Kumar *et al.,* 2012b) Essential oils have been used in sensitive areas, such as homes, schools, restaurants, and hospitals (Batish *et al*., 2008; Palacios *et al*., 2009b). Data on the insecticidal activity of EOs from *Citrus sinensis* and *C. aurantifolia* peel showed 70% fumigant effectiveness in killing house flies in a room after 60 min of spraying (Ezeonu *et al.*, 2001). Eos from plants reported to have insecticidal effects against adult houseflies include *Minthostachys verticillata*, *Hedeoma multiflora*, *Citrus sinensis*, *Citrus aurantium*, *Eucalyptus cinerea* and *Artemisia annua* with LC_{50} values of 0.5, 1.3, 3.9, 4.8, 5.5 and 6.5 mg/fly at 30 minutes, respectively (Palacios *et al*., 2009 a, b). The insecticidal properties of lemon peel, grapefruit, and navel orange citrus oils against adults and larvae of *M. domestica* have been investigated and grapefruit peel oil was toxic to *M. domestica* adults while lemon oil was toxic to larvae. Piper betel EO showed a fumigant LC_{50} of 10.3 mg/dm^3 in a 24-h exposure period (Mohottalage *et al.,* 2007). More recently, 34 EOs were screened against the housefly, and Pogostemon cablin EO was found to be the most potent topical insecticide, with an LD_{50} of 3 µg/fly; *Mentha pulegium* oil was the most potent fumigant insecticide (LC_{50} = 4.7 µg/cm^3) (Pavela, 2008; Soonwera, 2015) determined to study the effect of essential oils from herbal plants to control houseflies revealed that the essential oil from *S. aromaticum* showed the excellent larvicidal and oviposition deterrent activity against larvae and females of house flies and these results exhibited higher toxic than cypermethrin In this way, number of essential oils shows insecticidal properties against different insect pest so that there is an increase interest in developing plant origin insecticides.

Mode of action of essential oil

Essential oils interfere with basic metabolic, biochemical, physiological, and behavioral functions of arthropods. They inhale, ingest or absorb essential oils. The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine (Enan *et al*., 2001) or GABA-gated chloride channels (Khater, 2011) Some essential oils have larvicidal effects and the capacity to delay development and suppress emergence of adults of insects of medical and veterinary importance (Khater *et al.,* 2008, 2011; Koul *et al.,* 2008; Kumar *et al.,* 2010). The objective of the present study, was to assess the larvicidal, adulticidal, pupicidal effect of Lemon grass and Orange essential oil on housefly, (*M. domestica)*.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves of lemon grass and peels of sweet orange were used in this study. The fresh leaves of lemon grass were collected from Veterinary College and Research Institute, Namakkal farm campus, while peels of sweet orange were collected from fruit juice stall in Namakkal.

Extraction of essential oil

In this study, the essential oils were extracted by hydrodistillation method as described by (Radunz *et al.,* 2002) with some modifications. The fresh leaves and peels were cut into small pieces and approximately 100 g of cut leaves were placed into a 1000 ml boiling flask and 500 ml of water was added to this. The leaves containing flask was placed on heating mantle and then carefully fixed with Clevenger apparatus. The Clevenger apparatus was then connected with water tap to ensure continuous circulation of water during extraction of oil. The contents were boiled at 80° C for 3 – 4 hrs; the essential oil produced during this process got collected above the water in the graduated collecting tube. The oil was then collected in glass vials containing 4 % anhydrous sodium sulphate and stored at 4° C until use.

Chemical analysis of essential oils

The essential oils that were extracted from the plants chosen for this study were submitted to the Indian Institute of Crop Processing Technology (IICPT), Government of India, Thanjavur, Tamil Nadu, for chemical analysis. The given samples were diluted with chloroform and essential oil component analysis was performed by Gas Chromatography/ Mass spectroscopy (GC/MS) using the equipment GC Clarus 500 Perkin Elmer. The temperature programme was 110°-280° C, 5 - 10° C/ minute. Helium was used as carrier gas with flow rate of 1 ml / minute, split: 10:1. Chiral analyses were performed with the same temperature programme.

The mass spectra were obtained at an ionization voltage of 70 eV. The identification of compounds in the chromatographic profiles was achieved by comparison of their mass spectra with a library data base (NIST version 2005). The percentage composition of oils was computed by area normalization method GC peak areas calculated as the mean value of two injections at the rate of 2 µl from each oil.

