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Essential oil from *Citrus grandis* (Sapindales: Rutaceae) as insecticide against *Aedes aegypti* (L) (Diptera: Culicidae)

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Abstract
Study on the usage of essential oil for the control of mosquitoes transmitting vector borne diseases has been receiving renewed interest for last two decades as they are target specific and environmentally safe as compared to the synthetic products. In the present investigation, an attempt was made to evaluate the efficacy of essential oil from the leaves and peel of *Citrus grandis (C. grandis)* against different developmental stages of dengue vector *Aedes aegypti*. Results showed that the essential oils from the leaves possess more ovicidal activity (LC50 value 9.56ppm) than the peel at 72 hour. As larvicidal, the essential oil from the peel of *C. grandis* showed more effect with LC50 value 61.71ppm than the leaves (LC50 value 126.45ppm at 72 hour) of the plant. There was not much adulticidal potential recorded in oil extracted from leaves but the oil extracted from peel showed moderate adulticidal activity with LC50 value 174.96ppm against the mosquito. Thus it can be inferred that the essential oil from *C. grandis* is a potential candidate for the control of different developmental stages of *Aedes aegypti*.

Keywords: *Citrus grandis*, *Aedes aegypti*, Essential oil, mosquitocidal,

1. Introduction
Research relating to mosquitoes receives a great attention now-a-days in the whole world as they transmit certain pathogens to human. Among those diseases, dengue is an important one as its occurrence has been increasing year after year across the globe. Due to presence of 4 different serotypes of DEN virus, it is still difficult to develop effective vaccines against dengue as preventive measure. Hence to control this disease, the destruction of vector mosquito is a one and only useful step. *Aedes aegypti* is the common vector of dengue due to their prominent breeding capacity in very small or artificial containers [1].The approach to overcome the serious obstacles is based on the interruption of the disease transmission cycle either by targeting the early developmental stages or by killing or repelling the adult mosquitoes using insecticides [2]. Different chemical insecticides are available in market and are being used against the vector mosquitoes but they possess various side effects like long persistency, non-target specificity and development of resistance. Considering these disadvantages modern people seem to give more importance on the concept of natural products.

Plants are sources of alternative agents for control of various harmful insects as they are rich in bioactive compounds. Plant based natural insecticides mainly comprise of extract or essential oil, which are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment [3-5]. Thus in the present concept of green insecticide, some rational attempts have been made to include substances such as plant extracts, essential oils and toxins from organic origin. Several plants were used to study their efficacy against different development stages of vector mosquitoes [6-9].

Essential oils are volatile natural complex secondary metabolites having a strong odour. Due to lipophilic property, they can interfere with various physiological systems of an organism [10-12]. So, essential oils and their derivatives can be used as an alternative of synthetic insecticides.

Under the Rutaceae family, Citrus genera have attracted a great interest due to its prominent insecticidal properties. Among different constituent compound of essential oils from the

Rutaceace family, Limonoids have attracted greater attention as they possess growth regulating, feeding and oviposition deterrent activities [13-15]. Although, insecticidal, anti-microbial or antioxidant properties of extracts or essential oil from peel or seeds of some species of the Citrus genera had been studied [15-21], but study regarding the efficacy of the essential oil of *Citrus grandis* against mosquitoes are very few. Therefore, the present study was aimed at evaluating the potentiality of essential oil from the peel and leaves of *C. grandis* against different developmental stages of *Ae. aegypti.*

2. Methodology

2.1 Rearing of *Ae. aegypti*

The eggs of *Ae. aegypti* were collected from ICMR, Dibrugarh, Assam and reared in insect culture room, Dept. of Zoology, Gauhati University by following the method described by Arivoli *et al* [22] (25–29 ºC temperature and 80–90% relative humidity). Larvae and adults were fed on powdered dog biscuit and yeast (3:1) and 10% glucose solution respectively. After pupation, pupae were kept inside the cage and after 5th day from hatching, adult female mosquitoes were blood-fed on restrained albino rat.

2.2 Collection of plant materials

The fruits and leaves of *Citrus grandis* were collected from the Nalbari district, Assam.

2.3 Extraction of Essential oil

The essential oil was extracted from the plant with the help of hydro-distillation method using Clevenger apparatus.