Maintenance of house flies colony

Adult flies were collected from the poultry farms using sweep net and brought to the fly control laboratory of this department. The flies were then carefully released into a 30 × 30 × 30 cm wooden frame chamber covered on three sides with netlon while front side covered with glass in order to monitor the fly breeding and there is a provision to place food for flies and collection of larvae and pupae. The flies were reared at room temperature under a 12:12 light and dark cycle. Approximately 100 g of larval medium, made of cattle feed 50 g, yeast 1 g and water 49 ml, in a plastic container was placed into the breeding chamber as food for adult flies and as substrate for larval stages. The third stage larvae, pupae and adult flies produced in the colonies were used for conducting *in vitro* trials

Efficacy of essential oils against larvae

A total of 6 *in vitro* trials were conducted separately for each essential oil to evaluate their larvicidal properties against larvae of house flies. In these trials 50, 100, 150 and 200 microliter of essential oils were poured into four glass vials containing 1 ml of acetone each and mixed well. The oil acetone mixture was then transferred to specimen containers containing 24 ml of water in order to obtain 0.05, 0.1, 0.15 and 0.2 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 6 *in vitro* trials were conducted separately for each essential oil to evaluate their 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 medium and thoroughly mixed. Each trial was monitored for 4 days and adult fly emergence was recorded.

Pathological study

The dead and live larvae were collected from the treated and control group to determine the pathological changes caused by the essential oils. The dead and live larvae were processed and stained with haematoxylin and eosin stain and examined under binocular research photomicroscope.

Efficacy of essential oils against adult fly – Fumigation

A total of six trials were carried out to determine the adulticidal properties of each essential oils against house flies by method as described by (Wang *et al*., 2001) with some modifications. In these trials, the cotton balls soaked in 50, 100, 150 and 200 microliter of essential oil dissolved in 1 ml of acetone solution, were put 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_{50} values of oils against different stages of house flies (Denham, 2016).

RESULTS

In the present study, the fresh leaves of lemon grass yielded maximum of 2.0 -2.5 ml Eos. While the sweet orange peel yielded 5 - 7 ml of oil. The essential oil of lemon grass leaves and peels of sweet orange were analysed by GC – MS method at IICPT, Thanjavur. Lemon grass oil was characterized by the presence of 27 compounds which formed 98.88% of the total oil components. Chemical analysis of lemon grass oil showed β-Citral (21.03%) and Citral (32.49%), Eucalyptol (7.09%) geraniol acetate (5.46 5), camphor (2.7%) as the major components (Table 1). Analysis of sweet orange essential oil revealed 19 compounds, accounting for 94.42% of the total oil components with Limonene (53.31%), Carvol (7.74%), Cis-carveol (5.54 %) were the major constituents (Table 2).

Table 1: Major components of essential oil of lemon grass

Sl. 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

The results of the present study revealed that the essential oils of orange and lemon grass has significant larvicidal, pupicidal and adulticidal activity against *Musca domestica*. The different doses were used to obtain mortality. Orange and *lemon grass* Eos were tested against third instar larvae and 4th day old pupa of *M. domestica*. In larvicidal and pupicidal assay, it was found that lemon grass oil was highly effective as compare to orange oil. It was found that as the dose of both essential oils increased the % mortality increased. The results of larvicidal and pupicidal activity of Eos were shown in. The LD_{50} value of lemon grass and orange oil were 29.58 µl and 33.28 µl against larva of house fly respectively. The LD_{50} value of lemon grass and orange oil were 18.26 µl and 48.27µl against pupae of house fly respectively. It was found that with increase in dose, % mortality also increased it shows significant $P < 0.01$ when compare to control.

Table 2: Major components of essential oil of orange

Sl. No.	RT	Name of the compound	Percentage
1	2.17	β-Pinene	1.47
2	2.55	Limonene	53.31
3	3.36	Limonene oxide, cis	3.51
4	3.41	Limonene oxide, trans	5.02
5	4.33	*Cis*-carveol	5.44
6	4.65	Carvol	7.74
7	5.96	1,2-Cyclohexanediol, 1-methy-4 [methylethenyl	3.79
8	6.03	α- Farnesene	3.64
9	6.24	Tetrahydrofuran-2-ol,3,4-di [1-butenyl]-	2.90
10	7.10	7-Oxabicyclo[4.1.0] heptane	1.19

Dead larvae from the essential oil treated and control group were collected to determine the gross and histopathological changes produced by the essential oils. The dead larvae from treated group appeared shrunken and black in colour, while histopathological study revealed bleb formation and intestinal cell damage. The larvae from control group showed no such abnormal changes.