2.4 Bioassay of essential oils

2.4.1 Larvicidal assay

The larvicidal activity of individual essential oil was assayed according to WHO guidelines [23]. Twenty numbers of healthy 4th instar larvae were transferred to disposable cups (depth 5-10cm). A wide range concentration (10-1000ppm) was used in triplicate to record the sub lethal concentration (LC50) along with one negative control (water) and one positive control (DMSO). If the pupation occurred or 10% larvae died in the control group in the exposure time then the test was repeated. The mortality in the control groups if occurred between 5-10% then, the mortalities of treated group was corrected by using Abbot's formula [24].

$$\text{Mortality (\%)} = \frac{x-y}{x} \times 100$$

Where x =percentage survival in control group
y = percentage survival in treated group

2.4.2 Ovicidal assay

The ovicidal bioassay was performed following the Samidurai *et al* [25] with little modifications. For this bioassay, 50 eggs of the *Aedes aegypti* were transferred to disposable plastic cup (depth 2.5cm) and different concentrations (10-1000ppm) of the essential oil (using DMSO as solvent) were applied. Three replications were set against each concentration with one positive control (DMSO) and one negative control (water). The percentage of ovicidal activity after 72h was calculated by the following formula-

$$\text{Percent ovicidal activity} = \frac{\%\,of\ eggs\ hatched\ in\ control - \%\,of\ eggs\ hatched\ in\ treated}{\%of\ eggs\ hacted\ in\ control} \times 100$$

2.4.3 Adulticidal assay

For the adulticidal bioassay, the impregnated filter paper bioassay method described by Ramar *et al* [26] was followed with some modifications. For this assay, three replicates with10 numbers of 4-5 days old non blood fed female mosquitoes in each group were made. Based on the preliminary screening, the different concentration of selected essential oil was prepared in 2 ml of acetone and applied on Whatman no.1 filter papers (size 12 x15 cm^2) placed in exposure tubes (depth 10cm) where only acetone was used in control group. 4-5 days old sugar fed mosquitoes were transferred to each tube after the evaporation time of 10 minutes of acetone. The mortality was recorded upto 72 hours. If mortality exceeds 20% in the control batch, the whole test was rejected. Again, if mortality in the controls was above 5%, results with the treated samples were corrected using Abbott's formula [24]

$$\text{Mortality (\%)} = \frac{x-y}{100-y} \times 100$$

Where X = the percentage mortality in the treated sample and Y = the percentage mortality in the control.

2.5 Statistical analysis

The LC50 values of different bioassays of this oil were calculated by probit analysis with the help of the SPSS and METLAB software.

3. Results

3.1 Larvicidal assay

During the study of larvicidal activity of essential oil from peel and leaves of *Citrus grandis*, different concentrations (10-1000ppm) of the oil were applied against 4th instar larval stages of *Ae. aegypti*. In the study, it was observed that the larval mortality was directly related to the exposure time and concentration of the essential oil. Larval mortality was recorded upto 72 hours. The results showed that the essential oil from the peel was more effective than the oil from leaf of *Citrus grandis* as larvicides (Table-1).

Table 1: Larvicidal activity of essential oil of *Citrus grandis* against *Ae. Aegypti*

Essential Oil	Time (Hour)	Lc50 (Ppm)	Regression Equation	Lower Bound	Upper Bound	Chi-Square Value
Peel (*C. grandis*)	24	228.49	Y=0.20+2.03x	1.688	2.402	60.990
	48	135.07	Y=0.38+2.17x	1.798	2.518	58.094
	72	61.71	Y=1.83+1.76x	1.805	2.526	37.656
Leaves (*C. grandis*)	24	157.51	Y=-2.43+3.38x	2.589	4.266	7.828
	48	142.83	Y=-2.67+3.56x	2.709	4.571	10.147
	72	126.45	Y=-2.74+3.73x	2.526	4.271	12.529

3.2 Ovicidal assay

Results of the ovicidal efficacy showed that the essential oil from leaf possess more ovicidal activities against *Ae. aegypti* as it showed less LC50 value than the peel of *Citrus grandis* (Table-3). During the ovicidal bioassay, hatching of eggs of target mosquitoes were observed from 24 hour to 72 hour but no more hatching of larvae was recorded after 72h after application of both essential oils. Therefore LC50 values were calculated at 72h of exposure. After applying 1000ppm concentration of these two oils, no hatching was observed in case of essential oil from the leaves while in the oils of peel, 28.58%, 51.73% and 52.8% hatching was recorded at 24 hour, 48 hour and 72 hour exposure periods respectively (table-2).