Whereas in the adulticidal assay, the efficacy of oil was determined based on the minimum time taken for 100 per cent fly mortality by the different concentration used. Among the two oils, Lemon grass oils at the concentration of 200 µl took minimum time of 4.3±0.11 minute to cause 100 per cent fly mortality while orange oil took 10.5±0.22 minutes. The essential oils of lemon grass were found to possess high fumigant toxicity than orange oils, because lemon grass oils took only 4 minutes to cause 100 per cent mortality.

In the present study, the adult flies showed behavioural changes such as convulsion, hyperextension of legs and abdomen, lying on their back and died.

DISCUSSION

Kumar (2010) stated that a maximum of 0.5 ml of essential oil was extracted from lemon grass using steam distillation method. The difference in the oil yield may be due to method of distillation employed. These observation do not corroborate with the findings of Zewde and Jenebere (2010) who extracted an average of 7.4 ml from 1 kg of fresh peels of orange by hydrodistillation. This wide variation in the oil yield might be due to variation in the origin (geographical region), climate and age of the plants. Saeb *et al.* (2012) reported that the amount and composition of essential oil strongly dependent on the developmental stage of plant (ontogeny) and therefore harvesting time is one of the most important factor influencing the oil quality.

In this study, 1,8- Cineole was not recorded in lemon grass oil, is highly in agreement with the findings of Kumar *et al*. (2013) who also identified Citral and 1,8-Cineole as the major components in the lemon grass. In the present study orange oil revealed high limonene content, this finding is in consonance with finding of Michaelakis (2009) and Karamaouna *et al.* (2013). The quantity and composition of essential oils may be influenced by various factors such as distillation method, climate, soil composition, plant organ, age and vegetative cycle. Further, the occurrence of quantitative and qualitative variations in the essential oil is attributed to the isomerization, saponification and other reactions that may occur under distillation conditions (Kumar, 2012 a). A earlier reports showed that the major chemical component of citrus oils was limonene, ranging from 32 to 98%, with sweet orange containing 68 98%, lemon 45 76% and bergamot 32 45% In another study on the characteristic odour components in the essential oil from *F. japonica* peel, limonene was the most dominant component, amounting to 93.73% (Choi, 2005).

Begum *et al.* (2011) reported that larvicidal activity of crude extracts of *Calotropis procera* and *Annona squamosa* against *M.domestica* $(LC)_{50}$ = 282.5 and 550 ppm respectively). Seo and Park (2012) studied the larvicidal activity of extracts from 27 plant species against *M.domestica*, and results revealed that *Phryma leptostachya* showed 100% larvicidal activity at 10 mg/g concentration. Paveala *et al*. (2008) reported that *Satureja hortensis*, T*hymus vulgaris*, *Salcia offenalis* and *Mentha piperita* caused 50 % mortality in fourth instar larvae of *M. domestica* at the concentration of 193, 256, 500 µl. Morey and Khandagle (2012) revealed that *M. piperita* essential oil had the highest larvicidal properties with a LC_{50} value of 104 ppm at 1% concentration. Mohottalage *et al*. (2007) investigated the insecticidal properties of lemon peel, grape fruit and navel orange oils against adult and larvae of *Musca domestica* and found that grape fruit oil was toxic to adult *Musca domestica* while lemon oil was toxic to larvae.

In the pupicidal assay, Sinthuri and Soonwera (2010) obtained 100 per cent pupal mortality through *in vitro* method by direct spraying of oils on the pupae. Despite pupae being seeded into oil incorporated medium as has been done in the present study, 98 per cent mortality was observed. This indicates that the efficacy of essential is not only determined by various factors pertaining to the plant species but also the method used for testing.

In contrast to the observations of the present study Samarasekara *et al*. (2006) recorded knocking down of flies in 1 hr after topical application of essential oils of lemon grass. Although oils were directly applied on the flies in their study, the time required to cause mortality in flies was very long. This significant variation in time taken to cause mortality may be due to difference in the chemical constituents of oils used in present study and earlier studies. Park and Shin (2005) reported that fumigation toxicity, clove oil showed 100 % mortality in Japanese termites at 0.5 µl/ L of air. The orange peel oil has been reported to have fumigant toxicity 13 times more than that of methyl bromide (Tripathi *et al*., 2003).

CONCLUSION

In the present study, it was found that lemon grass and orange essential oils from Indian herbs showed high potential as larvicide, pupicide and adulticide for house fly control. In this study the two essential oils have high potential for development of new product or green product to house fly management. Finally, the green products based on herbal essential oils is considered environmentally safe and safer human health and alternatives to insect pest control with chemical insecticides, and could be a good option for Indian people.

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