Table 2: Hatching percentage of eggs of *Ae. aegypti* at two different concentration of the essential oil of *C. grandis*(Peel and leaves)

Essential oil	Serial no	No of individual/replica	Concentration	24 hour (%±SE)	48 hour (%±SE)	72 hour (%±SE)
Peel (*C. grandis*)	1	50	1000 ppm	28.58±1.55	51.73±2.11	52.8±2.45
	2	50	100 ppm	42.86±0.58	70±0.33	72.5±0.58
Leaves (*C. grandis*)	1	50	1000 ppm	0±0	0±0	0±0
	2	50	100 ppm	25±1.17	32.07±1.79	32.07±1.79

Table 3: Ovicidal activity of essential oil from *C grandis* against *Ae. Aegypti*

Essential oil	Time (Hour)	Lc50 (Ppm)	Regression Equation	Lower Bound	Upper Bound	Chi-Square Value
Peel(*C. grandis*)	72	-	-	-	-	-
Leaves(*C. grandis*)	72	9.56	Y=4.00+1.02x	0.900	1.135	129.965

Cannot determined

3.3 Adulticidal assay

No effective adulticidal activity was observed of the essential oil from *Citrus grandis* (leaves) while little adulticidal efficacy was shown by the essential oil from peel against *Ae. aegypti* with LC50 value 174.96 ppm after 72 hour exposure time (table-5). The results of the adulticidal bioassay of both essential oils against the target species have shown that the efficacy was directly related to the concentration of the essential oils (table-4).

Table 4: Mortality percentage of the adults of *Ae. aegypti* at two different concentrations of the essential oil of *C.grandis*

Essential oil	Serial no	No of individual /replica	Concentration	24 hour (%±SE)	48 hour (%±SE)	72 hour (%±SE)
Peel (*C grandis*)	1	10	1000 ppm	100±0	100±0	100±0
	3	10	100 ppm	23.3±0.33	23.3±0.33	23.3±0.33
Leaves (*C grandis*)	1	10	1000 ppm	46.6±1.01	46.6±1.01	46.6±0.67
	3	10	100 ppm	0±0	0±0	0±0

Table 5: Adulticidal activity of essential oil of *Citrus grandis* against *Ae. Aegypti*

Essential Oil	Time (Hour)	Lc50 (Ppm)	Regression Equation	95%confidence level	95%confidence level	Chi-Square Value
				Lower Bound	Upper Bound	
PEEL (*C grandis*)	24	220.49	Y=-1.75+2.88x	2.125	3.342	78.175
	48	174.96	Y = - 2.59 + 3.38 x	2.727	4.440	11.808
	72	174.96	Y = - 2.59 + 3.38 x	2.727	4.440	11.808
LEAVES (*C grandis*)	24	-	-	-	-	-
	48	-	-	-	-	-
	72	-	-	-	-	-

Cannot determined

4. Discussion

Results of the current study revealed that the essential oil from the leaves and peels of *C. grandis* has promising insecticidal activity against the *Ae. aegypti* mosquitoes. The essential oils from leaves of *C. grandis* showed highest toxicity against the eggs of *Ae. aegypti* followed by the larvicidal and adulticidal activity. Previous studies reported that the eggs of mosquitoes were more susceptible to any stress than other developmental stages as the encapsulation of the eggs actually increased exposure to stresses by holding embryos in stressful condition while larvae or other stages could easily avoid these through passive dispersal or vertical migration [27].

Among the all bioassay, the essential oil from the peel of *C. grandis* showed the highest activity as larvicides followed by the adulticidal and ovicidal activity. Like other insects, the integument of the mosquito larva in their structure and biochemical constituents differs from the shell of eggs which may add difference in the penetration rate of different insecticides to the body [28]. Variation in the response to same essential oil by the same mosquito species in different developmental stages was due to physiological as well as morphological variations [29].

The major constituent compounds of the essential oil generally one of the important factors responsible for insecticidal activity of the particular essential oils [30]. Though Mansour *et al.* [31] already reported limonene as the dominant compound in different *Citrus* species but our previous study regarding the constituent compounds of essential oil of *C. grandis* revealed nootkatone and ß citronellol as probable major constituents of the essential oil from the peels and leaves respectively [32]. Geographic locations, method of

extraction, time of harvesting are some factors which influence those variations of the profile of constituents of an essential oil[14]. The variation of constituent compounds in different organs of the same plant was also supported by previous studies [33]. Variation in constituent compound of these two essential oils may be one of the responsible factors for their variation in mosquitocidal activity against the same target species- *Ae. aegypti*.
So, further study regarding the toxicity of the constituent compounds is necessary for more clear and fruitful result in the field of mosquito control.

5. Conclusion
The results indicated that the *C. grandis* (leaf and peel) derived essential oil can be safely incorporated in *Ae. aegypti* management programme to prevent the spread of dengue. However further studies regarding the mode of action and field trials are necessary to make them as promising candidate for dengue control.

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